NUTRITION THERAPY TO TREAT ZINC DEFICIENCY IN CELIAC DISEASE

A PILOT STUDY TO EVALUATE THE FEASIBILITY OF A ZINC-OPTIMIZED GLUTEN-FREE DIET COMPARED WITH ZINC SUPPLEMENTATION TO TREAT ZINC DEFICIENCY IN CELIAC DISEASE

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TITLE: A Pilot Study to Evaluate the Feasibility of a Zinc-Optimized Gluten-free Diet Compared with Zinc Supplements to Treat Zinc Deficiency in Celiac Disease

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Lay Abstract

Low Zinc (Zn) is common in patients with Celiac Disease (CeD). Zn supplements are often prescribed to treat low Zn; however, they have been associated with side effects and reduced absorption of other nutrients. Some patients prefer to make changes in their diet; however, this is challenging as anti-nutrients such as phytates block Zn absorption. Therefore, a diet that improves Zn would contain foods high in Zn and low in phytates, however it is unclear whether this diet is practical. I have been investigating how possible it is to implement a diet to improve Zn compared to Zn supplements in CeD patients with Zn deficiency. The preliminary analysis shows that patients can adopt this Zn diet for 3 months, however, the number of participants enrolled are less than expected, and adherence to the diet intervention is less than supplements. We have implemented strategies to improve recruitment of participants and adherence for the remainder of the trial.

Abstract

Background: Nutritional deficiencies are frequent in celiac disease (CeD), and one of the most common is zinc (Zn) deficiency. Supplements are often prescribed to treat Zn deficiency; however, they have been associated with adverse events and reduced absorption of other minerals. Data collected in our clinic showed that 38% of CeD patients would opt for a diet to improve Zn, however, such a diet may be challenging due to food interactions with phytic acid, which blocks Zn absorption. Therefore, the feasibility and efficacy of a Zn-optimized diet compared to supplementation is unknown.

Aims: To assess the feasibility of the protocol and collect data on estimated effect sizes for secondary outcomes to plan a properly powered randomized controlled trial (RCT).

Methods: We conducted an open-label, pilot RCT. CeD patients were randomized to Zn supplementation (Zn gluconate 25mg) or a Zn-optimized diet for 3 months and followed up with a 3-month pragmatic approach. We evaluated enrollment rates and adherence to both interventions. Plasma and urine Zn, stool samples, and questionnaires were collected pre- and post-intervention.

Results: We enrolled 28 participants and 16 of them have completed the study. Interim analysis shows an enrollment fraction of 26% (i.e. 28/108 eligible participants), and a dropout rate of 17.9%. Eighty-two % of participants allocated to the Zn-supplement intervention and 50% in the dietary intervention were compliant at 3 months. Based on the effect size for normalization of plasma Zn at 3 months, 142 participants are required for an adequately powered RCT in the future. There were no significant differences in

gastrointestinal or extra-intestinal symptoms, quality of life, anxiety and depression or adverse events between interventions.

Conclusion: Based on this preliminary analysis, recruitment of participants will take 6 months longer than expected. Assessment of reasons for diet non-adherence will allow implementation of strategies to improve feasibility.

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List of Symbols and Abbreviations

AE	Adverse Event
ALT	Alanine Transaminase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
APC	Antigen Presenting Cell
ASA24	Automated Self-Administered 24-Hour
BMI	Body Mass Index
CBC	Complete Blood Count
CeD	Celiac Disease
CeDAT	Celiac Disease Adherence Test
CeD-QoL	Celiac Disease Quality of Life
CI	Confidence Interval
CNS	Central Nervous System
CRF	Case Report Form
CRP	C Reactive Protein
CSI	Celiac Symptoms Index
DV	Daily Value
DGP	Deamidated Gliadin Peptide
DMT1	Divalent Metal Transporter 1
ELISA	Enzyme-Linked Immunosorbent Assay
ES-VAS	Extraintestinal Symptoms-Visual Analogue Scale
FNDDS	Food and Nutrient Database for Dietary Studies
GERD	Gastroesophageal Reflux Disease
GF	Gluten free
GFD	Gluten Free Diet
GI	Gastrointestinal
GIP	Gluten Immunogenic Peptide
HADS	Hospital Anxiety and Depression Survey
HiREB	Hamilton Integrated Research Ethics Board
HLA	Human Leukocyte Antigen
IgA	Immunoglobulin A
IBS-D	Irritable Bowel Disease-Diarrhea
IEL	Intraepithelial Lymphocyte
IFN- γ	Interferon-gamma
IgG	Immunoglobulin G
IL-21	Interleukin-21
MHC	Major Histocompatibility Complex

MUMC	McMaster University Medical Center
NCC	Nutrition Coordinating Center
OR	Odds Ratio
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PERMANOVA	Permutational Multivariate
PPI	Proton Pump Inhibitor
pZn	Plasma Zinc
PZMR	Phytate/Zinc Molar Ratio
RCT	Randomized Controlled Trial
RDA	Recommended Dietary Allowance
ROC	Receiver Operating Curve
SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
TCR	T Cell Receptor
TSH	Thyroid Stimulating Hormone
TG2	Transglutaminase 2
tTG	Tissue Transglutaminase
USDA	United States Department of Agriculture
V1	Visit 1
V2	Visit 2
V3	Visit 3
Zn	Zinc

Declaration of Academic Achievement

I declare that I have completed all required work included in this thesis under the supervision of Dr. Maria Ines Pinto-Sanchez. The progress and results of this thesis were presented annually at the Farncombe Research in Progress in 2022-2023 and 2023-2024. Additionally, in collaboration with Dr. Anil Verma we published "Comment on Chao, H.-C. Zinc Deficiency and Therapeutic Value of Zinc Supplementation in Pediatric Gastrointestinal Diseases. *Nutrients* 2023, *15*, 4093". This was in response to a paper on zinc deficiency in the pediatric CeD patients to spread awareness of zinc deficiency in the adult CeD population.

Preliminary results of this thesis, which include the zinc-pragmatic study were also presented at the Farncombe Institute Research Day (September 2023), the Canadian Digestive Disease Week Conference (March 2024) and the Digestive Disease Week Conference (May 2024). All other work that is not original, has been cited.

Chapter One: Introduction and Literature Review

1.11 Celiac Disease

Celiac disease (CeD) is a complex, multi-systemic, immune-mediated condition developed through a combination of environmental, immune, and genetic factors (Voisine & Abadie, 2021). To develop CeD certain conditions must exist such as exposure to the antigen gluten and the presence of Human Leukocyte Antigen (HLA) DQ2 or DQ8. However, additional co-factors are required to trigger the development of CeD such as pro-inflammatory gut microbes (i.e. bacteria, viruses), non-immunogenic proteins such as wheat amylase trypsin inhibitor, or other non-HLA genes such as IL-2 and IL-21 (Verdu & Schuppan, 2021).

After gluten digestion, peptides of gliadin, the alcohol-soluble fraction of gluten, cross the submucosa in the small intestine and become deamidated by the enzyme transglutaminase 2 (TG2) (Abadie et al., 2024). Deamidated gliadin peptides (DGP) bind to major histocompatibility complex (MHC) class II HLA variants DQ2/DQ8 on antigen presenting cells (APC) (Voisine & Abadie, 2021). APC's bind to specific CD4+T cells through the T cell receptor (TCR) eliciting a pro-inflammatory response characterized by the secretion of cytokines such as IFN-γ and IL-21 (Tye-Din et al., 2018). This results in the activation of intraepithelial lymphocytes (IELs) leading to injury of the small bowel mucosa and stimulation of a B-cell response producing CeD-specific antibodies such as anti-gliadin and anti-TG2 (Tye-Din et al., 2018).

1.12 Clinical Presentation of CeD

Clinical presentation of CeD includes gastrointestinal symptoms such as diarrhea, bloating, abdominal pain and distension (Adams et al., 2024). Extra-intestinal manifestations refer to non-intestinal symptoms associated with CeD including dermatitis herpetiformis, fatigue, headache, iron deficiency anemia, osteoporosis, delayed puberty, and short stature (Therrien et al., 2020). Many patients remain undiagnosed due to insufficient awareness and highly variable presentation of the disease. Despite adopting the GFD, around 30% of people with CeD will have persistent symptoms, and less than 10% of patients will achieve mucosal healing after one year on a GFD (Kivelä et al., 2021).

1.13 Diagnosis of CeD

Diagnostic criteria for CeD requires a combination of mucosal atrophy found in duodenal biopsies and positive serological testing for anti-tTG, anti-endomysium, and/or anti-DGP (Rubio-Tapia et al., 2023). There is currently no test that is 100% specific and sensitive, which is one reason that CeD must be confirmed by duodenal biopsies for an accurate diagnosis. However, there are many limitations of duodenal biopsies due to variability in size, site, and number of biopsy samples taken (Beig et al., 2021).

The relevancy for duodenal biopsies for diagnostic purposes has been challenged especially in the pediatric population. Pediatric patients with titers >10 times the upper normal limit for anti-tTG, anti-endomysium and/or anti-DGP antibodies with CeD

presenting symptoms may opt out of duodenal biopsies as established by the European Society of Paediatric Gastroenterology Hepatology and Nutrition (Husby et al., 2012).

1.14 Treatment of CeD and Limitations of GFD

The only available management of CeD is a strict avoidance of gluten, i.e., a gluten-free diet (GFD) which is difficult to maintain as it is expensive, socially inconvenient, and nutrient lacking. (Marciniak et al., 2021).

A study by Lee at al. (2019) showed that the cost of a well-balanced GFD is approximately 240% more than a wheat based well-balanced diet. This is because, products that naturally contain gluten such as bread, pasta, cereal etc. require further processing to remove the protein, gluten while ensuring it is still palatable (Aljada et al., 2021). Although gluten free (GF) processed foods are available at larger grocery stores, a study by Singh & Whelen (2011), identified that gluten-free processed foods at smaller budget and convenient stores lacked gluten-free alternative products, which poses as an additional barrier to patients with CeD.

Additionally, acclimating to the GFD may have a negative impact on one's quality of life. Qualitative research studies have shown that CeD patients on a GFD experience discomfort and psychological distress as they are constantly on alert for gluten cross-contamination, must plan all their meals in advance, and face unwanted visible awareness at social events and/or social isolation (Bacigalupe & Ploche, 2015). Patients with CeD should be provided with support through a psychologist to mitigate the psychological impact of CeD and improve long-term GFD adherence (Simón et al., 2023).

Lastly, numerous studies have shown that GFDs tend to be nutritionally imbalanced. Macro and micronutrients such as protein, iron, niacin, riboflavin, vitamin b12, zinc, selenium and fiber are deficient, while higher levels of saturated fats, cholesterol and sugars are often reported in gluten-free processed products (Taetzsch et al., 2018) This has resulted in patients with CeD having an increased risk of developing weight gain, obesity, and heart disease after implementation of the GFD (Niland & Cash, 2018).

1.21 Causes of Nutrient Deficiency in CeD

Nutrient deficiencies in treated and newly diagnosed CeD are common due to several reasons which include 1) malabsorption of nutrients due to villous atrophy and intestinal inflammation (Martín-Masot et al., 2019) 2) nutrient deficiency due to lack of fortification of GF processed products, (Bledsoe et al., 2019) and 3) food interactions such as phytic acid (Gibson et al., 2018) (Figure 1.1.).

Firstly, small bowel inflammation in CeD may lead to malabsorption of nutrients when the disease is active (Catassi et al., 2022). CeD affects the duodenum and proximal jejunum in the small intestine, the site where nutrients such as zinc, copper, iron, folate, biotin and vitamins (A,D,E,K) are absorbed (Basile et al., 2024). Nutrient deficiencies due to mucosal damage is common in newly diagnosed patients CeD, but also after the GFD has been introduced, as mucosal inflammation can take several years to heal (Rubio-Tapia et al., 2010). Intestinal inflammation in patients with CeD causes different degrees of malabsorption, depending on the extent of inflammation at diagnosis, in conjunction

with the continuous voluntary or inadvertent exposure to gluten while on a GFD (Wieser et al., 2021).

In addition, restrictive diets such as a GFD are often associated with nutritional deficiencies (Bledsoe, et al., 2019). GF substitutes are higher in sugars, fats, and lipids to increase palatability and are less fortified with added minerals and nutrients compared to their gluten-counterpart (Jamieson et al., 2018). Thus, individuals adopting a GFD do not consume adequate amounts of various micronutrients; particularly vitamin D, vitamin B12, folate, iron, magnesium, calcium, and zinc (Zn) (Melini & Melini, 2019).

Finally, an area of research not explored and a potential reason for nutrient deficiencies in patients with CeD adopting a GFD can result from food interactions, specifically with phytic acid. Phytates are a food component that bind to minerals such as Zn, copper, and calcium preventing its absorption. High phytic acid foods include whole grains, nuts, seeds, and legumes (Zhou & Erdman, 1995). GF substitutes such as sorghum, maize, quinoa, and brown rice are very rich in phytic acid, compared to all-purpose white flour, whereas other GF substitutes such as tapioca and potato starch are low in phytic acid (Brouns, 2021). Depending on the GF substitutes used in typical wheat-based products (i.e. bread, pasta, cookies) this may result in decreased absorption and deficiencies of these micronutrients in the CeD population.

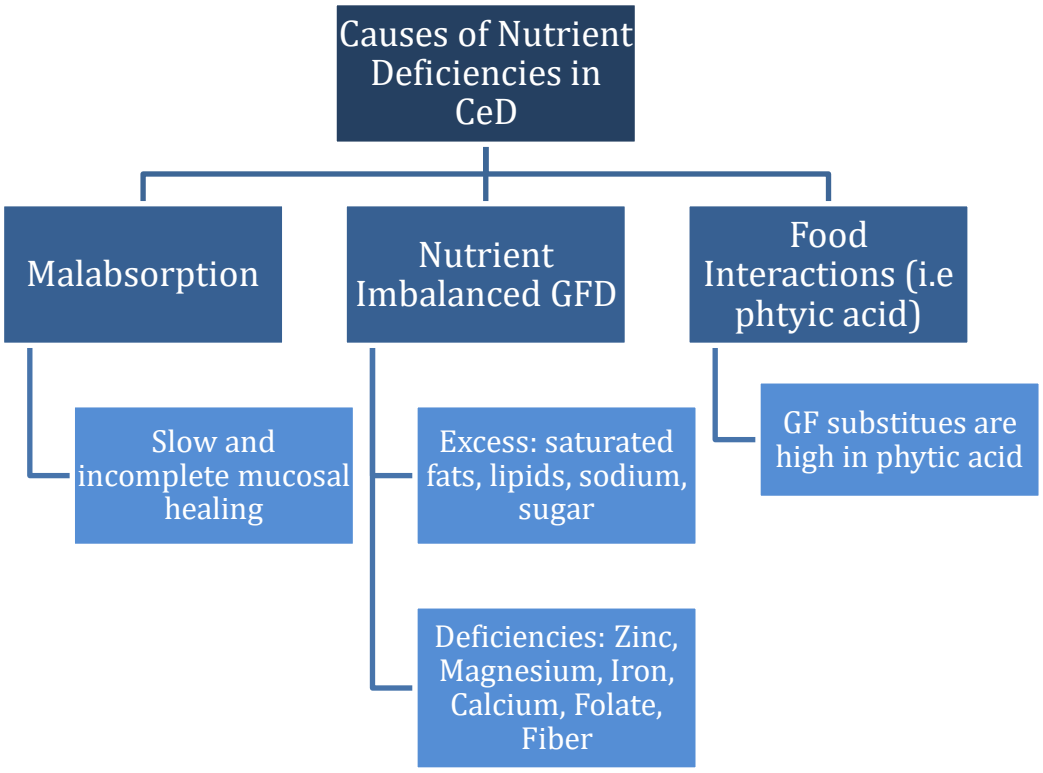


Figure 1.1 Causes of Nutrient Deficiencies in Patients with CeD

1.22 Nutrient Deficiency at the McMaster Celiac Clinic

Data from 182 CeD patients from Ontario attending our Celiac Disease Clinic at McMaster University show that 64% of patients on a GFD have nutrient deficiencies, and the most common micronutrient abnormality was Zn deficiency (plasma Zn levels below normal range), affecting 45.8% of the CeD population, followed by ferritin (16.9%) and vitamin D (33.3%) (Jivraj et al., 2022). Based on a subgroup analysis by GFD adherence, the results showed that Zn deficiency was greater in patients not strictly complying to a GFD (59.2%) compared to patients on a strict GFD (35.1%) (p=0.003). The persistence

of Zn deficiency in CeD patients strictly adherent with the GFD suggest that a nutrient imbalanced GFD diet plays a significant role in Zn deficiency, at least in a proportion of patients.

Additionally, results from this study showed that Zn deficiency persisted in patients both on a short term-51.8% (<2 years on GFD) and long term GFD-45.1% (>2 years on GFD) and was associated with higher anxiety and depression, which suggests that Zn deficiency has further clinical implications in CeD. Therefore, regardless of short or long term, strict or non-strict adherence to a GFD, Zn deficiencies persisted in patients with CeD, suggesting that Zn deficiency may be related to nutritional inadequacies in the GFD.

1.31 The Role of Zinc in the Human Body

After iron, zinc (Zn) is the second most abundant trace mineral in the human body. Zn plays a key role in several important biological processes including cell turnover and immune system function (Maret, 2016). Approximately 2800 proteins bind to Zn (Andreini et al, 2005) and the divalent cation plays catalytic, structural and regulatory functions in the human body (King, 2011). Zn is important in gene expression whereby Zn has a structural function in transcription fingers in zinc finger proteins (Hara et al., 2017). Zn is also involved in apoptosis, cell differentiation, and proliferation in cellular processes (Maret & Sandstead, 2006). Additionally, Zn can act as a synaptic neuromodulator triggering signal transduction pathways (Fukada et al, 2011).

Zn is released from food as the Zn^{2+} cation which binds to ligands secreted endogenously and is transported to the small intestine (Maares & Haase, 2020). At the

intestinal brush border membrane Zn binds to the Zrt-, Irt-like protein (ZIP)4, which imports ionic Zn from the lumen into the enterocyte (Kury et al., 2002). Within the enterocyte, Zn is found as cytoplasmic free Zn, Zn-ligand bound, metallothionein-bound Zn, and free Zn stored in vesicles (Wellenreuther et al., 2009). At the basolateral side of the enterocyte, Zn is exported by the ZnT-1 protein (McMahon & Cousins, 1998). Additionally, ZnT-5B protein is present at the apical side of the membrane to export Zn+ into the lumen and ZIP5/14 are present at the basolateral side to import zinc back into the enterocyte (Wang et al., 2004). Once Zn enters the lamina propria it enters the blood stream and binds to albumin (Scott & Bradwell, 1983). It then enters the portal system which carries Zn to the liver which is followed by systemic circulation where Zn is dispersed to organs across the body (Krebs, 2000).

Zn is absorbed in the duodenum proximal to the jejunum (Lee et al., 1989) which is the site where malabsorption occurs in patients with CeD (Freeman, 2008). The human body contains about 2.6 g of Zn and is distributed across various organs. Zn is primarily localized in the bone and muscle (86%), skin (4.2%) and liver (3.4%), while plasma Zn accounts for only less than 1% of the total Zn (Maares & Haase, 2020). There is no dedicated storage site of Zn (Rink & Gabriel, 2000). Zn is tightly regulated by homeostasis and the main regulatory mechanisms involve absorption of exogenous Zn, the secretion and excretion of endogenous Zn and partial reabsorption (Krebs, 2000). Zn must be continuously replenished by consumption of dietary Zn to replace endogenous losses of Zn (Rink & Gabriel, 2000) which is excreted in the form of bodily waste (i.e. feces) and fluids (i.e. urine, sweat and semen) (Brown et al., 2004).

1.32 Clinical Manifestations of Zn Deficiency

It is well-known that Zn deficiency impacts health negatively and can manifest in a variety of clinical symptoms such as delayed sexual development in adolescents, increased risk of infections, and decreased wound healing (Maxfield et al., 2023). Zn deficiency can also manifest with skin lesions, anorexia, diarrhea, hair loss, loss of appetite, sensory impairments, and neurological changes such as lethargy and depression (Brown et al., 2004; Maxfield et al., 2023; Roohani et al., 2013).

There has been no research conducted on Zn deficiency symptoms specifically in CeD patients. There is an overlap in clinical manifestations related to both Zn deficiency and active CeD which include extraintestinal symptoms such as skin rashes, foggy mind, headaches, and gastrointestinal symptoms such as diarrhea and loss of appetite (Figure 1.2). Therefore, when patients simultaneously present with Zn deficiency (plasma levels of zinc; pZn < 9.4 µmol/L) and have active CeD (i.e. increased TTG, mucosal atrophy), it is poorly understood whether clinical symptoms are due to Zn deficiency or CeD disease activity.

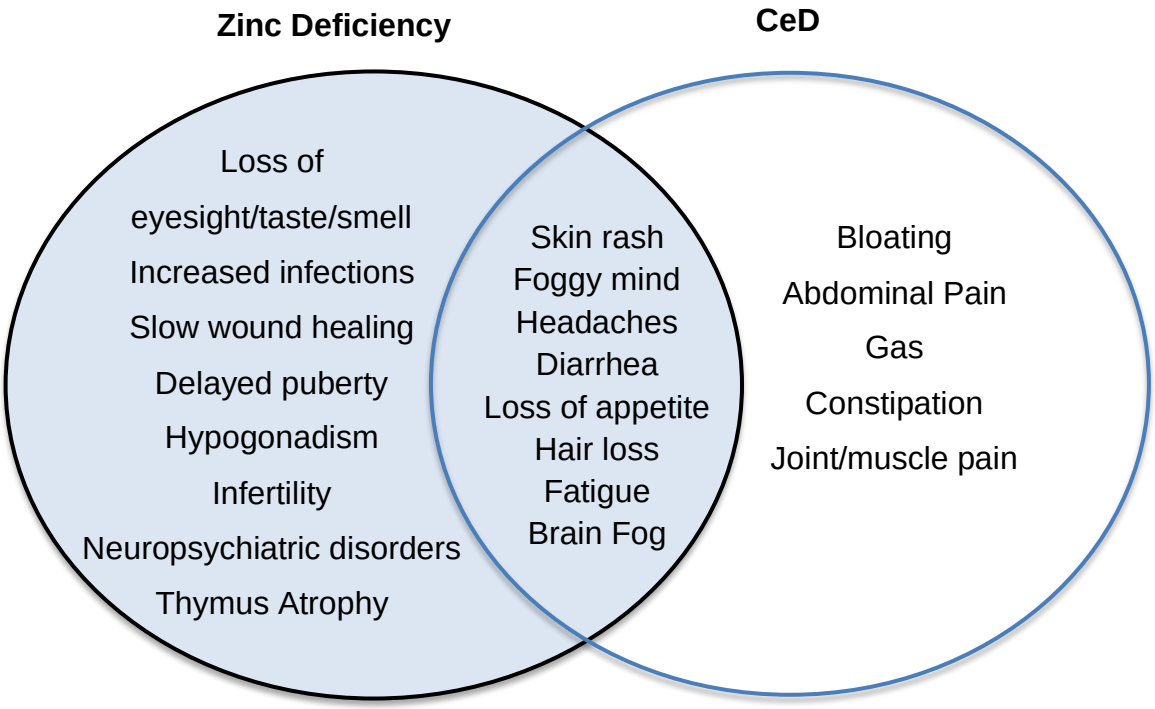


Figure 1.2 Clinical Manifestations of CeD compared with Zn Deficiency
(Brown et al., 2004; Maxfield et al., 2023; Roohani et al., 2013)

1.33 Zinc Deficiency in CeD

Zn deficiency in CeD has been studied in both untreated (i.e. newly diagnosed) and treated CeD patients on a long-term GFD. In untreated CeD patients, malabsorption of Zn deficiency is a result of damaged epithelial membrane (Crofton et al., 1990). A study by Jameson (1999), found that Zn deficiency was correlated with villous atrophy in patients with CeD. Their results showed that 60% of patients with partial villous atrophy, 80% with subtotal villous atrophy, and 92% with complete villous atrophy had Zn deficiency. A study by Wierdsma et al. (2013) at the VU University Medical Center in the Netherlands, assessed nutritional status of eighty newly diagnosed/untreated adult CeD

patients. In this study, 87.5% of patients had at least one nutrient deficiency and the most prevalent nutrient deficiency found at the time of diagnosis was Zn (67%), followed by decreased iron storage (46%) and anemia (32%). These results were also confirmed in a study by Bledsoe et al. (2019) in 309 newly diagnosed CeD patients at Mayo Clinic, where there was a significantly higher prevalence of Zn deficiency (59%) compared with age and sex matched controls (33%) ($p<0.001$).

In patients with treated CeD on a long-term GFD, Zn deficiency may be due to a combination of nutritional inadequacy of the GFD in addition to malabsorption. In addition to the study conducted at the McMaster Celiac Clinic (Jivraj et al., 2022) where Zn deficiency was found in 48% of patients, other studies have shown persistent Zn deficiency in 30% of patients with CeD after 1 year on GFD (Kemppainen et al., 1998) and 20% after 2 years on a GFD (Crofton et al., 1983).

Compared to the CeD population, 17.3% of the global population are at risk for inadequate Zn dietary intake (Wessells & Brown, 2012). However, the prevalence of Zn deficiency as determined by pZn is most common in low- and middle-income nations such as sub-Saharan Africa (20-83%) and South Asia (5-45% of population) likely due to malnourishment, higher consumption of plant-based diets, and phytic acid (Hess, 2017; Gupta et al., 2020). The prevalence of Zn deficiency in Canada is unknown, however it is estimated that 10-35% of Canadians are consuming less than the recommended dietary amount (RDA) of Zn, and this is more prevalent in older adults where 40% of people 70 and over are not consuming adequate dietary Zn (Health Canada, 2012). Compared to the prevalence of Zn deficiency in the general population (17.3%), untreated and treated CeD

have a 1-4x higher prevalence of Zn deficiency which is concerning and must be addressed.

1.34 Zinc Deficiency in CeD: McMaster Celiac Clinic

At the McMaster Celiac Clinic, we conducted a pragmatic observational study in Zn deficient patients with CeD to understand symptomatic responses to supplements and patient willingness to treat Zn deficiency with a diet. Participants (n=44) completed questionnaires to assess gastrointestinal symptoms measured through the Celiac Symptoms index (CSI) (Leffler et al., 2009) and extraintestinal symptoms measured by a visual analogue scale at baseline and follow up (3-6 months after clinic visit). All patients were treated with 25 mg/day of Zn gluconate. We found that there were no significant differences found in overall CSI scores and individual gastrointestinal symptoms such as diarrhea, headache, and low appetite. However, there was a significant decrease in brain fog ($p<0.001$) and joint and muscle pain ($p=<0.001$) after Zn supplementation.

Table 1.1 Plasma Zn, Celiac and Extraintestinal Symptoms at Baseline and Follow up

	Baseline Median (IQR)	Follow up Median (IQR)	P value
Zinc plasma(n=19)	8.55 (0.75)	10.35 (2.07)	**<0.001**
CSI score (n=25)	37 (16)	34 (9)	0.43
Skin Rash (n=24)	0 (3)	0 (2)	0.495
Headache (n=24)	3 (5)	2 (3)	0.46
Brain fog (n=24)	4 (3)	3 (2)	**0.014**
Fatigue (n=24)	5 (4)	4 (4)	0.3
Numbness of limbs (n=24)	1 (3)	0 (1)	0.9
Joint or muscle pain (n=24)	3.5 (4)	2 (5)	**0.040**
Fainting (n=24)	0 (0)	0 (0)	0.23
Canker Sores (n=24)	0 (2)	0 (1)	0.054
Feeling Anxious (n=24)	6 (2)	4 (4)	0.38
Feeling Depressed (n=24)	4 (5)	2 (4)	0.53

Additionally, there was a weak negative correlation between plasma zinc level and brain fog ($r=-0.26$, $p<0.04$), however no other extraintestinal symptoms were significantly correlated (See Appendix A).

1.35 Role of Microbiota in Zinc Absorption

Previous studies demonstrate that Zn levels modulate the relative abundance of bacteria (Li et al., 2020) and the accumulation of intracellular Zn has a bactericidal effect (Harbison-Price et al., 2020). Zn deficiency was associated with low relative abundances of Clostridiales and Verrucomicrobia, increased levels of Enterobacteriaceae and Enterococcus (Lopez & Skaar, 2018), and increased growth of the pathogen Group A Streptococcus (Makhtal & Kumaraswami, 2018). Furthermore, studies in a mouse model showed that Zn deficiency impacts the host and increases E. coli virulence, with a pro-inflammatory effect (Bolick et al., 2014).

Interestingly, in a collaborative study with Dr. Verdu, we found that Enterobacteria that express FimH, a mannose adhesin subunit on type 1 fimbria expressed by some E. coli ssp, were increased in newly diagnosed and treated CeD compared to controls (unpublished). In addition to the pro-inflammatory effect, FimH expressing bacteria E. coli produces phytase (Kozlowski et al., 2010); which could also influence Zn bioavailability. Although Zn homeostasis has been proposed as a key factor driving host-microbial interactions, the relationship between FimH expressing bacteria and Zn has not been investigated in CeD. Furthermore, the effects of Zn supplementation on the gut microbiota have been limited to studies in rodent models (Davis et al., 2020; Sauer et al., 2019), and

therefore, there is a need for studies evaluating the effect of Zn supplementation in human gut microbiota.

1.36 Zinc Supplementation Treatment and Adverse Effects

The main strategies for combating Zn deficiency include dietary modification and supplementation (Bledsoe et al., 2019; Roohani et al., 2013). In clinical practice, patients with Zn deficiency are treated with oral Zn supplements and pZn levels are monitored every 3 months until the levels normalize. Mild Zn deficiency should be treated with two-three times the RDA and four-five times for severe Zn deficiency (Walravens et al., 1989; Beers, 2006). The proper dose of Zn supplement to treat patients with CeD has not been standardized, however the recommended upper tolerable limit for Zn in adults is 40 mg of elemental Zn per day (Institute of Medicine, 2001). Research on the efficacy of Zn supplementation in addition to a GFD is limited an unapplicable to the clinical management of CeD. Although Zn supplements are well tolerated in general, they have been associated with side effects including gastrointestinal symptoms such as abdominal pain, diarrhea, nausea, and gastric hemorrhage when consumed long-term and in excess (Skalny et al., 2021) which may lead to non-compliance with treatment. Zinc supplementation is available in different formulations such as Zn oxidate, Zn sulfate, Zn acetate, Zn aspartate, Zn gluconate and Zn orotate (Table 1.2) (Brnic, et al., 2016).

Additionally, Zn, iron and copper compete for binding to a divalent metal transport protein (DMT1) involved in moving metals across the luminal membrane for intestinal absorption (Scheers, 2013). Excess Zn intake competes with copper absorption

and may lead to copper deficiency (Hoffman et al., 1988), whereas similar amounts of iron supplementation can have an inhibitory effect on Zn absorption when given together (Arredondo et al., 2006; Solomons and Jacob, 1981; Whittaker, 1998).

Table 1.2 Common Zinc Supplement Formulations (Saper & Rash, 2009; Ośko et al., 2023; Wegmüller et al., 2014)

Zn Formulation	Elemental Zinc (%)	Side Effects
Zn Gluconate	14	Nausea, vomiting, abdominal pain, metallic taste, dry mouth
Zn Citrate	34	Nausea, abdominal discomfort
Zn Acetate	35	Abdominal discomfort, metallic taste
Zn Sulfate	23	Nausea, abdominal discomfort, low bioavailability, metallic taste
Zn Orotate	13	Nausea, abdominal discomfort
Zn Oxide	80	Nausea, abdominal discomfort, low bioavailability

1.41 A Zinc Diet

The recommended dietary elemental amount (RDA) of Zn in adults is 11 mg/day in males and 8 mg/day in females. In pregnant women, Zn intake increases to 11-12 mg/day whereas children require less dietary Zn consumption than adults (Institute of Medicine, 2001).

The richest food sources of Zn include seafood, meat, wholegrain cereals, and legumes. Oysters contain the highest amount of Zn than any other food as one serving of oysters (3 ounces) is equivalent to 291% Daily Value (DV) or 32 mg of Zn. (Appendix B). However, red meat such as beef contributes to 20% of Zn intake in the United States as it is more commonly consumed (Huth et al., 2013). Zn is fortified in breakfast cereals as one serving is equivalent to 25% DV/ 2.8 mg of Zn, which is an important source of Zn in children (Berner et al., 2014). In wheat, Zn is found in the outer layer of endosperm and

the embryo/germ, therefore unrefined grains will have higher amounts of Zn compared to refined grains (Geissler & Powers, 2005). Zn is also available in plant-based foods such as legumes, nuts, and whole grains albeit the concentration of phytic acid is higher in these foods, reducing Zn bioavailability (Gibson et al., 2018). Foods with the lowest amount of Zn include fruits and vegetables due to their high-water concentration (Gibson, 1994).

1.42 Bioavailability of Dietary Zinc-Inhibitors and Enhancers

Dietary Zn consumed is not equivalent to the bioavailable Zn that is absorbed. There are several inhibitors and enhancers that can decrease and/or increase Zn bioavailability. The main inhibitor of Zn absorption is phytic acid, an "antinutrient" which is found in plant-based foods such as legumes, seeds, nuts, and grains (Adams et al., 2002) (A list of phytate foods in found in Appendix C). Inositol hexaphosphates and pentaphosphates are the forms of phytate which chelate with Zn, iron, and calcium forming an insoluble complex (Türk et al., 1996). The enzyme phytase is responsible for breaking down phytic acid and releasing any binding minerals such as Zn. Phytase is highly prevalent in uncooked and intact wheat, rye, and barley which breakdown phytic acid diminishing its inhibitory effects on Zn absorption (Mayer et al., 2023). Unfortunately, GF substitutes such as corn, sorghum, oats, and millet have little to no phytase activity (Eeckhout & De Paepe, 1994). These foods are also high in phytic acid concentration which may be an additional reason for decreased Zn absorption in CeD.

Additionally, amino acids that contain sulphur such as cysteine and methionine (found in grains, nuts, seeds, and vegetables) and hydroxy acids (such as lactic acid, tartaric acid, malic acid, and citric acid) enhance the absorption of Zn and are formulated into Zn

supplements (Lönnerdal, 2000). As well, increased amounts of protein concentration have a positive effect on Zn absorption; however, this is highly dependent on the type of protein present. Casein protein in milk has an inhibitory effect on Zn absorption, whereas soy protein does not (Lönnerdal, 2000). Furthermore, when iron and/or copper are present with Zn in a meal there is no inhibitory effect on Zn absorption (Chiplonkar & Agte, 2006; August et al., 1989), but high dietary calcium will inhibit Zn absorption (Wood & Zheng, 2007).

Alkaline Phosphatase (ALP) is an enzyme used to measure liver and bone function. Zn^{2+} is a constituent ion that activates ALP and studies have shown that Zn deficiency is negatively associated with ALP activity (Cho et al., 2007; Ray et al., 2017). However, only 50% of infant patients with serum Zn deficiency (n=4) also had low-ALP (Hattner 1993). Therefore, the use of serum ALP as an indicator of Zn deficiency is controversial and needs to be further investigated in both the adult and pediatric population.

1.43 Patient Preference for a Zinc Diet: Zinc Pragmatic Study

In the pragmatic observational study (HIREB#14108) conducted at the McMaster Celiac Clinic, we also assessed patient knowledge of nutrient deficiencies and their treatment preference for Zn supplementation compared to a Zn diet. Out of 44 Zn-deficient CeD patients, we found that 38.1% of CeD patients would prefer a Zn-optimized GFD compared to 61.9% that prefer Zn supplements. Furthermore, when given yes or no options, 88.6% of patients reported that they were likely to treat Zn-deficiency through a Zn-diet and 86.4% were likely to strictly comply with a Zn-diet. However, only 40.9% reported

that they were familiar with a Zn diet and 15.9% reported that the additional cost of a Zn-GFD would be a barrier to adopting this diet (Appendix D). Based on these results, dietary enhancement seems to be a desirable approach to treat and prevent Zn deficiency associated with the GFD but requires additional education. The results of this study support the need for a Zn-optimized diet to treat Zn deficiency in CeD and these results were presented this year at CDDW 2024 and DDW 2024 (Tandon et al., 2024). The manuscript is currently under preparation.

1.44 Development of a Zinc-Optimized Diet

A proposed Zn-optimized diet is one that would include foods high in Zn and low in phytates. The phytate to Zn molar ratio (PZMR) is a useful measure that determines Zn bioavailability, and a lower molar ratio indicates increased optimal Zn status (Gibson & Ferguson, 2008). The complete elimination of phytate rich foods may be unrealistic, however the inhibitory effect of phytic acid can be diminished by several food processing techniques such as baking, soaking, sprouting, and fermenting (Gupta et al., 2015). By employing these food preparation techniques in high phytate foods, this can increase the bioavailability of Zn in the diet.

Unfortunately, there have been no clinical dietary intervention studies that have implemented this specific diet in the general or CeD population, therefore, the efficacy of a Zn-optimized diet compared to Zn supplementation is unknown.

1.5 Study Rationale

Zn deficiency is a prevalent micronutrient deficiency amongst treated CeD (Fathi et al., 2013; Kemppainen et al., 1998; Crofton et al., 1983). Zn supplementation continues to be the primary clinical treatment to improve Zn deficiency but may result in gastrointestinal side effects and compete for absorption with other minerals (Maxfield et al., 2023; Maret & Sandstead, 2006). Dietary optimization to improve Zn levels is preferred in a portion of patients with CeD (Tandon et al., 2024), however, there have been no studies exploring the efficacy and feasibility of a Zn-optimized diet compared to Zn supplementation. A non-inferiority randomized controlled trial (RCT) to evaluate this will require many participants, and it is anticipated that a large multicenter study will be expensive. A pilot study asks the same question as a feasibility study (i.e. whether something can be done, and should we proceed with it), has a similar study design to the full RCT but conducted on a smaller scale (Thabane et al., 2010). Therefore, the proposed pilot study will assess the feasibility of the proposed protocol and calculate estimates of effect size to plan a larger clinical trial.

1.51 Study Hypotheses

1. A Zn-optimized GFD may be a feasible alternative to Zn-supplements to treat and prevent Zn deficiency in patients with CeD adopting a GFD.
2. Restoring Zn levels will improve symptoms and intestinal function/mass parameters in patients with treated CeD.
3. Restored plasma Zn levels will be associated with a decrease in pro-inflammatory

microbiota, such as FimH-expressing bacteria.

1.52 Study Aims/Outcomes

Primary outcome and endpoint: To assess the feasibility of the protocol (adherence and acceptability of treatment and protocol, recruitment, and retention rates), and provide estimated effect sizes (point estimates with confidence intervals) for all measures of interest (secondary outcomes) to plan a properly powered RCT.

Secondary outcomes and endpoints:

To assess the efficacy of Zn-optimized GFD compared to Zn supplements at:

1) normalizing plasma Zn levels (pZn ≥9.4 μmol/L) after 3 months of treatment

2) maintaining normal Zn levels (9.4 ≤ pZn ≤ 15 μmol/L) at 6-month follow-up

3) improving CeD symptoms (decrease in CSI score) at 3 and 6 months

4) improving extraintestinal symptoms (reduction of headaches, skin, tiredness, and brain fog scores measured by a visual analog scale (VAS)

5) reduction in anxiety and depression scores measured by HADS at 3 and 6 months

6) improving intestinal function/mass (citrulline plasma levels >40 μmol/L) at 3 and 6 months.

We will also: 7) assess predictors of normalization of plasma Zn levels at 3 months (albumin, dietary Zn intake, dietary phytate intake, alcohol intake, exercise, Zn supplements, citrulline levels, CeD activity measured by TTG IgA and/or DGP IgG (in IgA deficiency), GFD adherence, FimH expressing bacteria, weight, BMI, sex), and

8) assess whether a Zn-optimized diet is better tolerated than Zn supplements (reduced number of adverse events)

Chapter Two: Methods

2.1 Study Design

This study is an open-label, pilot, randomized controlled trial, with two arms in parallel. The study is currently conducted at the McMaster University Medical Center. The total study period is 6 months and involves 3 visits. At the screening visit (Visit 1), participants sign informed consent and are randomized to one of the two interventions, Zn-supplementation or a Zn-optimized diet. Demographic characteristics are collected, participants complete questionnaires, and blood, urine, and stool are collected.

The treatment period finishes at Visit 2 (3 months after V1) where symptoms are re-assessed, blood, urine, and stool samples collected, and adverse events are documented in the case report form (CRF). At Visit 2 (V2), compliance to the intervention assigned is also assessed through pill counting in the supplement group and analysis of food journals in the diet group.

A follow up Visit 2.5 is conducted 1-2 weeks after Visit 2, where we instruct participants to either a) start Zn supplements or increase their dosage of supplements if pZn levels measured at Visit 2 are below the normal level, or b) remain on the same intervention if their pZn are within normal range. This pragmatic approach of follow-up provides further information on a) feasibility of extending the treatment period up to 6 months and b) whether normal pZn levels can be maintained with a Zn-optimized GFD.

In the last visit (Visit 3-3 months after Visit 2), participant symptoms are reassessed, blood, urine, and stool samples collected, adverse events are documented and compliance to the pragmatic intervention is evaluated (Study Design shown in Figure 2.1)

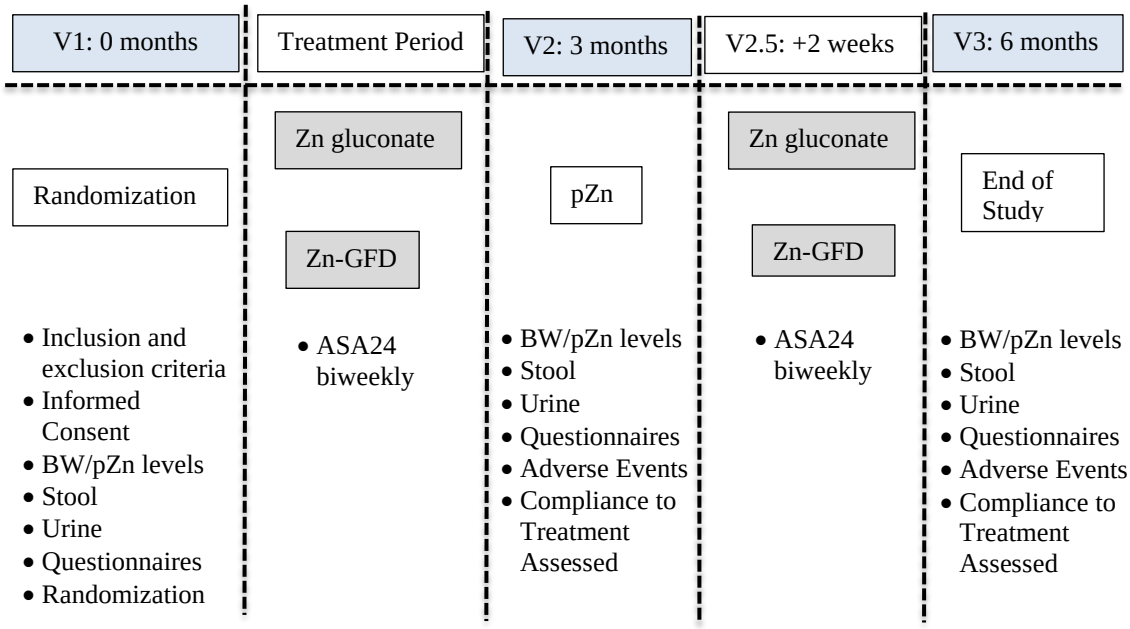


Figure 2.1 Study Design

2.2 Subject Recruitment

Subjects were recruited from the McMaster Adult Celiac Clinic and the Digestive Diseases Clinic. Over 500 patients per year are followed up in the Adult Celiac Clinic at McMaster and a majority are on a stable GFD. As part of the clinical practice, CeD patients have bloodwork done annually to measure pZn levels within other tests. Potential candidates are identified if they have recent blood work within 3 months indicating low pZn. After bloodwork is done, it takes approximately 2-3 weeks to receive the results. If pZn is below normal, the patient is contacted about the study by a member of the medical

staff through direct electronic messaging (MyChart). If the patient is interested in the study their contact information is passed along to the study coordinator who proceeds with further communication. We aim to recruit 25 patients per year during this 2-year trial.

Eligible subjects are identified based on the following inclusion criteria: a) adults over 18 years old with a confirmed diagnosis of CeD based on serology (elevated anti-TTG IgA, DGP IgG, or anti-endomysial IgA) and biopsy showing villous atrophy (Marsh 3a or greater), b) Adopting a GFD for over 6 months, and c) Untreated Zn deficiency (pZn levels ≤9.3 μmol/L). Participants are excluded based on the following exclusion criteria: a) being on a Zn-optimized diet, b) medical history of exocrine pancreatic insufficiency (fecal elastase < 200), intestinal obstruction, short gut, perforation, toxic megacolon, massive GI bleed or any serious illness that may interfere with study results, c) treatment with antibiotics or probiotics in the last 30 days, d) being pregnant or lactating, e) had an infection in the last 30 days

2.3 Interventions

1) Zn supplements. Those allocated in this arm are provided with 90 capsules of gluten-free Zn gluconate 25 mg (7 mg of elemental Zn; Jamieson®) and are instructed to take 1 tablet daily with a meal and at least 2 hours apart from other medications, calcium, iron, or copper supplements. The proposed dose is based on current recommendations for supplement initiation in clinical practice. Zn gluconate was chosen compared to other formulations because it is well absorbed, has high bioavailability at low doses (Osko et al.,

2023), and acceptable tolerance. The Zn supplements are provided by the study coordinator. Jamieson has provided in kind support 200 bottles of Zn 25 mg.

2) *Zn-optimized GFD*. Instructions provided by a dietitian to establish a target of 11 mg/day (female) and 14 mg /day (male) provided by Zn-rich food sources, adjusted for dietary phytate intake. These target values are based on a previous study showing that the absorptive capacity of Zn from the diet significantly reduces above these numbers (Sian et al., 1993). (See Appendix E for Dietary Handout and Appendix F for Zn-GFD Recipes).

2.4 Study Measurements: Primary and Secondary Outcomes

The primary outcome for this pilot study is to assess feasibility of the protocol, and therefore, a feasible study will be considered successful. Feasibility is estimated based on recruitment rates, retention rates at 3 months, and adherence to the intervention.

The trial will be considered feasible without protocol deviations if the enrollment fraction (i.e., number of enrolled patients / numbers of eligible patients) is 60% or above. If the enrollment fraction is between 40-59%, the trial would be feasible if protocol modifications are implemented. If the enrollment fraction is <40%, the study would require longer than expected to finish and therefore, not feasible with current resources. In addition, this study would be considered feasible with a minimum recruitment of 25 patients per year, retention rates of 75% at 3 months, and a drop-out rate less than 20%.

Adherence to the Zn supplement arm is measured by tablet counting. Compliance to the Zn-supplementation intervention is met by greater than 75% adherence (68/90 pills taken). Adherence to the Zn-optimized diet was assessed by the ASA24 recall

questionnaire, which was completed by the patient once bi-weekly. Patients met with our dietitian who provide instructions on a Zn-optimized diet at visit 1 and assessed adherence to the diet at visit 2. Compliance to the Zn-optimized diet is measured by an increase in bioavailable Zn from baseline. Assessing bioavailable Zn rather than total dietary Zn for intervention compliance allows us to account for the impact of phytic acid on Zn absorption. A study that is feasible based on compliance will have at least 75% of total participants in each arm adhere to the intervention assigned. A summarized version of primary and secondary outcomes with their associated measurements are included in Table 2.1.

Table 2.1 Study Outcomes and Measurements

Outcome	Measurement
Primary Outcome: Feasibility of the Protocol at 3 months	**Adherence to Treatment: > 75%** Zn-optimized Diet → ASA 24 Zn Supplementation→ Pill Counting **Retention Rate**→ # of dropouts (<20%) & 75% retention at 3 months **Recruitment Rate** → 25 patients per year + 10 patients to account for dropouts & enrollment fraction of 60%
Secondary Outcomes	
Normalize and maintain pZn level at 3 and 6 months	**Plasma Zn≥9.4 µmol/L**
Change in CeD symptoms at 3 and 6 months	**CSI Questionnaire** **CeD-QoL Questionnaire**
Change in Extraintestinal Symptoms at 3 and 6 months	**ES-VAS Questionnaire**
Change in Anxiety and Depression at 3 and 6 months	**HADS Questionnaire**
Predictors of pZn Normalization	- **Demographic information**→ sex, age, BMI - **Blood**→ albumin, CeD markers - **Stool**→ fecal elastase, microbiota, Fim-H expressing bacteria - **Urine Zn** - **GFD Adherence**→ GIP, CeDAT - **Dietary Zn**→ ASA24 - **Phytate intake** → NCC database
Tolerance of Diet vs Supplements	**Severity and Frequency of Adverse Events**
Other measurements	
Knowledge of Nutrient Deficiencies and Preference to Treat Low Nutrients	**Patient Perception Survey**

2.5 Dietary Zinc and Phytic Acid Calculation

The Automated Self-Administered 24-Hour (ASA24) is a validated dietary assessment tool that provides the nutritional output for Zn consumed in mg (Subar et al., 2024) (See Appendix G for a sample ASA24 Report). To determine the concentration of phytates (mg) consumed per food journal, a database was purchased from the Nutrition

Coordinating Center (NCC) at the University of Minnesota (https://www.ncc.umn.edu/). This is the only database that provides phytic acid values and it also contains over 19,500 foods and 8,100 brand name products. The ASA24 gives each food item a specific 8-digit code from the Food and Nutrient Database for Dietary Studies (FNDDS).

The FNDDS provides nutrient values for all foods reported from a dietary intake in What We Eat in America, included in the National Health and Nutrition Examination Survey (NHANES). However, the NCC database use codes provided by the United States Department of Agriculture (USDA) which include a larger and more comprehensive database of food. The USDA uses FoodData Central, a web-based data system composed of five different types of nutrient composition data. This includes Foundation Foods (basic, unprocessed/lightly processed foods), SR Legacy (was the primary food composition database in the United States), FNDDS (foods reported in NHANES), Experimental Foods (food published in peer-reviewed journals associated with USDA) and Branded Foods (branded and private labeled foods). To locate the foods from ASA24 in the NCC database and find the total phytic acid consumed required multiple steps which include:

1) Change each food code in ASA24 from FNDDS to USDA.
2) An additional file was purchased from the NCC to link the FNDDS codes to USDA, however, many foods could not be located and therefore the following website was used to find USDA codes (https://fdc.nal.usda.gov/).
3) Once each food was provided a USDA code, each food was given a different food ID to locate their phytic acid per 100 g in a separate database purchased

from the NCC.

4) Foods that we were unable to find in the NCC database (i.e. gluten-free processed foods), were given an estimated equivalent (Appendix H).
5) After all foods where located, the total phytic acid concentration per food diary was calculated, as well as the phytate/Zn molar ratio and the bioavailable Zn (a detailed explanation of how Zn bioavailability was calculated is provided in Appendix I).

2.6 Data Collection: Case Report Form and Questionnaires

A case report from (CRF) was developed on REDCap to securely document participant information. This includes demographic information (sex, age, years diagnosed with CeD), weight, height, BMI, medical history, medications, adverse events, intervention assigned, blood work, urine and stool sample results. Questionnaires including CSI (Appendix J) , HADS (Zigmond & Snaith, 1983) (Appendix K) , CeD-QoL (Dorn et al., 2010) (Appendix L) , ES-VAS (Appendix M) , CeDAT (Lefler et al., 2009) (Appendix N), and the Patient Perception survey (Appendix O) were collected through Zamplo which is a secure-health technology platform used to monitor and collect patient reported outcomes. In addition to completing study questionnaires through this platform, Zamplo also allows participants to record in real-time changes in symptoms, medication, adverse events, physical activity and any other health information the participant would like to monitor.

2.7 Collection and Analysis of Blood Work, Stool and Urine Samples

Participant blood and urine samples were analysed by the Core Lab at the McMaster University Medical Center (https://ltig.hrlmp.ca/).

Bloodwork: Includes CBC, CRP, alkaline phosphatase, cholesterol, ALT, albumin, TSH, ferritin, iron, copper, ceruloplasmin, amino acid profile including citrulline, total IgA, anti-DGP IgG, anti tTG IgA and zinc in plasma (pZn). pZn is the recommended biomarker of Zn status as it reflects dietary intake, responds to Zn supplementation and there is available reference data based on age and sex (IZiNCG, 2012). Venous blood was collected at least 2 hr apart from a meal in a 3 ml royal blue trace element vacutainer tube (K2-EDTA plasma). After collection, the sample was dropped at the central laboratory at MUMC within 8 h of blood extraction.

Zn in random urine: Participants were asked to collect a random urine sample in a sterile container with an amount of urine of at least 50 ml. Sample was either collected at home within the previous 24 hrs and stored in the fridge, or on-site on the day of the visit.

Stool samples for microbiota analysis, fecal elastase, and gluten immunogenic peptides (GIP). For microbiota analysis, patients were instructed to collect a stool sample anaerobically. Samples are sequenced for microbiota composition (16S sequencing Illumina) and FimH expressing bacteria (quantitative PCR) which is performed in collaboration with Dr. Michael Surette (16S sequencing) and Dr. Verdu (FimH expressing bacteria) at McMaster University. Additional aliquoted stool (5 gm) was sent to the central laboratory for testing fecal elastase (a marker of pancreatic function) using ELISA protocol (for laboratory reference see: https://lrc.hrlmp.ca/ViewTestLRC.aspx?testID=1130).

Values <200 µg/g of stool indicated pancreatic insufficiency. Additional aliquoted stool sample is provided to Dr. Caminero (collaborator) to measure the concentration of GIP in stools by sandwich ELISA (iVYLISA GIP-S kit; Biomedal SL, Seville, Spain). The results are expressed as µg GIP per g feces. Measurements of GIP >0.25 µg/g is considered as gluten exposure, and higher levels indicate a higher amount of GIP.

2.8 Randomization Process

Subjects are randomized 1:1 ratio to each arm. The sequence of the treatments is randomly generated using a computer-based random number generator (RStudio, Boston, MA). A student experienced in the randomization process but not involved in the study performs the randomization to preserve treatment allocation concealed from participants and study staff. Blinding is not possible due to the differences in intervention, and therefore the outcome assessor is blinded to reduce detection bias.

2.9 Sample Size and Power Calculation

There is no previous estimate effect size of a Zn-optimized GFD vs Zn supplementation in CeD. Based on preliminary data, the response to normalization of Zn levels with supplementation is 70%. Considering a similar response rate for Zn-optimized diet (60%), a non-inferior and adequately powered RCT (alpha 0.05 and power 0.8) will require a sample size of 356 patients per arm. This samples size calculation will be refined after obtaining additional data from the pilot trial. In this pilot study, we will include 7% of the estimated sample size and we will re-assess sample size based on the pilot study. We

will recruit an additional 10 patients to account for 20% dropout rates. The total estimated sample size for this pilot trial is 60 patients. If we decide to move forward with the larger study, patients from the pilot study will be included in the larger study.

2.10 Statistical Analysis

The preliminary analysis for this report was carried out using SPSS v.27 and figures were created using GraphPadPrism v.10. The analysis was performed per protocol and missing data <10% was replaced with mean for continuous variables and mode for categorical variables. Differences between categorical variables was determined using the Chi-squared and/or Fisher's exact test for smaller sample sizes. Feasibility outcomes were analyzed using descriptive statistics reported as percentages with a 95% confidence interval [CI]. Estimated effect sizes are included as means ± SD for continuous variables and frequency (n, %) for dichotomous variables. The proposed sample size per secondary outcome was calculated using a power of 0.8 and an alpha of 0.05. Differences in secondary outcomes between and within intervention groups were analyzed using the Mann Whitney U test and the Wilcoxon signed-rank test, respectively. Simple logistic regression was performed to determine predictors which normalized pZn levels at 3 months, presented as odds ratio (OR) with 95% confidence interval [CI]. All tests were significant if $p < 0.05$.

In the future, R Statistics will be used for microbiota statistical analysis in collaboration with Dr Marco Constante from Dr. Verdu lab. Differences between bacterial communities will be tested by permutational multivariate ANOVA (PERMANOVA)

calculated using an unweighted UniFrac distance. Once we have recruited 60 participants and the study is complete, we plan to perform the final analysis in collaboration with Dr. Lehana Thabane. We will perform analysis according to the intention to treat and per-protocol. We also plan to perform an ANCOVA, with testing for effect modifiers (dietary Zn) and potential covariates (sex) in 3- and 6-month periods. Multiple imputations will be used to handle >20% missing data and a sensitivity analysis will be conducted for participants who completed the study but did not comply to the study intervention assigned.

2.11 Summary

This chapter provides a summary of the materials, and methods used to execute this research study. This includes the study design, participant recruitment, clinical measurements, data collection, and statistical analysis.

Chapter 3: Results

3.1 Study Population and Recruitment

Recruitment started immediately after obtaining HIREB approval, and the study is ongoing. The preliminary analysis for this thesis includes participants recruited between November 2022-May 2024 from the McMaster Celiac Clinic. Details on study population including the number of eligible, excluded, no response, and refused participants (i.e. with reasons for refusal) are included in Figure 3.1. To date, 28 participants have been enrolled in the trial and their demographic characteristics at baseline (Visit 1) can be found in Table 2. There were no significant differences in demographic characteristic between the Zn-

optimized diet (n=14) and Zn supplement (n=14) group. A timeline including past and projected participant enrollment is included in Figure 3.2. With our current rate of enrollment, the recruitment of 60 participants will be completed by the end of August 2025.

The status of enrolled patients in the trial is included in Figure 3.3. This diagram shows the number of participants who have completed each visit, assigned to each intervention, withdrawals, and those ongoing. In total, 28 patients have completed Visit 1 of which 21 participants have completed Visit 2, 16 participants have completed the entire trial (Visit 3), 5 have withdrawn, and 8 are ongoing. After the 3-month intervention assigned at V1, 5/10 (50%) of participants in the Zn-optimized diet group proceeded with taking Zn supplements for the pragmatic portion of this study (3-6 months). The other five participants had low pZn levels at Visit 2 which is why we recommended they switch to Zn supplementation for the remainder of the study. Out of 11 participants in the Zn-supplement intervention at V2, only 1 (9.1%) participant with normal pZn decided to switch to the diet intervention after 3 months of treatment. Seven (63.6%) participants remained on the same dosage of Zn supplements (25 mg/day) as their Zn levels were normal and 3 (27.3%) participants were required to increase their dosage of Zn supplements to 50 mg/day due to persistently low levels of pZn.

A timeline of the study including HiREB approval, amendments, and protocol deviations are included in Table 3.2. Our strategy to recruit participants changed several times over the course of the study period (Table 3.3). The strategy that resulted in the largest enrollment/month (3 patients/month) was having both the medical administrative staff and the study coordinator proactively calling patients. The strategy that resulted in the least

number of patients recruited/month (one patient enrolled/month) was having only the medical administrative staff contacting the potential candidates. In addition, we noted several patients were excluded due to taking multivitamins or Zn supplements and still had Zn deficiency. Therefore, we amended the protocol to include these patients as they will still benefit from the treatment.

3.2 Results of Primary Outcome: Assess Feasibility & Provide Estimated Effect Sizes

3.21 Recruitment Rate

Over the past 17 months, 28 participants have been enrolled in the trial. A feasible study considered recruitment of 25 participants per year and an enrollment fraction (i.e. number of eligible patients/numbers of enrolled participants) of 60%. Our interim analysis showed that based on our current recruitment, our enrollment fraction (i.e. 28/108 eligible participants) is 25.9% at this time, indicating that the targeted date for enrolment may not feasible based on current recruitment rate as it took 16 months to recruit 25 participants, which is 4 months over the predicted time. One of the barrier's impacting recruitment was inconsistency of patient approach across the study, as previously described. Therefore, to improve recruitment, the clinical team will be proactively contacting potential candidates and provide study information as appropriate. Based on previous data, we estimate the recruitment rate will be tripled by implementing this approach.

3.22 Retention Rate

In total, 5 out of 28 participants have withdrawn from the trial. The dropout rate of 17.86% and a retention rate at 3 months of 82.14% is within estimated (drop out <20% and

the 3-month retention rate >75%), confirming feasibility in terms of retention rates. Two out of five participants withdrew due to randomization errors, as we realized after enrolment that one participant was already taking Zn supplements, and the other participants pZn had normalized at baseline. This protocol deviation was informed to HiREB. Based on this, we decided to ensure baseline laboratory is collected closer to the visit rather than relying on laboratory done in clinic. The other three participants withdrew due as they were unable to continue with the study due to personal commitments.

3.33 Compliance to Intervention

We considered this study feasible if >75% adherence to Zn-supplement and Zn-optimized diet intervention is achieved. Compliance was determined based on 1) pill counts: taking at least 68/90 (75%) of tablets in the supplement group; and 2) an increase in bioavailable Zn compared to baseline in the diet group. Nine out of eleven participants (81.8%) allocated to the Zn-supplement group were compliant at 3 months and 5/10 participants were compliant in the diet group (Table 3.4). We were not able to assess dietary compliance in 2 participants (2/10) as they did not complete any food diaries but completed all other study procedures. Therefore, 5/8(62.5%) of the patients who did complete food diaries were compliant with the Zn-optimized diet. Participant-specific dietary Zn, phytate intake, bioavailable Zn and pZn levels are included in Table 3.5 and delta pZn is correlated with delta bioavailable Zn in Figure 3.4. Based on these results, compliance with treatment is within expected for the Zn-supplementation group but slightly below in the Zn-optimized diet. An ROC curve is included in Appendix R which shows the classification of normalized pZn based on bioavailable Zn.

Table 3.4 shows that participants in the Zn-optimized diet group consumed a higher amount of Zn through diet compared to the Zn supplement group (median: 18.19 mg vs 8.65 mg). Also, participants in the Zn-optimized diet group consumed increased phytates compared to the supplement group (971.5 mg vs 660.5 mg), however, when accounting for phytates, the bioavailable Zn consumed was greater in the Zn-optimized diet group compared to the Zn supplement group (median: 5.46 mg vs 2.45 mg). To get a better understanding of food preparation techniques used to decrease phytate content in food we will proactively collect this information (See qualitative questionnaire for Zn supplement and diet in appendix P).

3.34 Estimated Effect Sizes of Secondary Outcomes

Additionally, our primary outcome included an analysis of estimated effect size of all secondary outcomes to plan a properly powered RCT in the future (α= 0.05, power=80.0%). The point estimates (mean ± SD) for continuous variables and frequency (n, %) for dichotomous variables are presented in Table 3.6. At 3 months, 50% of participants in the Zn-optimized group achieved normal pZn, compared to 72.7% in the Zn supplement group. The results show that to be confident in our results in those who normalized pZn at 3 months we require a sample size of 142 participants (See Appendix Q for sample size calculation). Effect sizes and proposed sample size of secondary outcomes for a non-inferior RCT is outlined in Table 3.6.

3.3 Secondary Outcomes: Assess Differences in Zn-Optimized Diet vs. Zn-Supplement

It is important to note that this study is not properly powered to assess differences, but we are rather looking for trends of response in both groups.

3.31 Normalizing pZn at 3 months of Treatment

As expected, pZn levels significantly increased in both the Zn-optimized diet ($p=0.03$) and Zn-supplement group ($p <0.01$) after 3 months of treatment (Figure 3.5). At Visit 2 (3 months post-randomization), there is no significant difference in pZn levels between the Zn-optimized diet compared to the Zn-supplementation group ($p=0.44$) (Table 3.7).

3.32 Maintaining Normal pZn levels at 6-month Follow-up

At Visit 3, there was no significant difference in pZn levels between the Zn-optimized diet compared to the Zn-supplementation group ($p=0.26$) (Figure 3.5). Participants were able to maintain their Zn levels at 6 months with no significant increase or decrease in pZn within the Zn-optimized diet group ($p=0.63$) and the Zn supplementation group ($p=0.41$) (Figure 3.5).

3.33 Improving CeD Symptoms and Quality of Life at 3 and 6 months

There were no significant differences in CSI scores at 3 months ($p=0.53$) and at 6 months ($p=0.07$) between the Zn-optimized diet compared to the Zn-supplementation group (Table 3.7). However, there is a significant difference in nausea reported between interventions at 3 months ($p<0.01$). There was also a significant difference in reported stomach rumbling ($p <0.01$) and hunger pains ($p=0.02$) between interventions at 6 months.

Within the same intervention, there was no significant improvement in celiac symptoms at 3 and 6 months (Figure 3.6).

Additionally, there was no significant difference in CeD-QoL scores at 3 months (p=0.57) and at 6 months (p=0.96) between the Zn-optimized diet compared to the Zn-supplementation group (Figure 5). Within the same intervention group, there was no significant improvement in Ced-QoL scores at 3 and 6 months (Figure 3.7).

3.34 Improving Extraintestinal Symptoms at 3 and 6 months

There were no significant differences in any of the extraintestinal symptoms between the Zn-optimized and Zn-supplement group at 3 and 6 months (Table 3.7).

3.35 Reduction in Anxiety and Depression Score at 3 and 6 months

There was no significant difference in HADS scores between the Zn-optimized diet and the Zn-supplementation group at 3 months (p=0.46) and 6 months (p=0.83). Figure 3.8 shows that within the Zn-supplementation group there was a significant difference in HADS scores (p=0.04) after 3 months, but not at 6 months (p=0.39). However, there was no significant difference in the Zn-optimized diet group at 3 (p=0.20) or 6 months (p=0.38)

3.36 Assess Predictors of pZn Normalization at 3 months

We investigated several different independent variables that may be associated with normalizing Zn levels at 3 months (Table 3.8). Out of all the variables assessed, only albumin levels were associated with normalization of pZn levels (OR= 1.40 (0.86-2.67), p=0.01).

3.37 Tolerance to Intervention at 3 months

In the Zn-optimized diet group, 8/10 (80%) of participants experienced at least one AE at 3 months compared to 7/11 (63.6%) participants in the supplement group (p=0.06). The total number of AEs reported was 11 in the diet group and 12 in the supplement group which equally equates to 1.1 AE/person. The most common AE experienced was diarrhea (16.7%) in the supplement group, and cold (18.2%) and muscle soreness (18.2%) in the Zn-optimized diet group. AEs reported in each group are shown in Table 3.9.

3.4 Differences in Microbiota Composition at 3 Months

From 56 fecal samples in 27 participants, a principal coordinate analysis for beta diversity plot found that there were no significant differences in the microbial communities between or within the same intervention at 3 months (Figure 3.9).

3.5 Patient Knowledge and Treatment of Nutrient Deficiencies

All patients received the same information on available treatment for Zn deficiency, and the potential for diet therapy to treat Zn deficiency. Therefore, as anticipated, in terms of overall knowledge of nutrient deficiencies, treatment preferences, and likeliness to comply with either supplementation or diet, there were no significant differences between groups at baseline and follow-up (V2). However, after randomization, participants in the diet intervention were more familiar with the Zn-diet compared to the Zn-supplement ($p<0.01$) (Table 3.10). This is most likely due to the educational instructions provided to participants at randomization, and these results are similar at V2 (p=0.02). Interestingly, at

baseline 78.6% of participants randomized to the diet preferred to treat their Zn deficiency through diet, but this steeply dropped to 40.0% at follow-up. This drastic drop in preference for the diet treatment within the diet-intervention group, acknowledges that there are barriers or obstacles that patients are facing in this group, that needs to be further explored. To address this, we included a questionnaire that we will be administering for this purpose (see appendix P)

3.6 Summary

This chapter depicts the results of each study objective. The primary objective of feasibility was met regarding overall retention rates and compliance to the Zn-supplementation intervention. However, based on recruitment rates and compliance to the Zn-optimized diet group, this study would not be feasible in the time frame proposed. In addition to feasibility, the point estimates of our secondary outcomes have determined the number of participants needed to plan a non-inferior powered RCT in the future and outcomes to measure in a larger study.

Chapter Four: Discussion

4.1 Main Findings

There are currently no studies exploring a Zn-optimized diet in the general population or CeD patients and this study is the first to explore this novel dietary option to manage Zn-deficiency. Our preliminary analysis shows that the current recruitment rate and compliance to the Zn-optimized diet intervention is below expected, potentially

compromising feasibility of the study. Therefore, other strategies need to be explored to improve recruitment rates and dietary compliance. On the contrary, retention rate and compliance with the Zn-supplementation intervention are within expected, and therefore, the study would be feasible in this regard.

We acknowledge that a high enrollment fraction proposed for this study of 60% is too ambitious and unrealistic based on experience of similar clinical trials conducted in our McMaster Adult Celiac Clinic, which have lower enrollment fractions estimated ~25%. Therefore, a 20-25% enrollment fraction will be a realistic goal for future studies.

Based on the point estimates and effect size of pZn at 3 months, we estimate a larger study will require 142 participants to be confident in our results between the Zn-optimized diet and Zn-supplement intervention in pZn. Based on our preliminary results, we would require ~81% fewer participants (n=142) than our originally anticipated future RCT sample size of 712. Our preliminary analysis shows that the effect size (i.e. normalization of pZn) in the Zn-optimized diet is slightly smaller (50%) than originally anticipated (60%), and the effect size was as expected (72.7%) in the Zn supplement group. However, this is only a preliminary analysis based in 10 patients.

An interesting finding is that out of the 8 participants that completed dietary recalls in the Zn-optimized diet, 2/3 patients who were non-compliant to the diet still had normalized pZn levels 3 months post-randomization (Figure 3.5). This may be due to under-reporting their dietary intake in ASA24, or adherence to the cooking methods provided to remove phytates in food thus diminishing it inhibitory effect on Zn absorption. On the other hand, 2/5 participants that were compliant to the Zn diet, did not normalize

pZn, which may be a result of over reporting in ASA24 or due to high phytates intake (Figure 3.5). One of these patients was close to a normalized pZn at 9.3 umol/L (increase of 1.3 umol/L from Visit 1). The other participants decreased in pZn by one umol/ which may be a result of a COVID infections. These options should require further analysis at the end of the study.

Based on the results of this pilot study, it seems that the Zn-supplement and Zn-diet intervention may have similar effect size at improving Zn-levels at 3 months, which is an interesting finding. If this effect is sustained, this information would indicate that the Zn-optimized diet is similar effective than the Zn-supplement group at improving pZn levels. However, our preliminary data is based on a small population of completed patients (supplement, n=11 and diet n=10), with missing data of dietary compliance in two patients due to time commitments. Once all 60 participants complete the study, effect sizes and sample size will be re-calculated to confirm these results.

Regarding our secondary outcomes, there were no differences in pZn levels, in overall CeD symptoms, quality of life, extra-intestinal symptoms, anxiety and depression, and adverse events between the Zn-optimized diet and Zn-supplement group at 3 and 6 months, which is expected for a low powered pilot study. However, we did find a difference in nausea reported at 3 months between intervention groups. Nausea is a side effect of both excess Zn consumption and active celiac (Plum et al., 2010; Taylor et al., 2008) therefore it is difficult to differentiate the cause of symptoms. Additionally, when assessing predictors that normalize pZn, albumin was the only variable to have a significant effect. Serum albumin acts as the primary transport protein that binds to pZn (60%), followed by

α-macroglobulin (30%), and transferrin (10%) (Lu et al., 2008; Scott & Bradwell, 1983). A study by Namikawa et al. (2023) confirmed similar results to our study showing a significantly positive correlation with serum Zn levels and albumin in patients with gastric cancer. Based on this, albumin should be included as a parameter to explore in this study. Pilot and feasibility studies are not properly powered to assess differences between groups, but estimating effect size and assessing trends in difference between groups in each secondary outcome will be key to plan a proper powered study.

4.2 Strengths

This study has several strengths, the most notable being that this is the first study to ever introduce a Zn-specific dietary intervention as an alternative to Zn-supplementation in patients to treat Zn deficiency. No other dietary study has been conducted implementing a Zn-optimized diet in the general population, nonetheless with CeD patients. Our study is further strengthened by implementing a Zn-optimized diet, to reduce phytic acid consumption to improve Zn absorption. As many GF substitutes are high in phytates, this could be a reason for poor absorption of Zn in the diet.

Another strength of this study is the 3-month pragmatic design implemented after the randomized intervention. This pragmatic approach allows us to provide similar instructions to clinical practice that meet the nutritional needs of each patient. In this second portion of the trial, we combine both the patient preference for the intervention that they prefer while recommending an intervention based on their pZn status.

4.3 Limitations

Although this study has its strengths, it is acknowledged that there are several limitations, particularly with recruitment. Firstly, patients who have low pZn are not targeted during their clinic visit as most patients get the results of their blood work several weeks after their appointment. These results are delivered electronically to the PI after 2-3 weeks. Once the PI reviews the blood work, sends a message to patients through myChart, and the study coordinator hears back that the patient may be interested, several weeks to months can go by creating a significant delay in recruitment. Some patients may start taking a multivitamin or Zn supplement on their own as they have access to their blood work during this time. Others could unknowingly be eating high Zn foods, all of which will raise patients Zn levels and thus they may not be eligible for the study anymore. To contact potential patients faster, we will have both the clinical administrative staff and study coordinator make initial contact with patients. This provides more opportunity for patients to hear about the trial and can potentially decrease the time required for a patient response, which is a significant barrier for recruitment, as the proportion of no-response is 68/176=38%.

Additionally, we faced several issues using the ASA24 food journals when analyzing the phytic acid concentration of patient's records. This interface has a very limited number of gluten-free items for participants to choose from which could significantly impact the levels of phytic acid calculated as certain GF substitutes (i.e. corn, quinoa and brown rice flour) have higher amounts of phytates compared to wheat. To combat this limitation, we asked participants the brand of their GF products and matched

them to a suitable GF product in the NCC database to calculate phytates (Appendix L). Additionally, two patients in the dietary intervention group did not complete the food diaries due to time constraints. Moving forward, to get better accuracy and completion of patient dietary recall, it would be recommended to test another food entering software such as Keenoa (https://keenoa.com/) which is more efficient, user-friendly and has a larger database of GF foods.

Also, another limitation of this study is difficult for us to understand whether the symptoms experienced by patients is due to their CeD activity or related to Zn deficiency, as clinical manifestations can overlap. To differentiate between the cause of symptoms, we will be assessing other markers of CeD activity such as CeD autoantibodies, GFD dietary compliance and intestinal inflammation through the amino acid citrulline. It would also be worth exploring to have an additional group of non-CeD Zn-deficient controls to accurately compare symptoms between groups.

4.4 Future Directions

In the future, we will continue recruitment until we reach 60 participants, which we aim to complete by August 2025. We also plan to introduce an additional questionnaire to understand the challenges that participants faced in both interventions and further dietary assessment of food preparation (i.e. phytic acids). In collaboration with the Dr. Verdu Lab, we are currently working on the preliminary results of 16S RNA sequencing of 56 fecal samples from 27 participants in the trial to obtain effect estimates of microbiota composition and expression of Fim-H bacteria between intervention groups. Once all 60

participants have been recruited, final analyses will be done in collaboration with Dr. Lehana Thabane such as an intention to treat analysis, determining effect modifiers and potential covariates (sex, age, microbiome) and sub-group analyses to evaluate sex differences and adherence to GFD. We also plan to incorporate our qualitative assessment survey to help us receive participant feedback on the diet and supplement intervention so we can improve compliance in the future.

4.5 Conclusion

In conclusion, the preliminary results from our interim analysis study is feasible based on retention and compliance to the Zn-supplement intervention. There needs to be improvement in recruitment rate and compliance to the Zn-optimized diet intervention. Additionally, we identified further areas of improvement such as food recording software, and calculation of Zn bioavailability. This study is ongoing and therefore, with this knowledge we aim to employ the most efficient strategy to improve recruitment and dietary compliance. Additionally, we were able to provide estimated effect sizes of secondary outcomes and predict sample size for an adequately powered RCT in the future. Overall, interim analyses are important in clinical trials, including pilot and feasibility studies, and should be incorporated when planning future studies.

Table 3.1 Participant Demographics at Visit 1

	Zn-Optimized Diet (n=14)	Zn Supplement (n=14)	p-value
Age (years), median (IQR)	54.5 (17.5)	48 (33.5)	0.42
Gender-female, n (%)	11 (78.6)	13 (92.9)	0.60
Years Diagnosed, median (IQR)	4 (7.5)	2.5 (8)	0.90
BMI, median (IQR)	26.9 (8.3)	25.8 (8.2)	0.45
GFD Adherence, n (%)			
- CeDAT- Non-compliant[1]	3 (21.4)	3 (21.4)	1.0
- GIP-Gluten Detected[2]	1 (7.1)	0 (0.0)	1.0
Medications, n (%)			
- Levothyroxine	2 (14.3)	6 (42.9)	0.09
- PPI	1 (7.1)	5 (35.7)	0.17
- Progesterone/estrogen	1 (7.1)	3 (21.4)	0.60
- Anti-diabetic	1 (7.1)	2 (14.3)	1.0
- Antidepressants	2 (14.3)	6 (42.9)	0.09
- Cardiovascular	2 (14.3)	3 (21.4)	1.0
- Analgesics	1 (7.1)	2 (14.3)	1.0
- Immuno-globulin	2 (14.3)	1 (7.1)	1.0
- Bronchodilator	2 (14.3)	0 (0.0)	0.48
- Antipsychotic	1 (7.1)	0 (0.0)	1.0
- Low Dose Aspirin	0 (0.0)	1 (7.1)	1.0
- Amino salicylate	0 (0.0)	1 (7.1)	1.0
- Anticonvulsant	1 (7.1)	0 (0.0)	1.0
- Antihistamine	0 (0.0)	1 (7.1)	1.0
- CNS stimulant	0 (0.0)	2 (7.1)	0.48
Supplementation, n (%)			
- Multivitamin	7 (50.0)	3 (21.4)	0.24
- Vitamin C	3 (21.4)	4 (28.6)	1.0
- Vitamin D	7 (50.0)	8 (57.1)	1.0
- Iron	3 (21.4)	7 (50.0)	0.24
- Calcium	1 (7.1)	2 (14.3)	1.0
- Magnesium	3 (21.4)	1 (7.1)	0.60
- Omega-3	2 (14.3)	3 (21.4)	1.0

- Selenium - Zinc - Potassium	0 (0.0) 5 (35.7) 0 (0.0)	1 (7.1) 2 (14.3) 1 (7.1)	0.48 0.39 0.48
Zn Plasma (µmol/L), median (IQR)	8.7 (0.9)	9.0 (1.0)	0.58
Zn Urine (µmol/L), median (IQR)	3.0 (3.7)	2.85 (4.9)	0.99
Zn Urine-Abnormal[3], n (%)	4 (28.6)	3 (21.4)	1.0
Celiac Activity, n (%) anti-tTg-IgA abnormal[4] anti-DGP-IgG abnormal[5]	 4 (28.6) 4 (28.6)	 3 (21.4) 1 (7.1)	 1.0 0.33
Other Current GI disorders, n (%) - GERD - Esophagitis - Hemorrhoids - Ulcerative Colitis - IBS-D - Cirrhosis - Fatty Liver - Diverticular Disease	 3 (21.4) 1 (7.1) 0 (0.0) 0 (0.0) 0 (0.0) 0 (0.0) 0 (0.0) 1 (7.1)	 2 (14.3) 0 (0.0) 1 (7.1) 1 (7.1) 1 (7.1) 1 (7.1) 1 (7.1) 0 (0.0)	 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0
Fecal Elastase Abnormal, n (%)	0 (0.0)	0 (0.0)	NA

[1] Non-compliance (>13 on CeDAT)
[2] GIP detected in stool (>0.25 µg GIP/g sample)
[3] Normal range (0.9 µmol/L -6.1 µmol/L)
[4] anti-tTg-IgA abnormal (>4.0 U/ml)
[5] anti-DGP-IgG abnormal (>20 units)

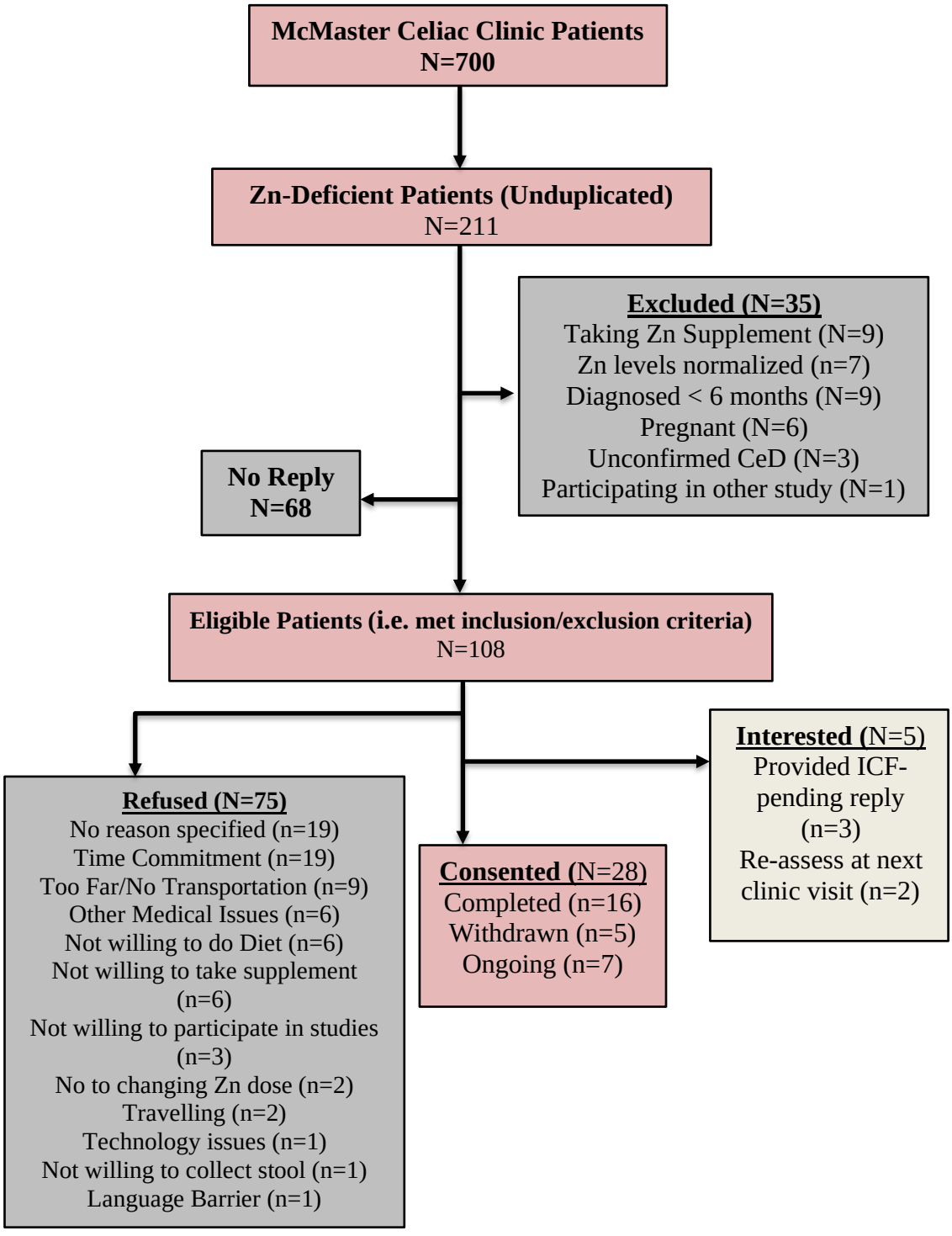


Figure 3.1 Study Recruitment of Patients in McMaster Celiac Clinic (Nov 2022-May 2024)

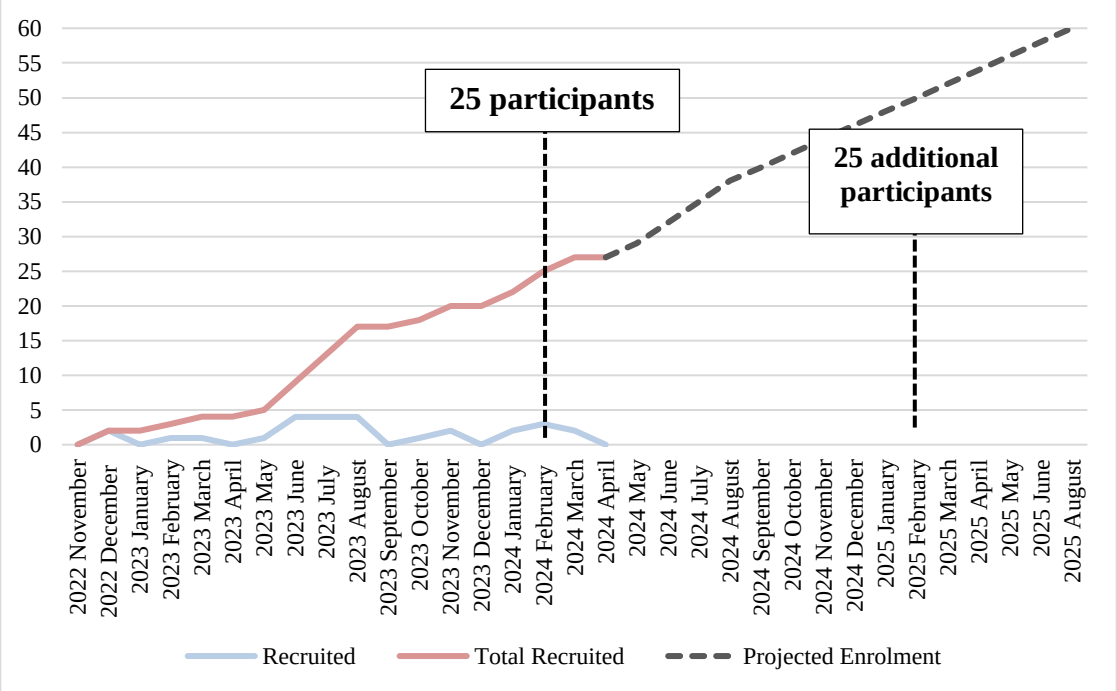


Figure 3.2 Participant Recruitment Over Time and Projection for the Future

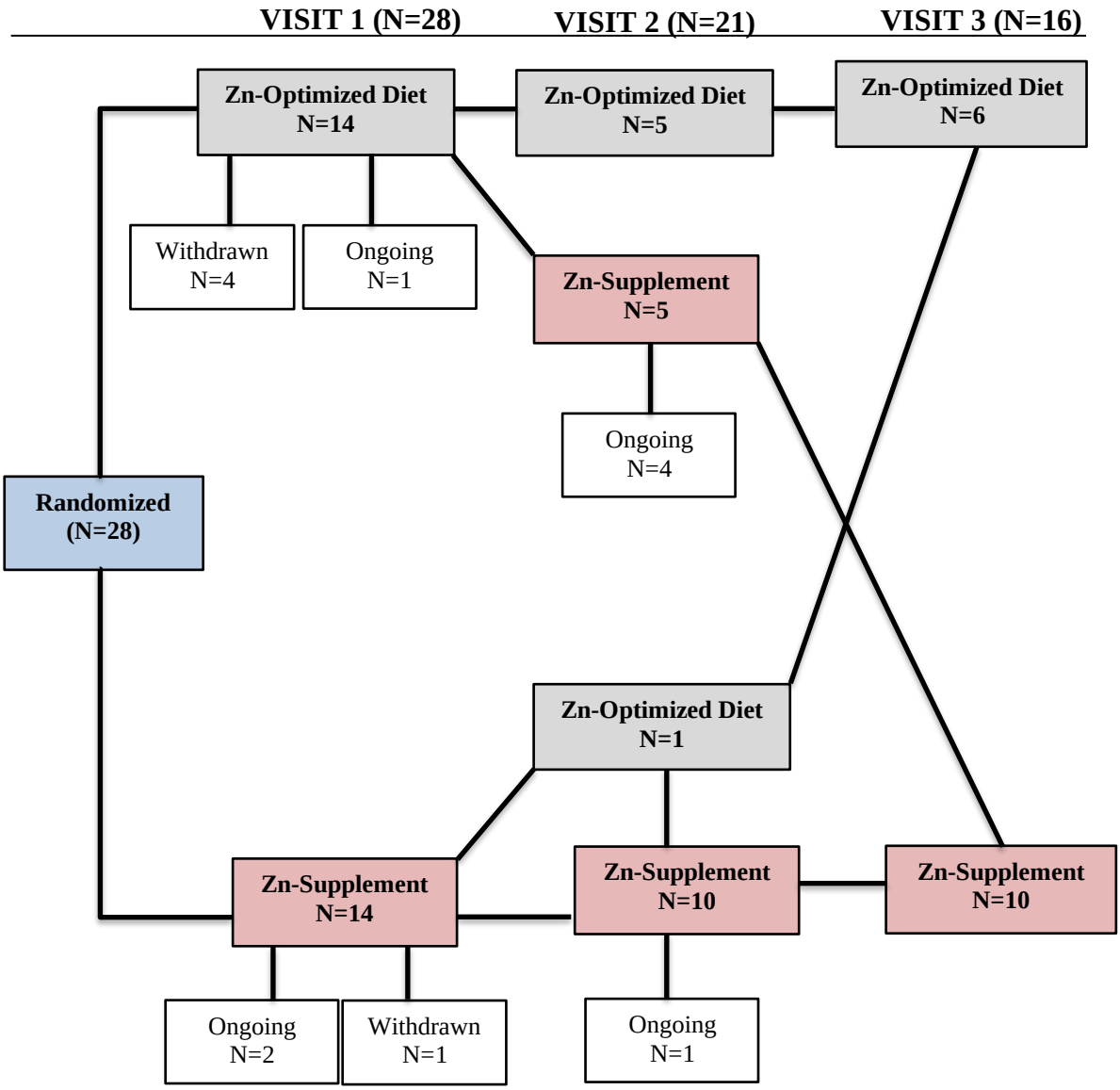


Figure 3.3 Flowchart of Participants Per Intervention and Visit Completion (May 25, 2024)

Table 3.2 Timeline of Study Events (May 2022-Present)

Date	Event Occurred
May 31 2022	Study Submitted to HiREB
July 7 2022	Provisional Approval Granted
September 2022	Started MSc
November 7 2022	Final Approval Granted- recruitment started
December 19 2022	Amendment 1: Use Zamplo Instead of RedCap for Participant Questionnaires
May 4th 2023	Amendment 2: Approval to remove "previous Zn supplementation in the last 30 days" from exclusion criteria to increase recruitment
August 2023	Protocol Deviation: Participant Zn006 stopped taking the Zn supplementation (50 mg/day zinc gluconate) provided by study and started taking their own brand of Zn supplement (25/day zinc bisglycinate)
December 5th 2023	Amendment 3: Approval to move Visit 1 to remote visit to increase recruitment
April 8th 2024	Amendment 4: Additional Questionnaire "Qualitative Assessment of Zn Diet/Supplementation approved

Table 3.3 Changes in Patient Recruitment Over Time (November 2022-Present)

Date	Method of Recruiting	Patient(s)/Month
November 2022-March 2023	Dr. Pinto-Sanchez's secretary (Sherry) would contact eligible patients through MyChart and/or email for the Zn pragmatic and/or Zn pilot study. Those who were interested would be forwarded to the study coordinator for follow-up.	0.8
April 2023-August 2023	Dr. Pinto-Sanchez's secretary (Tamara) would contact eligible patients through MyChart and/or email for the Zn pragmatic and/or Zn pilot study. Those who were interested would be forwarded to the study coordinator for follow-up. Study coordinator would also make first contact with potential patients on behalf of Dr. Pinto-Sanchez to increase recruitment.	2.6
September 2023-November 2023	Only secretary (Tamara) contacted eligible patients, however patients were only contacted for the Zn pilot study as Zn pragmatic study recruitment ended. Therefore, patient was not approached by coordinator.	1
December 2023-April 2023	Dr. Pinto-Sanchez would directly message eligible patients for the study through MyChart. Those interested were provided the study coordinator's email address to directly contact. Patients were not approached by coordinator. Study Coordinator would proactively contact participants who recently completed the oats study with low zinc.	1.4

Table 3.4 Compliance to Interventions at 3 Months

	Zn Supplement N=11	**Zn-Optimized Diet[1,4] N=10**
Pills Taken, Median (IQR)	76 (17)	NA
Additional Zinc Supplementation (mg), median (IQR)[2]	19.5 (9)	11 (16.5)
Daily Zn (mg), Median (IQR)	8.65 (3.61)	18.19 (17.95)
Phytates (mg), Median (IQR)	660.5 (382)	971.57 (794.3)
Bioavailable Zn (mg), Median (IQR)	2.45 (1.09)	5.46 (5.15)
Compliance, n (%)[3]	9 (81.8)	5 (50.0)

[1] Data for ASA 24 Excludes Baseline for Zn-Optimized Diet Group

[2] Additional Zinc Supplementation (mg): Includes multivitamin and previous Zn supplementation

[3] Compliance: Zn Supplement= 75% of pills taken at V2; Zn-Optimized Diet= N patients increase in Bioavailable Zn at V2

[4] Two out of the total ten participants in the Zn-optimized diet intervention did not complete food records. Data is not available for Zn and phytates.

Table 3.5 Bioavailable Zn and pZn in Zn-Optimized Diet at 3 months

Study ID	PZn-3 months (μmol/L)	PZn Normalized-Y/N	Compliant with Diet-Y/N	Baseline Bioavailable Zn (mg)	Post Intervention Bioavailable Zn (mg)	Zinc Diet (mg)
Zn001	11.3	Y	N	6.91	6.05	20.18
Zn010	9.3	N	Y	2.63	7.07	23.57
Zn011	10.5	Y	N	4.26	3.14	10.47
Zn012	13.2	Y	Y	2.91	4.86	16.20
Zn013	10.2	Y	Y	1.93	10.92	36.41
Zn017	13	Y	Y	2.63	3.57	11.89
Zn020	8	N	N	4.83	4.11	13.71
Zn022	8.1	N	Y	1.71	9.45	32.54

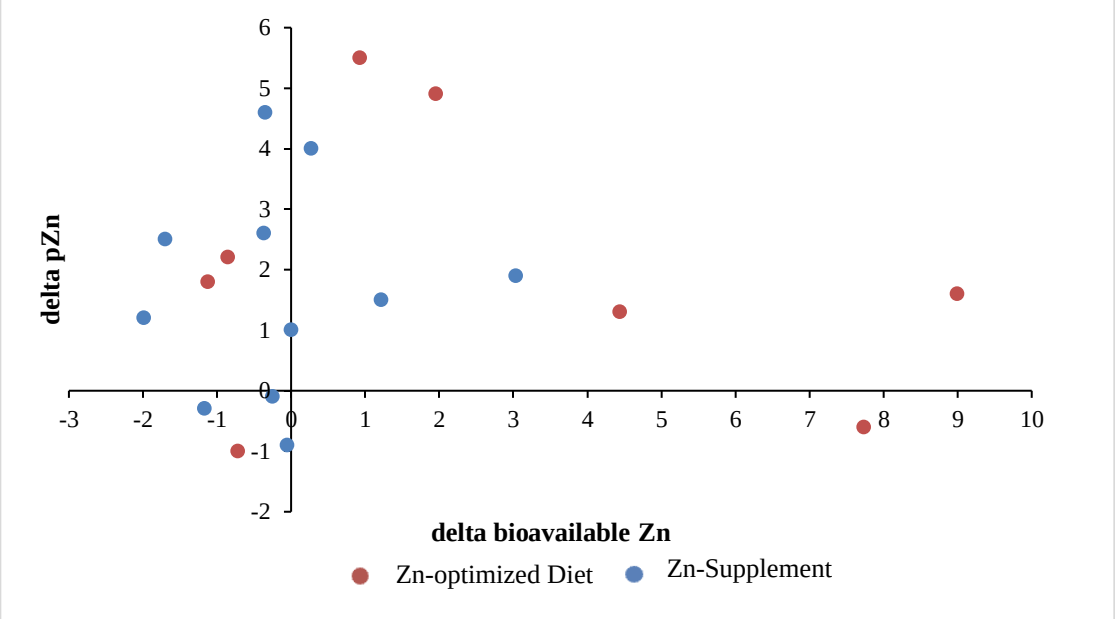


Figure 3.4 Delta bioavailable Zn vs. Delta pZn in Zn-Supplement and Zn-Optimized Diet at 3 months

Table 3.6 Point Estimates for Secondary Outcomes at 3 months

	Zn-Optimized Diet N=10	**Zn Supplement N=11**	**Proposed sample size for RCT[1]**
Number of patients that normalized pZn (n,%)	**5 (50.0)**	**8 (72.7)**	**142**
Number of patients maintaining normal Zn levels at 6 months[2]	**5 (83.3)**	**8 (88.9)**	-
Plasma Zinc (Mean ± SD)	10.08 ± 1.9	10.77 ± 2.03	-
CSI (Mean ± SD)	29.4 ± 4.88	33.18 ± 10.05	-
HADS Anxiety (Mean ± SD) HADS Depression (Mean ± SD)	6.7 ± 3.83 4.3 ± 1.95	7.8 ± 3.19 4.8 ± 2.14	-
ES-VAS (n, %) Skin Rash Headache Foggy Mind Fatigue Numbness of Limbs Joint or muscle pain Fainting Canker Sores Feeling Anxious Feeling Depressed	 4 (40.0) 5 (50.0) 7 (70.0) 10 (100.0) 3 (30.0) 9 (90.0) 1 (10.0) 2 (20.0) 5 (50.0) 4 (40.0)	 2 (18.2) 8 (72.3) 7 (63.6) 10 (90.9) 5 (45.5) 9 (81.8) 0 (0.0) 3 (27.3) 7 (63.6) 6 (54.5)	-
Ced-QoL (Mean ± SD)	45.3 ± 18.94	48.2 ± 14.95	-

[1] RCT (alpha 0.05, power=80%)
[2]Sample Size 3-6 months: Zin-Optimized Diet (N=6), Zn Supplement (N=9)

Table 3.7 Secondary Outcomes and Within Group Differences at V1, V2 and V3

	Visit 1		p	Visit 2		p	Visit 3		p
	D N=14	**S N=14**		**D N=10**	**S N=11**		**D N=6**	**S N=10**	
pZn levels, Median (IQR)	8.7 (0.9)	9.0 (1.0)	0.56	9.8 (3.5)	10.5 (1.4)	0.44	10.65 (2.9)	11.1 (3.2)	0.93
CSI Total, Median (IQR)	31 (11)	32.5 (16)	0.61	28.5 (8)	31 (19)	0.53	27.5 (11)	38.5 (15)	0.07
Stomach Pain	2 (1)	2 (2)	0.73	1.5 (1)	2 (2)	0.33	1 (1)	3 (2)	0.05
Nausea	1 (1)	1 (1)	0.91	1 (0)	2 (1)	**<0.01**	1 (0)	1 (1)	0.15
Stomach Rumbling	1 (1)	1 (1)	0.87	2 (1)	1 (1)	0.42	1 (0)	2 (0)	**<0.01**
Bloating	2.5 (2)	2 (2)	0.92	2 (1)	2 (3)	0.53	2 (1)	1.5 (3)	0.91
Diarrhea	1 (1)	1 (1)	0.52	1 (1)	1 (1)	0.72	1 (0)	1 (1)	0.29
Feel Bowels Not Empty	2 (1)	2 (0)	0.33	1 (2)	1 (1)	0.94	2 (1)	2 (3)	0.61
Hunger Pains	2 (1)	1 (1)	0.39	2 (1)	2 (1)	0.11	1 (1)	2 (0)	**0.02**
Low Energy	3 (1)	3 (2)	0.88	2 (0)	2 (3)	1.0	2 (1)	3 (2)	0.18
Headaches	1 (1)	2 (2)	0.06	1 (2)	2 (2)	0.27	1.5 (1)	2 (1)	0.05
Food Cravings	2 (2)	2.5 (1)	0.43	2 (0)	2 (2)	0.88	1.5 (2)	3 (1)	0.2
Low Appetite	1 (1)	1 (1)	0.61	1 (0)	1 (1)	0.33	1 (0)	1 (1)	0.08
CeD-QoL, Median (IQR)	46 (28)	42 (21)	0.71	42 (25)	45 (24)	0.57	45.5 (25)	44.5 (23)	0.96
HADS-Total, Median (IQR)	8.5 (9)	11 (4)	0.43	10.5 (9)	14 (3)	0.46	11.5 (13) 7.5 (8)	11.5 (5) 7.5 (5)	0.83 0.48
HADS-Anxiety	6 (4)	6.5 (5)	0.78	6.5 (8)	8 (3)	0.39	4 (5)	4 (3)	0.70
HADS-Depression	3.5 (4)	4 (4)	0.34	3.5 (2)	5 (3)	0.43			
ES-VAS, n (%)									
Skin Rash	7 (50)	6 (42.8)	1.0	4 (40.0)	2 (18.2)	0.36	4 (66.6)	3 (30.0)	0.30
Headache	7 (50)	10 (71.4)	0.44	5 (50.0)	8 (72.7)	0.39	4 (66.6)	10(100.0)	1.0
Foggy Mind	12 (85.7)	12 (85.7)	1.0	7 (70.0)	7 (63.6)	1.0	6 (100.0)	8 (80.0)	0.5
Fatigue	13 (92.9)	14 (100.0)	1.0	10 (100.0)	10 (90.9)	1.0	6 (100.0)	9 (90.0)	1.0
Numbness of Limbs	6 (42.9)	5 (35.7)	1.0	3 (30.0)	5 (45.5)	0.66	2 (33.3)	4 (40.0)	0.12
Joint/Muscle Pain	11 (78.9)	9 (64.3)	0.68	9 (90.0)	9 (81.8)	1.0	6 (100.0)	8 (80.0)	0.50
Fainting	3 (21.4)	1 (7.1)	0.60	1 (10.0)	0 (0.0)	0.48	1 (16.7)	1 (10.0)	1.0
Canker Sore	3 (21.4)	3 (21.4)	1.0	2 (20.0)	3 (27.3)	1.0	2 (33.3)	3 (30.0)	1.0
Feeling Anxious	10 (71.4)	10 (71.4)	1.0	5 (50.0)	7 (63.6)	0.67	4 (66.6)	9 (90.0)	0.52
Feeling Depressed	8 (57.1)	9 (71.4)	1.0	4 (40.0)	6 (54.5)	0.67	4 (66.6)	7 (70.0)	1.0
Adverse Events Reported (n)	-	-	-	11	12	-	1	10	

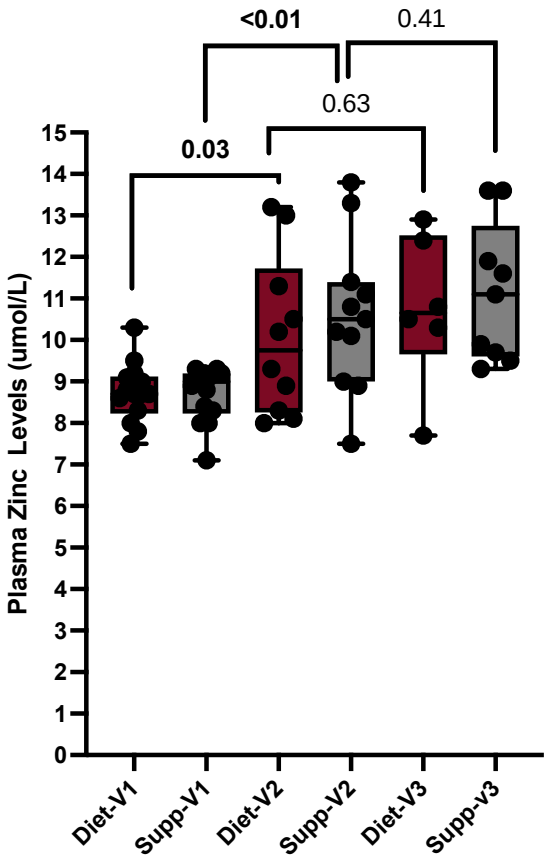


Figure 3.5 Plasma Zinc Levels Before and After 3 (V2) and 6 (V3) Months of Intervention. Box plots of pZn (µmol/L) in the diet and supplement intervention at Visit 1, Visit 2 and Visit 3 Visit. P-values <0.05 were considered statistically significant.

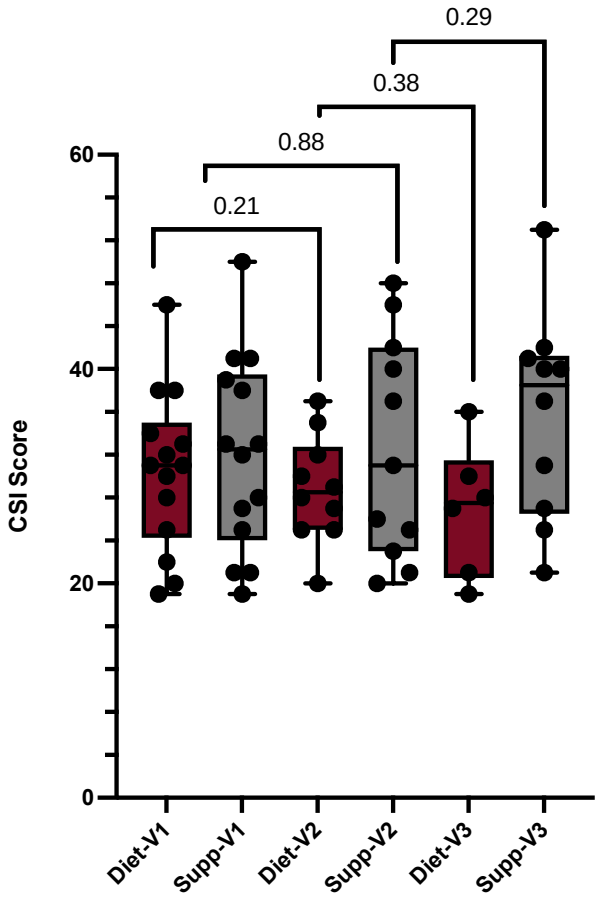


Figure 3.6 Celiac symptoms (CSI) before and after 3 (V2) and 6 (V3) months of intervention. Box plots of CSI scores in the diet and supplement intervention at Visit 1 , Visit 2, and Visit 3. P-values <0.05 were considered statistically significant.

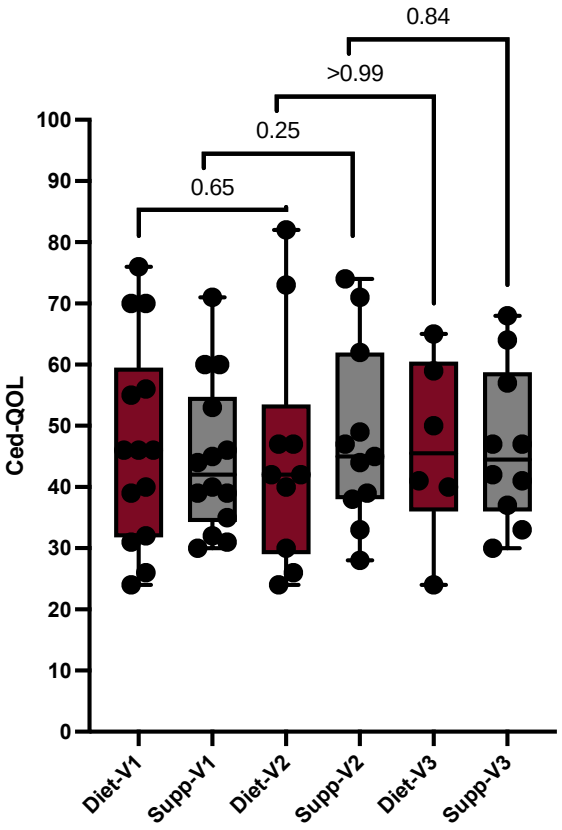


Figure 3.7 Quality of life (CeD-QoL) before and after 3 (V2) and 6 (V3) months of intervention. Box plots of CeD-QoL scores in the diet and supplement intervention at Visit 1, Visit 2, and Visit 3 Visit (B). P-values <0.05 were considered statistically significant.

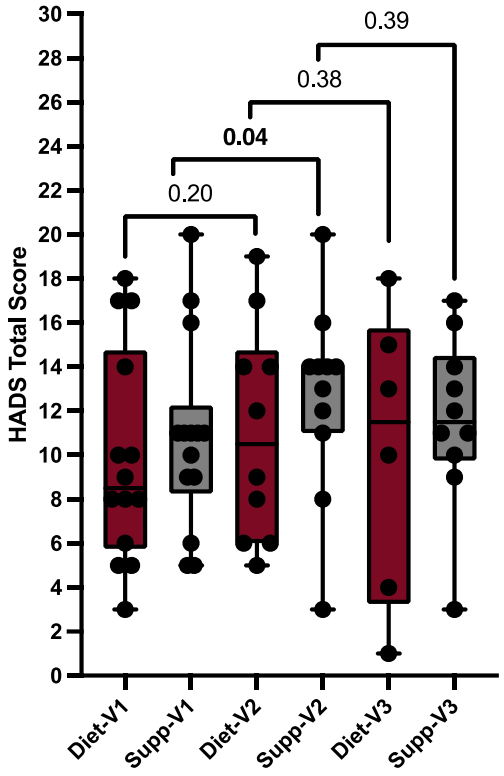
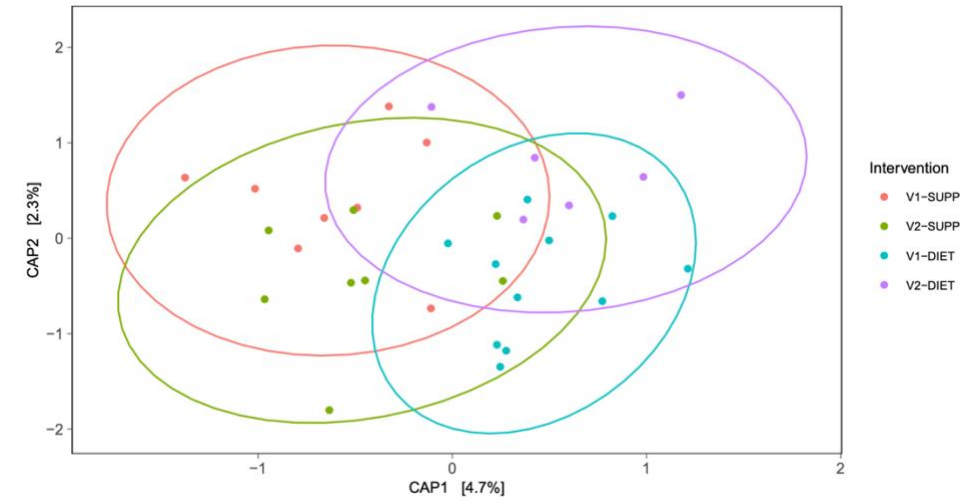


Figure 3.8 HADS Score before and after 3 (V2) and 6 (V3) months of intervention
Box plots of HADS scores in the diet and supplement intervention at Visit 1, Visit 2, and Visit 3. P-values <0.05 were considered statistically significant.

(A)



(B)

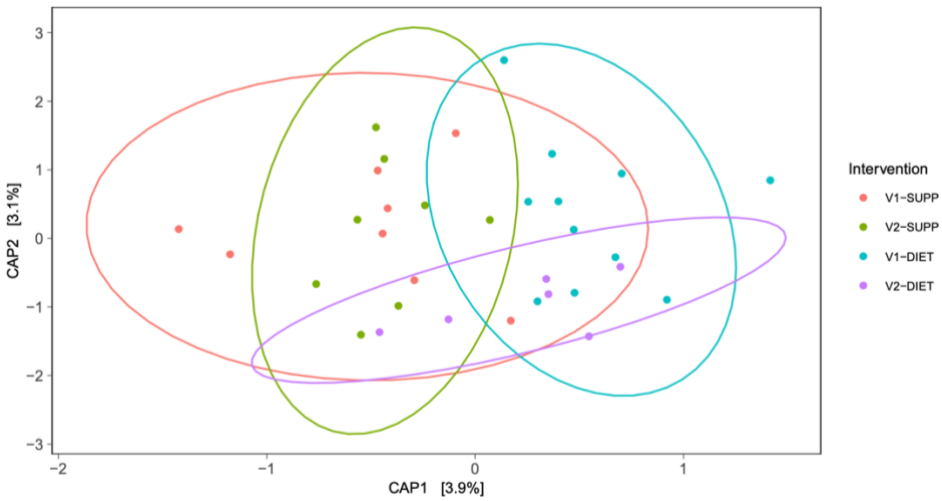


Figure 3.9 Beta-Diversity PCoA analysis with (a) Unweighted and (b) Weighted UniFrac distance

Table 3.8 Predictors on Normalization of pZn at 3 months

Predictor	OR (95% CI)	p-value
Urine Zinc	1.27 (0.88-2.38)	0.26
Albumin	1.40 (0.86-2.67)	**0.01**
Zinc Dietary Intake	0.95 (0.84-1.07)	0.25
Phytate Dietary Intake	1.00 (0.99-1.00)	0.79
Number of Zn Supplements Taken	0.91 (0.75-1.06)	0.31
TTG-IgA-Abnormal (Y/N)	2.1 (0.22-47.46)	0.69
DGP-IgG-Abnormal (Y/N)	0.37 (0.01-10.67)	0.71
CeDAT	1.23 (0.82-1.99)	0.39
GIP Detected (Y/N)	NA	-
Weight (kg)	1.01 (0.96-1.08)	0.71
BMI	0.99 (0.85-1.18)	0.61
Sex-Female	0.79 (0.03-9.80)	0.91

Table 3.9 Most Common Adverse Events from 0-3 months

	Zn-Optimized Diet, n (%) N=10	Zn Supplement, n (%) N=11	p-value	Casualty	Outcomes
Shingles	1 (9.1)	0 (0.0)	0.48	NR[1]	Recovered
Abdominal pain	0 (0.0)	1 (8.3)	1.0	NR	Recovered
Fatigue	0 (0.0)	1 (8.3)	1.0	NR	Recovered
Nausea	0 (0.0)	1 (8.3)	1.0	NR	Recovered
Diarrhea	0 (0.0)	2 (16.7)	0.48	NR	Recovered
Heartburn	1 (9.1)	0 (0.0)	0.48	NR	Recovered
Vomiting	1 (9.1)	1 (8.3)	1.0	NR	Recovered
Hair Loss	0 (0.0)	1 (8.3)	1.0	NR	Recovered
Blood in Stool	0 (0.0)	1 (8.3)	1.0	NR	Recovered
Stomach Flu	1 (9.1)	0 (0.0)	0.48	NR	Recovered
Dehydration	0 (0.0)	1 (8.3)	1.0	NR	Recovered
Headache	1 (9.1)	1 (8.3)	1.0	NR	Recovered
Sinus Infection	0 (0.0)	1 (8.3)	1.0	NR	Recovered
Cold	2 (18.2)	1 (8.3)	0.59	NR	Recovered
Arthritis	1 (9.1)	0 (0.0)	0.48	NR	Ongoing
Muscle Soreness	2 (18.2)	0 (0.0)	0.21	NR	Ongoing
Dizziness	1 (9.1)	0 (0.0)	0.48	NR	Recovered
Total # of Participants with AE	8 (80.0)	7 (63.6)	0.06		
Total # of AEs	11	12	-		

[1]NR=Not Related to Intervention

Table 3.10 Patient Knowledge and Perception of Zn-diet and Zn-supplementation Before and After Intervention

	D-V1 (n=14)	S-V1 (n=14)	p	D-V2 (n=10)	S-V2 (n=11)	p
Knowledge of Nutrient Deficiencies			.68			.87
Extremely	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Very good	1 (7.1)	2 (14.3)		3 (30.0)	3 (27.3)	
Somewhat	8 (57.1)	7 (50.0)		6 (60.0)	6 (54.5)	
Not very	4 (28.6)	5 (35.7)		1 (10.0)	2 (18.2)	
Not at all	1 (7.1)	0 (0.0)		0 (0.0)	0 (0.0)	
Importance to Treat Low Zinc Levels			.19			.55
Extremely	0 (0.0)	3 (21.4)		3 (30.0)	2 (40.0)	
Very	12 (85.7)	9 (64.3)		5 (50.0)	8 (72.7)	
Somewhat	2 (14.3)	1 (7.1)		2 (20.0)	1 (9.1)	
Not very	0 (0.0)	1 (7.1)		0 (0.0)	0 (0.0)	
Not at all	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Currently taking Supplements			.28			0.94
Yes	13 (92.9)	11 (78.6)		9 (90.0)	10 (90.9)	
No	1 (7.1)	3 (21.4)		1 (10.0)	1 (9.1)	
Preference to Treat Nutrients			.12			.81
Dietary Changes	11 (78.6)	7 (50.0)		4 (40.0)	3 (27.3)	
Supplement	3 (21.4)	7 (50.0)		5 (50.0)	6 (63.6)	
No preference	0 (0.0)	0 (0.0)		1 (10.0)	1 (9.1)	
Don't want to treat	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Familiar with Zinc Supplement			.21			.74
Extremely	0 (0.0)	1 (7.1)		0 (0.0)	1 (9.1)	
Very	1 (7.1)	3 (21.4)		3 (30.0)	3 (27.3)	
Somewhat	9 (64.3)	4 (28.6)		2 (20.0)	3 (27.3)	
Not so much	3 (21.4)	6 (42.9)		5 (50.0)	4 (36.4)	
Not at all	1 (7.1)	0 (0.0)		0 (0.0)	0 (0.0)	
Familiar with Zinc Diet			**<0.01**			**0.02**
Extremely	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Very	0 (0.0)	1 (7.1)		5 (50.0)	1 (9.1)	
Somewhat	12 (85.7)	3 (21.4)		5 (50.0)	5 (45.5)	
Not so much	1 (7.1)	9 (64.3)		0 (0.0)	5 (45.5)	
Not at all	1 (7.1)	1 (7.1)		0 (0.0)	0 (0.0)	

Likely to treat with Zinc Supplement			.35			0.05
Extremely	3 (21.4)	5 (35.7)		2 (20.0)	8 (72.7)	
Very	7 (50.0)	7 (50.0)		5 (50.0)	2 (18.2)	
Somewhat	4 (28.6)	1 (7.1)		3 (30.0)	1 (9.1)	
Not so much	0 (0.0)	1 (7.1)		0 (0.0)	0 (0.0)	
Not at all	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Likely to Treat with Zinc Diet			.10			.75
Extremely	0 (0.0)	1 (7.1)		1 (10.0)	0 (0.0)	
Very	9 (64.3)	5 (35.7)		4 (40.0)	5 (45.5)	
Somewhat	5 (35.7)	4 (28.6)		3 (30.0)	4 (36.4)	
Not so much	0 (0.0)	3 (28.6)		2 (20.0)	2 (18.2)	
Not at all	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Likely to Comply Zinc Diet			.41			0.35
Extremely	4 (28.6)	2 (14.3)		2 (25.0)	0 (0.0)	
Very	6 (42.9)	5 (35.7)		4 (40.0)	7 (63.6)	
Somewhat	4 (28.6)	5 (35.7)		2 (25.0)	1 (9.1)	
Not so much	0 (0.0)	2 (14.3)		2 (25.0)	3 (27.3)	
Not at all	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Likely to Comply Zn Supplement			.59			.44
Extremely	7 (50.0)	6 (42.9)		3 (30.0)	6 (54.5)	
Very	3 (21.4)	5 (35.7)		3 (30.0)	3 (27.3)	
Somewhat	3 (21.4)	1 (7.1)		4 (40.0)	2 (18.2)	
Not so much	1 (7.1)	2 (14.3)		0 (0.0)	0 (0.0)	
Not at all	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Zn-GFD Expensive			.45			.54
Yes	7 (50.0)	5 (35.7)		4 (40.0)	3 (27.3)	
No	7 (50.0)	9 (64.3)		6 (60.0)	8 (72.7)	
Zn-Diet Cost Limitation			.24			1.0
Extremely	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Very	0 (0.0)	0 (0.0)		1 (25.0)	1 (33.3)	
Somewhat	2 (28.6)	4 (80.0)		3 (75.0)	2 (66.7)	
Not so much	5 (71.4)	1 (20.0)		0 (0.0)	0 (0.0)	
Not at all	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	

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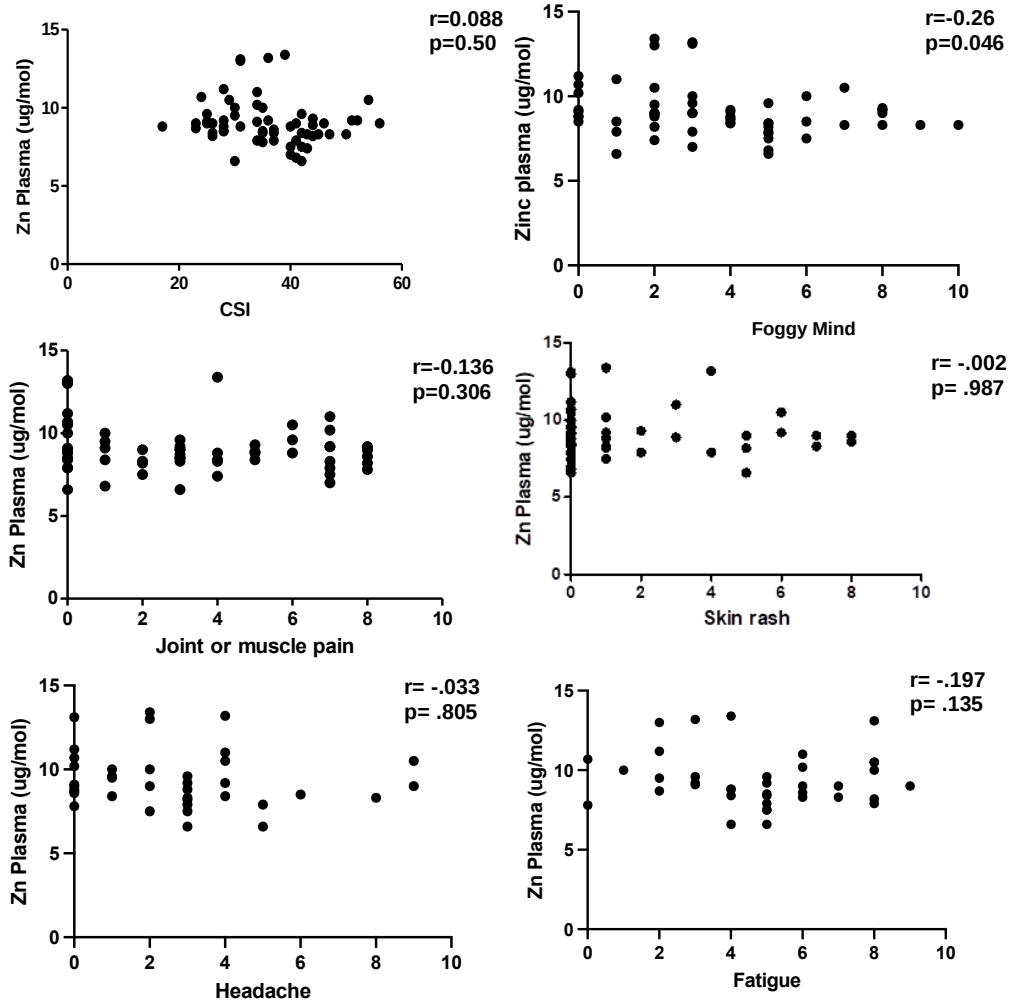
Appendix A: Zn Pragmatic Study: Spearman Correlations



Appendix B: Table of Zinc Foods

Food	Serving size	mg of Zn per serving	%DV
Oysters, farmed, raw	3 ounces	32.0	291
Oysters, cooked, breaded and fried	3 ounces	74.0	673
Beef, bottom sirloin, roasted	3 ounces	3.8	35
Beef chuck roast, braised	3 ounces	8.0	64
Crab, Alaska king, cooked	3 ounces	6.5	59
Beef patty, broiled	3 ounces	5.3	48
Lobster, cooked	3 ounces	3.4	31
Whey protein	1 scoop (30 gr)	2.9	26
Heart of palm	2 hearts	3	27
Baked beans, canned, plain	½ cup	2.9	26
Breakfast cereal, fortified with 25% of the DV for zinc	½ cup	2.8	25
Dark chocolate	30 g	2.5	22
Chicken, dark meat, cooked	3 ounces	2.4	22
Cereals, oats, regular and quick, unenriched, cooked with water	1 cup	2.3	21
Pumpkin seeds, dried	1 ounce	2.2	20
Lentils	¾ cup	2	18

Ricotta cheese	½ cup	2	18
Napa Cabbage	½ cup	2	18
Pork chop, loin, cooked	3 ounces	1.9	17
Yogurt, fruit, low fat	8 ounces (3/4 cup)	1.7	15
Turkey	75g (2 ½ oz)	1-2	8-15
Cashews, dry roasted	1.5 ounces (50 g)	3	15
Cheese, cheddar	1.5 ounces	1.5	14
Shrimp, cooked	3 ounces	1.4	13
Chickpeas, cooked, hummus	½ cup	1.3	12
Cheese, Swiss	1.5 ounces (50g)	1-2	11
Sardines, canned in oil, drained solids with bone	3 ounces	1.1	10
Oatmeal, instant, plain,	1 packet	1.1	10
Mushrooms	1 cup	1.3	10
Milk, low-fat or non-fat	1 cup	1.0	9
Almonds, dry roasted	1 ounce	0.9	8
Kidney beans, cooked	½ cup	0.9	8
Greek Yogurt, plain	6 ounces	1.0	9
Chicken breast, roasted, skin removed	½ breast	0.9	8
Egg	1 egg(63g)	0.6	5

Appendix C: Table of Zinc Foods with Phytate Values

Zinc Foods with Phytate Values
High=less availability of zinc **(bad)**
Low= more availability of zinc **(good)**

Food (100 gr)	Zn	Phytate	Phy/Zn	Phy/Zn interpretation <15 low; >30 high
Cereals				
Maize flour	2.2	792	36	High
Rice bran,	6.04	2560	42	high
Oats	3.97	1116	28	High
Maize Bran	3.7	1089	29	High
Sorghum flour	1.4	446	32	High
Corn	3.06	367	12	low
Legumes, seeds and nuts				
Ground nuts, flour	2.8	1297	45	High
Pigeon peas	2.2	727	33	High
Kidney beans	1.5	557	36	High
soybeans	4.89	2200	45	High
Sesame seeds	7.16	2500	35	High
Peanuts	1.83	952	52	High
Walnuts	3.37	982	30	High
Brazilian nuts	4.06	1719	42	High
lentils	3.27	779	24	moderate
Almonds	4.92	1400	28	moderate
Vegetables /roots (boiled)				
potato	0.25	1000	40	High

Cabbage	0.7	5	1	Low
Sweet potato	0.2	10	5	Low
Pumpkin	0.7	34	5	Low
Yam	0.3	50	13	Low
Fruits				
Mango	0.1	25	23	moderate
Avocado	0.3	11	3	Low
Banana	0.2	22	9	Low
Composed dishes with rice				
Rice and stew	0.6	118	21	moderate
Rice and beans	0.5	107	18	moderate

TIP: To reduce phytates, use sprouted beans and soak rice, beans and nuts for 24 hours and cook them for the longest time possible

Appendix D: Zinc Pragmatic Study: Treatment Preference

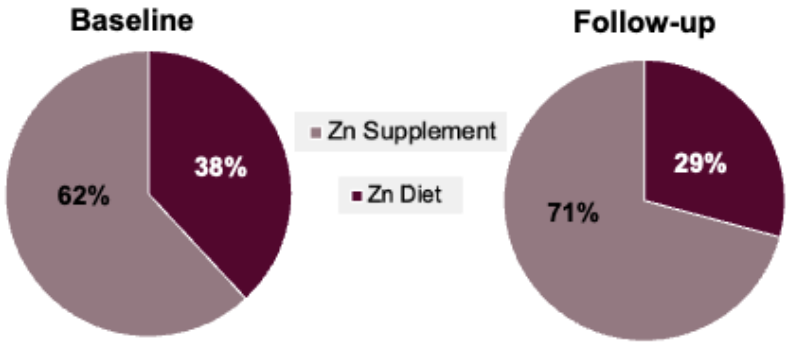


Figure 1. Preference for Zn Supplements vs. Zn Diet to Treat Zn Deficiency (%)

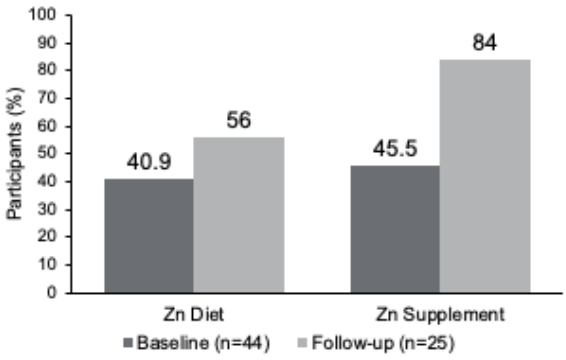


Figure 2. Familiar with Zn supplement vs diet

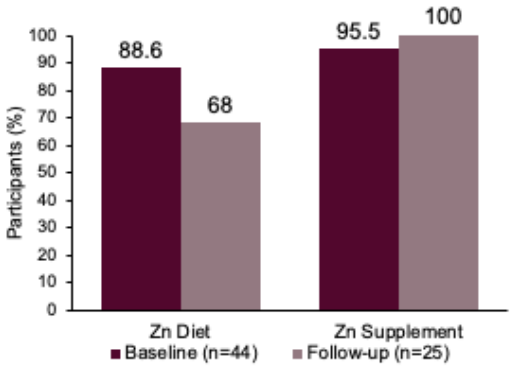


Figure 3. Likely to Adhere to treatments

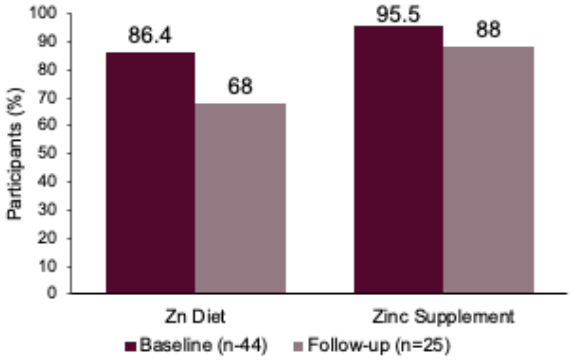


Figure 4. Likely to Strictly Comply to treatments

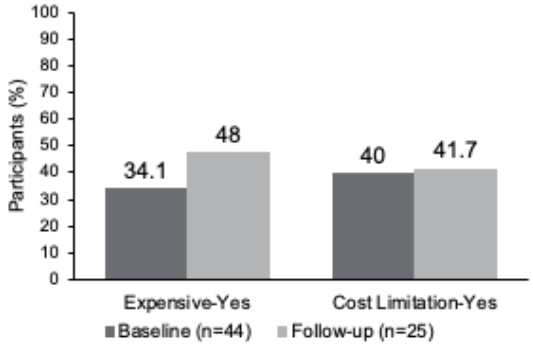


Figure 5. Cost Perception of a Zn-GFD (%)

Appendix E: Dietary Handout

Participant Dietary Information

Thank you for participating in our zinc pilot study. Today we will give you information on how you can increase your intake of zinc through diet.

What is Zinc and What Does it Do?

Zinc is a mineral that your body needs to be healthy. Zinc strengthens your immune system and helps with normal growth and development at all ages. Symptoms that are associated with zinc deficiency include: changes in taste and smell, loss of appetite, diarrhea, hair loss, impaired immune response, impotence, and tiredness.

Recommended Daily Intake

The daily recommended intake of zinc in adults is 11 mg for men and 8 mg for women. However, higher zinc levels may be needed when zinc blood levels are low. We recommend intaking **14 mg for males and 11 mg for females for this study.**

Food Sources of Zinc

The following table shows food sources of zinc, see **Table 1 (laminated copy).**

The best sources of zinc are oysters and beef, followed by crab, lobster, heart of palm, pork chop, baked beans, fortified breakfast cereal, dark meat of chicken, pumpkin seeds, yogurt, ricotta cheese, cashews, dark chocolate, chickpeas, Swiss cheese, lentils, oatmeal, milk, napa cabbage, mushrooms, almonds, kidney beans, chicken breast, turkey, eggs, whey protein.

Although zinc comes from a variety of different foods, the best sources are high protein foods like oysters, seafood, meat, beans and lentils. Some dairy products also have zinc.

Phytic Acid Blocks Zinc Absorption

Some foods that are rich in zinc are also high in phytates. Phytates are compounds in some vegetables that bind with minerals like zinc, and they prevent its absorption. For our study it is important that you keep a balance by monitoring your intake of high-phytate foods. You can do this by choosing mostly foods that have a low or moderate phytate to zinc ratio, and limiting foods with a high ratio. The foods highlighted in red are high in phytate, please limit your intake of these foods. If you do choose to eat phytic rich foods, try to eat them at different meals/times from zinc rich foods. Similarly, if you are taking iron or copper supplements they interfere with the absorption of zinc therefore spread them out 2 hours before or after meal.

Preparation Methods to Remove Phytates

Soak rice, beans and nuts for 24 hours before cooking or consumption. Cook rice and beans for the longest time possible (=maximum time indicated on the package). Please pay attention to this during your meal planning. After soaking please pat food dry, and store in the fridge.

Version 1, Oct 3 2023

Appendix F: Zn-GFD Recipes

Gluten-Free Recipes to Boost Your Zinc Intake

- Oysters are the best source of zinc. They are best enjoyed raw. Here are some other zinc boosting recipes to help you meet your daily zinc needs
- To reduce phytate content of your foods: soak rice, beans, and nuts for 24 hours and cook them for the longest time possible
- ** Denotes that these are zinc containing foods. Try not to omit or substitute these ingredients

Simple Grab & Go Snack Ideas
• Dark Chocolate (30 grams / 3 tbsp =2.5mg zinc) • Hard-Boiled Eggs (1 egg = 0.9mg zinc) • Yogurt (3/4 cup plain yogurt = 1.7mg) • Glass of cow's milk (1 cup = 1mg zinc) • Cheese slices/cubes (1.5 oz = 1.5mg) • Trail mix made of dark chocolate, raisins, almonds, gluten-free pretzels

Group	Daily Zinc Needs
Adult Male	> 11mg
Adult Female	> 8mg
Adult Pregnant	> 11mg
Adult Breastfeeding	> 12mg

Breakfast		
Veggie & Cheese Stuffed Omelet	**Breakfast Pizza**	**Yogurt Parfait**
Serves 1 • 2 eggs ** • 1/2 cup mushrooms** • 50g Swiss or Cheddar cheese, shredded** • 1 cup fresh spinach • 1 tbsp chopped onion • 1 tsp olive oil • Salt & pepper to taste 1) Saute veggies in oil 2) In another pan, cook egg. Fill omelet with veggies and cheese 3) **Can turn into scrambled eggs for ease** *Zinc per serving: 4mg*	Serves 1 • 2 eggs ** • 1-2 tbsp basil pesto • 2 tbsp ham, diced • 50g cheddar cheese, shredded** • 1 tbsp green onion • 1-slice tomato, diced • Salt and pepper to taste 1) Fry egg in a pan until fully cooked 2) Spread a layer of pesto over egg 3) Add other toppings, on low heat, put lid on pan to allow cheese to melt *Zinc per serving: 3.6mg*	Serves 1 • 3/4 cup plain yogurt (1 or 2%) ** • 1/4 cup fresh or frozen fruit (bananas, berries, pears) • 1 tbsp pumpkin seeds, toasted** • Honey or maple syrup *1)* Layer yogurt, fruit, pumpkin seeds and if desired a sweetener of choice in a bowl or mason jar *Zinc per serving: 2.2 mg*

Lunch		
Lentil Soup	**Grilled Cheese & Chicken Sandwich**	**Zinc Loaded Salad**
Serves 4 • 3 tbsp olive oil • 2 medium carrot, diced • 1 onion, diced • 1 stalk celery, diced • 3 cups mushrooms, chopped** • 1 1/2 cup lentils** • 2 tbsp tomato paste • 4 cups vegetable stock • 1 tsp thyme leaves (dried) • 1 bay leaf • 1/2 tsp oregano (dried) • Salt and pepper to taste • 4 tbsp lemon juice • Serve each with 50g Swiss cheese** 1) Saute carrot, onion, celery and mushrooms together in oil until caramelized (8-10 minutes) 2) Stir in remaining ingredients (except lemon juice). Bring to boil, reduce heat, cover and cook until lentils are softened (35-40 minutes). Use a blender to puree half of soup 3) Stir in lemon juice at serving *Zinc per serving: 3.5mg*	Serves 1 • 2 slices gluten-free bread • 2 slices Swiss cheese ** • 75grams (2.5oz) cooked chicken breast** • 1/2 cup sauteed mushrooms** • Butter/margarine 1) Coat outside of bread slices with butter or margarine 2) Layer bread, cheese, chicken, onion, bread 3) Grill over medium heat until crispy and cheese is melted. *Zinc per serving: 3.7mg*	Serves 1 • 1 hard-boiled egg** • 75grams (2.5oz) cooked chicken breast** • 50g (1.5oz) cheese (Swiss, Cheddar, Parmesan), cubed** • Leafy greens mix • Cherry tomatoes • Cucumber • Favorite gluten-free salad dressing *1)* On a bed of your favorite salad greens, mix in egg, chicken breast, cheese and your favorite salad add-ins *Zinc per serving: 4 mg*

Dinner			
Beef, Zucchini & Sweet Potato Skillet	**Chicken Pesto Pasta**	**Pork Chop Dinner**	**Other Dinner Ideas**

Serves 5
• 1 tbsp olive oil
• 1 pound lean ground beef**
• 1 clove garlic, minced
• 1 cup onion, diced
• 2 bell peppers, diced
• 2 large sweet potatoes, cubed, and roasted
• 2 medium zucchinis, chopped
• 1/4 cup water
• 1 tsp Dijon mustard
• 1/4 cup ketchup
• 1/2 tsp each of garlic powder, cumin, paprika
• Salt and pepper
1) Cook ground beef in oil. Add garlic, onion, zucchini and peppers cooking until vegetables are soft and beef is cooked through. Add potatoes, water, mustard, ketchup and seasonings.
2) Stir together, cook 5-10 minutes more to allow flavors to mix
Zinc per serving: 5mg
	Serves 4
	• 1.5 cups chicken, dark meat**, cooked, shredded
	• 1 tbsp butter
	• 3 cups mushrooms, sliced **
	• 2 cloves garlic, minced
	• 2 cups diced tomatoes (canned)
	• 3 cups baby spinach
	• 1/2 cup heavy cream
	• Gluten-free pasta (12 oz)
	• Salt and pepper
	• 1 cup (110g) parmesan cheese
	1) Cook pasta, keeping 1 cup of pasta water
	2) Saute mushrooms and garlic in butter until browned.
	3) Add spinach cooking until wilted. Add pasta, chicken, cream and 1/2 cup reserved pasta water (or use chicken broth instead of water). Stir together
	4) Serve with parmesan cheese
	Zinc per serving: 4.1mg
		Serves 1
		• 1 pork chop, lean, cooked (3 oz)**
		• 1/2 cup wild rice, cooked**
		• 1 cup steamed vegetables (carrots, broccoli, cauliflower, green beans)
		Zinc per serving: 4.2 mg
			• Beef burgers served with 2 slices of cheese (mozzarella, cheddar, Swiss)
			  • *Zinc per serving: 6.8mg*
			• Roast beef Sandwich with 3 ounce serving of beef
			  • *Zinc per serving: 8mg*
			  • Add cheddar cheese (1.5oz/50g) for more zinc (1.5mg)
			• Taco salad made with ground beef(2.5oz)**, cheddar cheese (1.5 oz)**, lettuce, tomato, salsa, sour cream, guacamole/ avocado slices
			  • *Zinc per serving: 7.5mg*

Appendix G: Sample ASA24 Report

MINERALS

Calcium (mg)	
EATEN	830
TARGET	1200

Copper (mg)	
EATEN	1
TARGET	0.9

Iron (mg)	
EATEN	10
TARGET	8

Magnesium (mg)	
EATEN	329
TARGET	320

Phosphorus (mg)	
EATEN	1196
TARGET	700

Potassium (mg)	
EATEN	1853
TARGET	2600

Selenium (mcg)	
EATEN	127
TARGET	55

Zinc (mg)	
EATEN	9
TARGET	8

Appendix H: Foods Not Found in NCC Database

Food Reported in ASA24	Food Equivalent from NCC Database
GF Multigrain cracker	Back to Nature GF Rice Thins
GF pasta	Based on primary ingredient of pasta
GF pizza	Domino's GF pizza cheese
GF cupcake/cake/donut/pie crust/muffin	GF pancake
GF breadcrumbs	Cooked white rice
GF graham crackers GF sugar cookies,	-Annie's cocoa and vanilla graham bunny -Girlscout toffeetastic cookie
GF saltine crackers	Ener-G cinnamon crackers
GF granola	Cascadian Farm organic French vanilla almond
Love Good Fats salted caramel almond bar	KIND granola bar caramel salted almonds
Hearts of Palm	Artichoke hearts

Appendix I: Bioavailable Zinc Calculation

Estimation of Zn intake was performed according to the International Zinc Nutrition Consultative Group (IZiNCG) algorithm[49] (available in www.izincg.org). Based on this we estimated:

1-Zn intake at baseline and after 3 and 6 months: The amount of Zn in food was be calculated using the ASA24®[45], which generates a micronutrient report that includes Zn consumption (*See trial registration in ASA24® website and* e*xample report in Supplementary*). We was estimate the average Zn intake at 3 months and 6 months, based on the median of all Zn intake weekly estimates in each period (0-3 months and 3-6 months);

2-*Estimate Zn bioavailability:* We used the WHO Model to estimate Zn bioavailability using the methodology described by Gibson et al[39]. For this, we retrieved information on dietary enhancers (meat, poultry, fish, seafood, eggs, whey protein, protein, organic acids including citric, lactic, maltic, and tartaric acids) and inhibitors (phytate, calcium, processed soy) of Zn in the 24-hr recall and we calculated the phytate /Zn molar ratio (PZMR) as follow[49]:

a) Calculate the total daily phytate intake (in milligrams). Phytate values for this USDA database are available from the University of Minnesota Nutrition Coordinating Center Nutrient Database (http://www.ncc.umn.edu/);

b) Divide the total daily phytate by the molecular weight of phytate (660) to give the phytate intake in terms of millimoles;

c) Divide the total daily Zn intake in milligrams by the atomic weight of Zn (65.4) to give Zn intake in millimoles; d) Divide the millimoles of phytate by the millimoles of Zn to find the PZMR. The atomic weight of iron= 55.85; the atomic weight of calcium= 40.08. Diet was classified as low (>15), moderate (5-15), or high (<5) bioavailability based on the PZMR. Diets rich in calcium or cereals are anticipated low bioavailability of Zn with estimated abruption of 15% of Zn, mixed diet containing animal fish protein are moderate bioavailability with estimated absorption of ~30%, and a diet rich in meat-fish are high-bioavailability with estimated absorption >50% of Zn;

3-Calculate intakes of available Zn from data on mean daily Zn intakes per individual depending on host-related factors *w*as calculated using Murphy's model using the algorithm: **Available Zn (mg/day) = total Zn (mg/day) × Zn availability factor (**set at 0.10 if PZMR is > 30; 0.15 for PZMR 15 - 30; and 0.30 for PZMR< 15). Calcium is not included in this model because the calcium content of most plant-based diets is too low to influence Zn bioavailability.

4- *Estimate adequacy of Zn intake* after calculating point 3, we estimated adequate Zn intake at each time point compared with the Recommended Dietary Allowance (RDA). In North America, RDA for an adult female is 8 mg/day and for men 11 mg/day[12].

Appendix J: CSI Questionnaire

Supplementary Table 2. Celiac Symptom Index (CSI)[a]

Question	1	2	3	4	5
1. Have you been bothered by pain or discomfort in the upper abdomen or the pit of the stomach during the past 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
2. Have you been bothered by nausea during the past 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
3. Have you been bothered by rumbling in your stomach during the past 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
4. Has your stomach felt bloated during the past 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
5. Have you been bothered by diarrhea during the past 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
6. When going on the toilet, have you had the sensation of not completely emptying your bowels during the past 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
7. Have you been bothered by hunger pains during the last 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
8. Have you been bothered by low energy level during the past 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
9. Have you been bothered by headaches during the past 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
10. Have you had food cravings in the last 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
11. Have you had loss of appetite during the past 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
12. Related to Celiac Disease, how is your health?	Excellent	Good	Fair	Poor	Terrible
13. Overall, how is your health?	Excellent	Good	Fair	Poor	Terrible
14. How much physical pain have you had during the past 4 weeks?	None	A little	Some	A good deal	Very much
15. I am comfortable	Strongly agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Strongly disagree
16. I am as healthy as anybody I know	Strongly agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Strongly disagree

[a]Leffler DA, Dennis M, Edwards George J, Jamma S, Cook F, Schuppan D, and Kelly CP. The Celiac Center, Beth Israel Deaconess Medical Center, Boston, Massachusetts.

Appendix K: HADS Questionnaire

Hospital Anxiety and Depression Scale (HADS)

Tick the box beside the reply that is closest to how you have been feeling in the past week.
Don't take too long over you replies: your immediate is best.

D	A		D	A	
		I feel tense or 'wound up':			**I feel as if I am slowed down:**
	3	Most of the time	3		Nearly all the time
	2	A lot of the time	2		Very often
	1	From time to time, occasionally	1		Sometimes
	0	Not at all	0		Not at all
		I still enjoy the things I used to enjoy:			**I get a sort of frightened feeling like 'butterflies' in the stomach:**
0		Definitely as much		0	Not at all
1		Not quite so much		1	Occasionally
2		Only a little		2	Quite Often
3		Hardly at all		3	Very Often
		I get a sort of frightened feeling as if something awful is about to happen:			**I have lost interest in my appearance:**
	3	Very definitely and quite badly	3		Definitely
	2	Yes, but not too badly	2		I don't take as much care as I should
	1	A little, but it doesn't worry me	1		I may not take quite as much care
	0	Not at all	0		I take just as much care as ever
		I can laugh and see the funny side of things:			**I feel restless as I have to be on the move:**
0		As much as I always could		3	Very much indeed
1		Not quite so much now		2	Quite a lot
2		Definitely not so much now		1	Not very much
3		Not at all		0	Not at all
		Worrying thoughts go through my mind:			**I look forward with enjoyment to things:**
	3	A great deal of the time	0		As much as I ever did
	2	A lot of the time	1		Rather less than I used to
	1	From time to time, but not too often	2		Definitely less than I used to
	0	Only occasionally	3		Hardly at all
		I feel cheerful:			**I get sudden feelings of panic:**
3		Not at all		3	Very often indeed
2		Not often		2	Quite often
1		Sometimes		1	Not very often
0		Most of the time		0	Not at all
		I can sit at ease and feel relaxed:			**I can enjoy a good book or radio or TV program:**
	0	Definitely	0		Often
	1	Usually	1		Sometimes
	2	Not Often	2		Not often
	3	Not at all	3		Very seldom

Please check you have answered all the questions

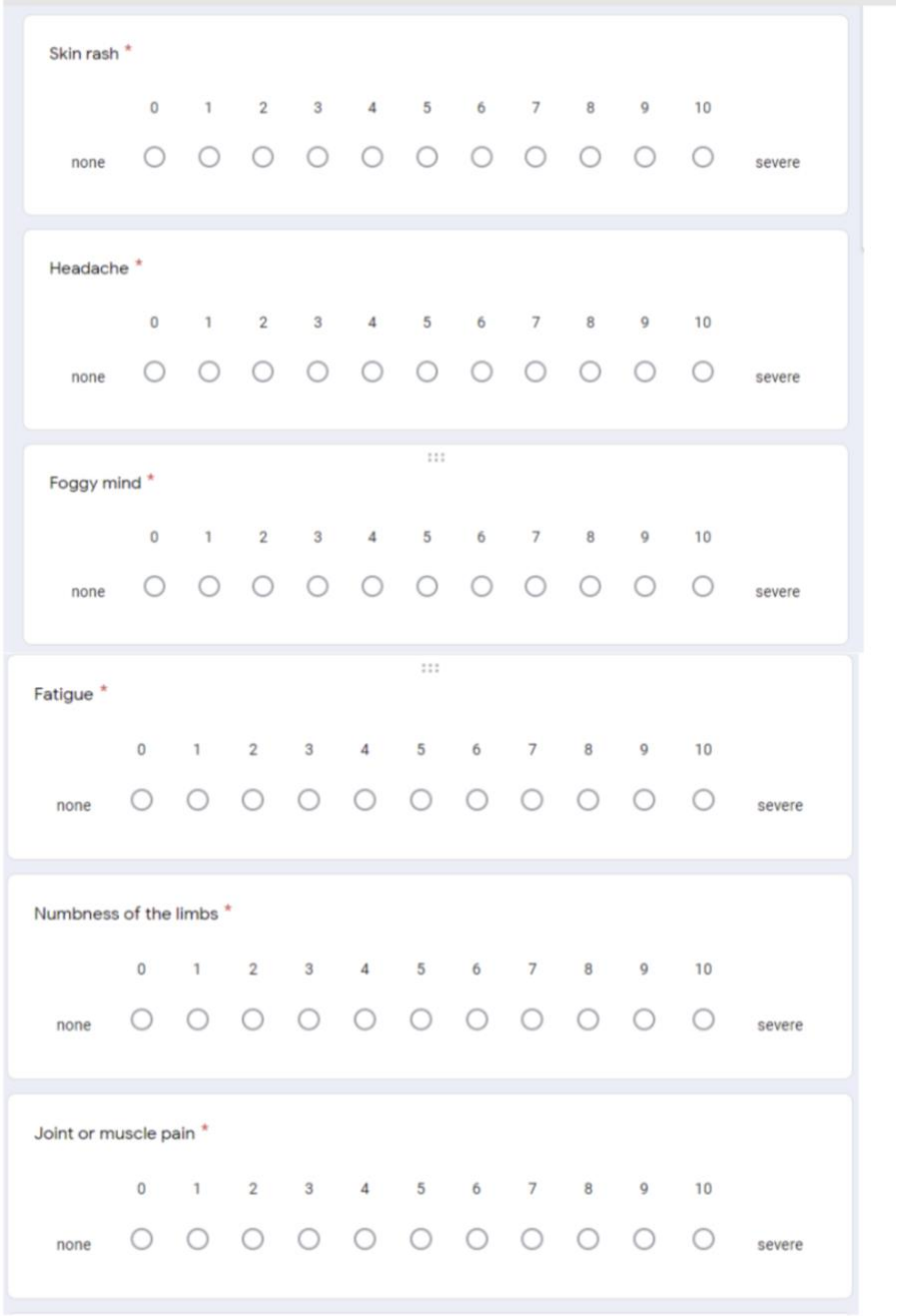
Scoring:
Total score: Depression (D) _____ Anxiety (A) _____
0-7 = Normal
8-10 = Borderline abnormal (borderline case)
11-21 = Abnormal (case)

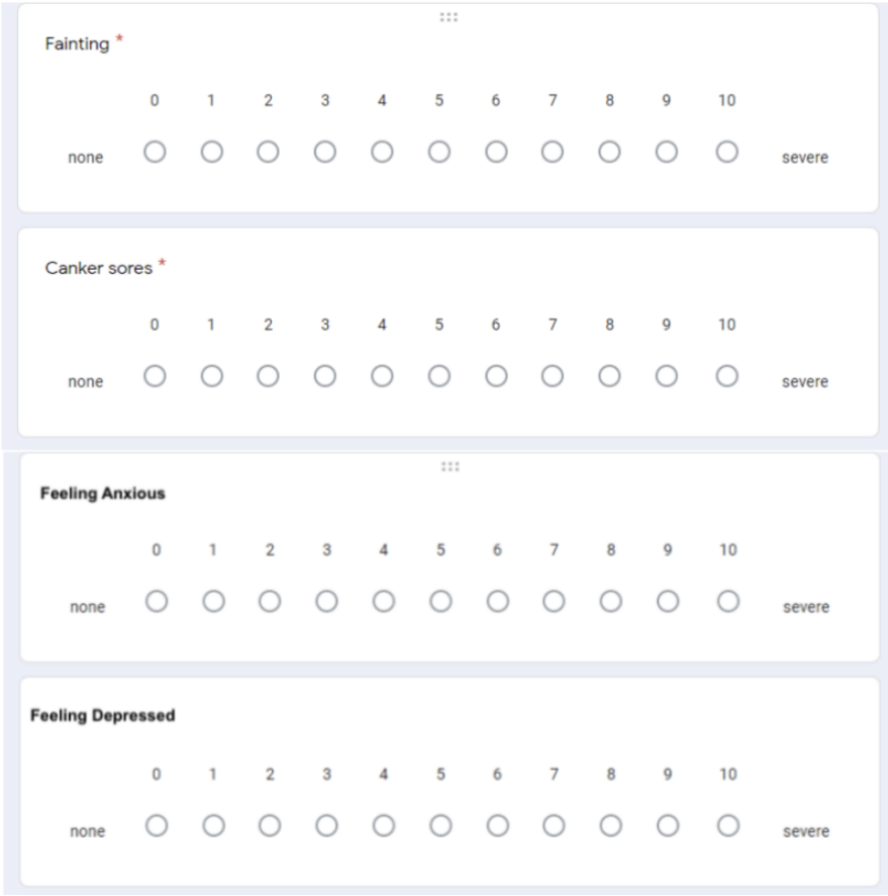
Appendix L: CeD-QOL Questionnaire

Please think about your life over the past month (30 days) and look at the statements below. Each statement has five possible responses. For each statement, please fill in one box in each row that best describes your feelings.

Statements	Not at All	A Little	Enough	Much	Very Much
1. I feel limited by this disease					
2. I feel worried that I will suffer from this disease					
3. I fell concerned that this disease will cause other health problems					
4. I feel worried about my increased risk of cancer from this disease					
5. I feel socially stigmatized for having this disease					
6. I feel like I'm limited in eating meals with co workers					
7. I feel like I am not able to have special foods like birthday cake and pizza					
8. I feel that the diet is insufficient treatment for my disease					
9. I feel that there are not enough choices for treatment					
10. I feel depressed because of my disease					
11. I feel frightened by having this disease					
12. I feel like I don't know enough about the disease					
13. I feel overwhelmed about having this disease					
14. I have trouble socializing because of my disease					
15. I find it difficult to travel or take long trips because of my disease					
16. I feel like I cannot live a normal life because of my disease					
17. I feel afraid to eat out because my food may be contaminated					
18. I feel worried about the increased risk of one of my family members having celiac disease					
19. I feel like I think about food all the time					
20. I feel concerned that my long-term health will be affected					

Appendix M: ES-VAS Questionnaire




Fainting *
0 1 2 3 4 5 6 7 8 9 10
none severe
Canker sores *
0 1 2 3 4 5 6 7 8 9 10
none severe
Feeling Anxious
0 1 2 3 4 5 6 7 8 9 10
none severe
Feeling Depressed
0 1 2 3 4 5 6 7 8 9 10
none severe

Appendix N: CeDAT Questionnaire

Question	1	2	3	4	5
Have you been bothered by low energy level during the past 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
Have you been bothered by headaches during the past 4 weeks?	None of the time	A little of the time	Some of the time	Most of the time	All of the time
I am able to follow a GFD when dining outside my home	Strongly agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Strongly disagree
Before I do something I carefully consider the consequences	Strongly agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Strongly disagree
I do not consider myself a failure	Strongly agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Strongly disagree
How important to your health are accidental gluten exposures?	Very important	Somewhat important	Neutral/unsure	A little important	Not at all important
Over the past 4 weeks, how many times have you eaten foods containing gluten on purpose?	0 (never)	1–2	3–5	6–10	>10

Appendix O: Patient Perception Questionnaire

1- **How would you rate your knowledge in nutrient deficiencies?**

   a) Extremely knowledgeable
   b) Very good knowledge
   c) Somewhat knowledgeable
   d) Not very knowledgeable
   e) Not knowledgeable at all

2- **How important is to you to treat low zinc levels (zinc deficiency)?**
   a) Extremely important
   b) Very important
   c) Somewhat important
   d) Not very important
   e) Not important at all

3- **Are you taking any nutrients or vitamins supplements?**
   a) Yes
   b) No

4- **If yes, please describe what supplements are you taking**
   (text box)

5- **What is your preference to treat low nutrients?**
   a) I prefer to make dietary changes and optimize nutrients in my diet to treat low nutrients
   b) I prefer to take supplement pills to treat low nutrients
   c) I don't care- I don't want to treat any low nutrients

6- How **familiar are you with zinc supplements?**
   a) Extremely familiar
   b) Very familiar
   c) Somewhat familiar
   d) Not so familiar
   e) Not at all familiar

7- **How likely would you treat your zinc deficiency with a supplement pill?**
   a) Extremely likely
   b) Very likely
   c) Somewhat likely
   d) Not so likely
   e) Not at all likely

8- **How familiar are you with diet to improve zinc?**

a) Extremely familiar
b) Very familiar
c) Somewhat familiar
d) Not so familiar
e) Not at all familiar

9- How likely would you treat your zinc deficiency with a diet?

a) Extremely likely
b) Very likely
c) Somewhat likely
d) Not so likely
e) Not at all likely

10-How likely is that you would comply strictly a treatment with a supplement pill?

a) Extremely likely
b) Very likely
c) Somewhat likely
d) Not so likely
e) Not at all likely

11-How likely is that you would comply with a diet that adjust food intake to improve nutrition?

a) Extremely likely
b) Very likely
c) Somewhat likely
d) Not so likely
e) Not at all likely

12-Do you perceive that the optimizing zinc in your GFD will more expensive than the GFD"?

a) Yes
b) No

13-If yes, how likely is that the extra cost will limit your choice of adopting a Zn-optimized GFD?

a) Extremely likely
b) Very likely
c) Somewhat likely
d) Not so likely
e) Not at all likely

14-Do you have any comments?

(text box)

Appendix P: Qualitative Assessment of Zn Supplements and Zn-Optimized Diet

Qualitative Assessment of Zinc Supplements

1. To what extent did you modify your diet during the study?
    a. Not at all
    b. A little
    c. Moderate
    d. A lot ()
- If yes (**answers 1b-1d**), please describe the changes you made to your diet. (open text)
2. Rate the palatability (i.e taste) of the zinc supplements?
    a. 1-unpalatable
    b. 2-no change
    c. 3-slightly more
    d. 4-highly palatable
    e. 5-more palatable compared to normal diet
    Branching logic answers (2a-2e): Explain reasoning for response to palatability (words)
3. How difficult was it to take the supplement each day?
    a. Not difficult at all
    b. A little difficult
    c. Difficult
    d. Very difficult.
4. If it was difficult **(answers 4b-4d),** what made the zn supplement intervention difficult to implement?
    - Remembering to take the zn supplement
    - Not being able to take zinc supplements with other medications
    - Gastrointestinal side effects (nausea, vomiting, diarrhea, abdominal pain)
    - Remembering to take zinc supplement with food
5. Was the zinc supplement more or less difficult to implement than you expected before enrolling in the study?
    a. More Difficult
    b. Same
    c. Less Difficult
6. Did you feel you received adequate instructions to implement the zinc supplement intervention? Yes / no. If no, please explain:
7. After the trial, would you continue consuming the zn supplement? yes/no
8. Did you consume any of the following foods?
    a. Legumes (beans,lentils,peas,chickpeas etc.)
    b. Nuts (peanuts, brazil nuts, walnuts, almonds etc.)
    c. Seeds (sesame, pumpkin, sunflower etc.)
    d. Grains (rice, oats, maize, etc.)

If they selected one of the answers above:

1. If yes, how often did you soak your food in water before eating?
    a. All the time

   b. Most of the time
   c. Sometimes
   d. Rarely
   e. Not at all
2. How often did you cook these foods for a longer period of time?
   a. All the time
   b. Most of the time
   c. Sometimes
   d. Rarely
   e. Not at all
3. How often did you consume these foods at the same time as the zinc supplement?
   a. All the time
   b. Most of the time
   c. Sometimes
   d. Rarely
   e. Not at all

Please provide any additional comments or feedback related to the study
(open text)

Thank you very much for your participation in the study and your valuable contribution to research.

Qualitative Assessment Zinc-optimized Diet

1. To what extent did you modify your diet during the study?
    a. Not at all (0 changes in meals)
    b. A little (1 meal or snack/day)
    c. Moderate (2 meals or snacks/day)
    d. A lot (3+ meals or snacks/day)
2. Rate the palatability (i.e taste) of the zinc diet?
    a. 1-unpalatable
    b. 2-no change
    c. 3-slightly more
    d. 4-highly palatable
    e. 5-more palatable compared to normal diet

    Branching logic answers (2a-2e): Explain reasoning for response to palatability (text)
3. Did you find the zinc diet more expensive to implement?
    a. Not at all
    b. A little more expensive
    c. Somewhat more expensive
    d. Much more expensive
4. How difficult was it to implement the diet on a daily basis during the study period?
    a. Not difficult at all
    b. A little difficult
    c. Difficult
    d. Very difficult.
5. If it was difficult (answers 4b-4d), what made the diet difficult to implement?(all that apply)
    - Having to soak high phytate foods
    - Avoiding combination of phytate foods with zinc foods
    - Not knowing what zinc-rich foods/meals to eat
    - Not suitable for my family
    - Difficult to eat out/socialize
    - Zinc-rich foods were not enjoyable
    - Higher food cost
    - Other:...
6. Was the diet more or less difficult than you expected before enrolling in the study?
    a. More Difficult
    b. Same
    c. Less Difficult
7. I felt that the zn diet restricted my overall diet?
        i. Strongly Agree
        ii. Agree
        iii. Neutral
        iv. Disagree
        v. Strongly Disagree
8. Did you feel you received adequate dietary instructions to implement the zinc diet? Yes / no. If no, please explain:

9. Did you use any of the gluten-free zinc recipes provided? Yes/No
10. After the trial, would you continue consuming the zn-diet? yes/no

11. Did you consume any of the following foods?
    a. Legumes (beans,lentils,peas,chickpeas etc.)
    b. Nuts (peanuts, brazil nuts, walnuts, almonds etc.)
    c. Seeds (sesame, pumpkin, sunflower etc.)
    d. Grains (rice, oats, maize, etc.)

If they selected one of the answers above:

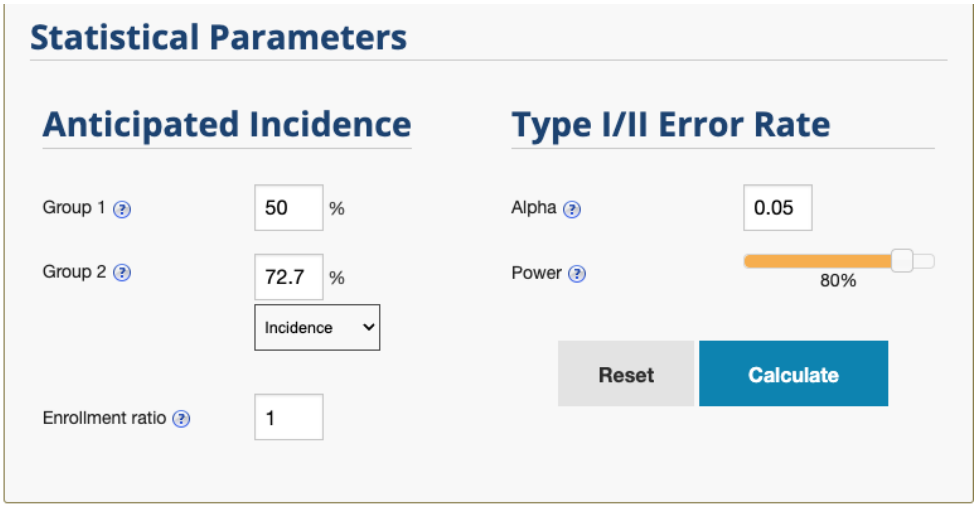
1. If yes, how often did you soak your food in water before eating?
    a. All the time
    b. Most of the time
    c. Sometimes
    d. Rarely
    e. Not at all
2. How often did you cook these foods for a longer period of time?
    a. All the time
    b. Most of the time
    c. Sometimes
    d. Rarely
    e. Not at all
3. How often did you consume these foods at the same time as a high zinc food?
    a. All the time
    b. Most of the time
    c. Sometimes
    d. Rarely
    e. Not at all

Please provide any additional comments or feedback related to the study

(open text)

Thank you very much for your participation in the study and your valuable contribution to research.

Appendix Q: Sample Size Calculation



RESULTS

Dichotomous Endpoint, Two Independent Sample Study

Sample Size	
Group 1	71
Group 2	71
Total	**142**

Study Parameters	
Incidence, group 1	50%
Incidence, group 2	72.7%
Alpha	0.05
Beta	0.2
Power	0.8

Appendix R: ROC Curve-Predicting pZn levels with Bioavailable Zn