Phase II feasibility study of the efficacy, tolerability and impact on the gut microbiome of a low residue (fibre) diet in adult patients with mitochondrial disease.

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Title: 
Phase II feasibility study of the efficacy, tolerability and impact on the gut microbiome of a low residue (fibre) diet in adult patients with mitochondrial disease.

Short Title: 
Low Residue Diet, Gut Microbiome and GI Dysmotility in Mitochondrial Disease

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Abbreviations:
- mtDNA, mitochondrial DNA; GI, gastrointestinal; IBS, irritable bowel syndrome; LRD, low residue diet; NMDAS, Newcastle Mitochondrial Disease Scale for Adults; NBD, Neurological Bowel Dysfunction; BSS, Bristol Stool Score; ROM, Radiopaque Markers; PAC-SYM, Patient Assessment of Constipation-Symptoms questionnaire; ES, effect size; BMI, body mass index; g, grams; n, number; SCFA, Short Chain Fatty Acids; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; VIP, vasoactive intestinal peptide; PYY, peptide YY; DNA, deoxyribonucleic acid; PERMANOVA, permutational analysis of variance; ANCOM, analysis of the composition of the microbiome; LDA, linear discriminant scores; LEfSe, linear discriminant analysis effect size

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Conflicts of Interest

The authors disclose no conflicts.

Data Transparency Statement:

Anonymous patient data will be made available for all variables beginning 9 months and ending 36 months after the Article’s publication. All proposals for sharing data can be submitted up to 36 months following Article publication. Requests for data sharing should be addressed to the corresponding author (GSG) and lead statistician (APB).

Ethical Statement: The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Author Contributions

GSG and PH conceived the study design.

GSG, PH, DH, YSN, CF, AB, RS and JN were involved in study execution, data collection, statistical analysis and manuscript preparation. MAJ, MMA, CL, CJS, CAL, DMT, RM and
APB analysed and interpreted the data. All authors drafted, critically revised and approved the final manuscript.

All authors drafted, critically revised and approved the final manuscript.

Abstract

Background and Aims

Gastrointestinal (GI) dysmotility is a common and debilitating clinical manifestation in patients with mitochondrial DNA (mtDNA)-related disease with no curative and few effective symptomatic therapies. A low residue diet (LRD) has been shown to be effective at reducing bowel urgency, pain, and distension in functional GI-related conditions. We assessed tolerability and effects of a LRD on bowel habits in patients with mtDNA-related disease.

Methods
A 12-week single-arm pilot study in patients with genetically determined primary mtDNA-related disease, meeting the ROME III constipation criteria. Co-primary outcomes were tolerability of a LRD (<10g fibre per day) assessed by food diaries and changes in stool frequency and consistency. Secondary outcomes included GI symptoms, disease burden, laxatives, physical activity levels, colonic transit time (CTT) using radiopaque markers, gut microbiome (patients and controls) and metabolomics. The gut microbiome of the mtDNA-related disease patients was compared against controls for observational purpose only.

**Results**

28 patients were enrolled and 24 completed the LRD intervention. The LRD was well tolerated with a mean fold change of -34% in dietary fibre (5.3 ± 10.4 grams) per day (p=0.03, confidence interval 0.7-9.9) with no adverse events. The proportion of stool samples with normal stool consistency increased from 36 to 49% (p=0.01), GI symptoms and laxative use was reduced. However, the LRD did not change stool frequency, stool output and CTT. The gut microbiome was significantly different between patients and controls but was not modulated by the dietary intervention.

**Conclusions**

LRD in patients with mtDNA-related mitochondrial disease and significant constipation is well-tolerated and a promising treatment for alleviating GI symptoms. These positive findings should be confirmed in a randomised controlled trial. ClinicalTrials.gov Identifier: NCT03388528.

**Key Words:** Gastrointestinal dysmotility, mitochondria, diet, residue, gut microbiome
**Word Count:** 3996 (inclusive of main text, references and table and figure legends)
Introduction

Mitochondrial diseases are a clinically diverse group of genetic disorders that are characterized by defects in oxidative phosphorylation caused by mutations in either the nuclear or the mitochondrial (mt) genome. Mitochondrial diseases are one of the most common groups of inherited neurometabolic disorders with mutations in mtDNA being the most common causative genetic defects in adult-related mitochondrial disease. The age of onset is variable and clinical expression of mtDNA-related diseases is wide ranging, but often results in significant morbidity and mortality. Gastrointestinal (GI) dysmotility is a frequent, debilitating manifestation and reported in up to 65% of patients with mtDNA-related disease, comparable with other common neurological disorders. GI symptoms consistently include dysphagia, abdominal pain, abdominal distention, bacterial overgrowth, constipation and, in severe cases, intestinal pseudo-obstruction, mimicking an acute surgical abdomen. Although the pathological mechanisms underlying the development of GI dysmotility and associated symptoms remain elusive, potential factors include visceral myopathy and/or impaired coordination of intrinsic and extrinsic pathways of the GI tract. Furthermore, mitochondrial dysfunction of GI smooth muscle as demonstrated in mitochondrial neurogastrointestinal encephalomyopathy patients and in mice with mtDNA polymerase gamma mutation, PolgD257A, is likely to contribute to GI motility, a key determinant of gut microbiome composition, although to date, no research into the gut microbiome in mtDNA disease has been conducted. Although the aetiology of the GI symptoms in mtDNA disease is likely to be multifactorial, insights from other disorders that share a clinical phenotype with mtDNA may provide further insight. For example, the gut microbiome has been implicated in the pathophysiology of various GI, metabolic and neurological disorders. Indeed the gut microbiome is crucial for GI integrity, immunity, drug metabolism, nutrient digestion and
absorption, and can facilitate gut motility, in part, through the synthesis of important neurotransmitters such as acetylcholine, essential for providing excitatory stimulation and smooth muscle contraction.

Management of GI dysmotility in patients with mtDNA-related disease is complex and often personalised to each patient, incorporating optimisation of nutrition, fluid intake, avoiding fasting and remaining active “https://www.newcastle-mitochondria.com/wp-content/cache/all/clinical-professional-home-page/clinical-publications/clinical-guidelines/index.html”). Alternatively, high-fibre diets are routinely implemented in bowel disorders, such as chronic idiopathic constipation and irritable bowel syndrome (IBS), although some evidence suggests that fibre can exacerbate GI symptoms including pain, distension and urgency. A low residue diet (LRD), a form of low fibre diet designed to minimise mechanical irritation caused by food residue and fibre, thereby, reducing GI workload and the associated GI symptoms such as abdominal pain and distension. A LRD has been reported to be well tolerated and efficacious for preparing patients pre/post-bowel-surgery. In addition, a LRD has been demonstrated to decrease bowel urgency, diarrhoea, pain and distension in IBS, idiopathic constipation and relieve GI symptoms in stricturing Crohn's disease. No trial of dietary manipulation in GI dysmotility associated with mtDNA disease has been conducted to date. We conducted a single-arm pilot study to test the tolerability and the effects of a LRD on bowel habits in mtDNA-related disease patients with GI dysmotility.
Materials and Methods

Participants

Patients were eligible for inclusion if they had a genetic confirmation of mitochondrial disease, aged ≥18 years, met the ROME III criteria of constipation (≤3 bowel movements/week, hard or lumpy stools with straining and sensation of obstruction and incomplete evacuations in at least 25% of bowel movements), as a measure of GI symptom severity, at least three months stable GI drug regimen prior to study inclusion, no known hypersensitivities to any of the ingredients in the preparations and not already implementing a LRD (inclusion/exclusion criteria are further detailed in Supplemental Appendix for patients and controls). mtDNA disease presents with significant variability within and between genetic mutations. However, of the 24 patients included in this study, 20 harboured the 3243A>G mtDNA mutation, the most common cause of adult-onset mtDNA disease, and given the rarity of this disorder, this represents a good sample size. Furthermore, all patients included in this study shared the same GI symptoms and were selected based on meeting stringent criteria described here. All patients were prescribed one Forceval® capsule, a multivitamin and mineral supplement, as part of routine care by a clinical dietician. Patients were advised to continue using laxatives as required and to keep records of their use. All participants provided informed written consent and the study was approved and performed under the ethical guidelines issued by our institution and complied with the Declaration of Helsinki.

Design and Procedures

We conducted a single-arm pilot study recruiting from the NHS Highly Specialised Service for Rare Mitochondrial Disorders in Newcastle upon Tyne and the UK Mitochondria Patient Cohort (Ref: 13/NE/0326). The trial protocol was approved by the National Research
Ethics Service Committee North East & Tyne and Wear South Research Ethics Committee (Ref: 17/NE/0193). The study was registered with ClinicalTrials.gov: NCT03388528.

Eligible patients were invited to attend a baseline visit (visit 1, Figure 1) and enrol onto the study (a full description of procedures is detailed in Supplemental Appendix).

**Intervention and Outcomes**

The co-primary outcomes were tolerability of a LRD and stool frequency and consistency. Tolerability was assessed by food frequency diaries and stool frequency and consistency using the Bristol Stool Score (BSS) according to the ROME III criteria. Secondary outcomes included: colonic transit time (CTT) using retention of radiopaque markers (ROM); self-reported symptoms as measured by Patient Assessment of Constipation-Symptoms questionnaire (PAC-SYM) and ROME III criteria, both assessments of GI symptom severity; anthropometrics; sleep duration, physical activity; gut metabolites; GI hormones and peptides and gut microbiome and dietary intake was recorded over 72 hours (one weekend and two week days) using INTAKE24, an open-source self-completed computerised dietary recall system based on multiple-pass 24-hour recall that has been validated against interview led dietary recall. All patients were provided with examples, support and guidance and following the LRD by a clinical dietician (PH). Further support for recording dietary intake or any other queries were supported by PH, DH, AB, who were contactable throughout the study duration. Safety was based on the number of adverse events reported. All outcomes were compared between baseline and following 12-weeks of the LRD, except for NBD score and Newcastle Mitochondrial Disease Scale for Adults (NMDAS). NMDAS is a qualitative rating scale that encompasses all aspects of mitochondrial disease, including gastrointestinal symptoms and quality of life that should be assessed every 6-12 months.
Gut Microbiome Profiling

DNA was extracted from 350mg of stool collected from ten patients at random and ten control subjects; detailed in the Supplementary Appendix. Briefly, sequencing quality was assessed using FastQC and quality scores were high (Phred >28) across the length of all paired-end reads in all samples. Illumina adapters were trimmed using Trim Galore. Sequences were then filtered to remove rRNAs using SortMeRNA v2.1b to identify reads aligning to any of the included rRNA databases. Host derived sequences were then filtered using Bowtie2 to remove reads aligning to the hg38 reference human genome. Filter summaries can be found in (Figure S1-3)). After filtering, samples, not including the negative control, had 11,935,479±1,605,354 reads (mean ± SD). A detailed description of methods for raw sequencing, profiling and functional relative abundances is included in Supplementary Appendix (Figures S1-S3).

Power and Statistics

Paired t-tests were used to compare within-group differences between pre and post LRD intervention. To investigate changes between pre and post LRD intervention, ordinal chi-square tests were performed on stool consistency (based on the ROME III cut off values for stool consistency) and PAC-SYM data, and standard chi-square was used to compare changes in ROME III criteria. Pearson correlation coefficients were used to investigate associations between total NMDAS and NBD score, PAC-SYM, ROME III, stool frequency and stool consistency. Sample size calculations for future studies were based upon the observed effect sizes for the chi-square tests (Cramér's V) and calculated using the pwr library in R and detailed in the supplementary (Page 6, Lines 173-179). A full description of metagenomic analysis, including data quality checks and correlations between study outcomes and gut
microbiome are presented in the Supplementary Appendix. All authors had access to the study data and reviewed and approved the final manuscript.
Results

Between September 2017 and July 2018, 28 patients were enrolled into the study; 24 patients completed 12-weeks of LRD intervention. Patient characteristics are summarised in Table 1, with additional clinical data presented in Supplemental Appendix Table S1. No significant differences in patients’ anthropometric, sleep duration or physical activity were observed between pre and post LRD intervention (Table 1).

LRD Tolerability

Tolerability was assessed by food diaries and demonstrated a significant -34% fold change in dietary fibre intake, reducing from 18 ± 8g/day vs. 12 ± 6g/day from baseline to study completion, respectively (mean reduction: 5 ± 10 grams per day, p=0.03) (Table S3, Supplemental Appendix). No changes were observed in any other dietary measures between pre and post-intervention (Supplemental Appendix Table S3, p>0.05). Four patients did not complete the study intervention, reporting the diet as too restrictive (n=2), or due to health-related problems that were not GI-related (anxiety (n=1) and muscle pain (n=1). No adverse events were reported in the remaining 24 patients who completed the 12-week LRD intervention.

Bowel Movements

NBD scores are detailed in Table 1, where the median NBD score for patients was 12 (range 5-27). There was no significant change in mean (p=1.00) or total stool frequency (p=0.40). There was a significant increase in the proportion of patients with normal stool and reductions in hard/constipated and loose stools when grouping stool consistency scores based on the ROME III cut-off values of 1-2 being constipation, 3-5 being normal and 6-7 being loose stool/diarrhoea (p=0.01) (Table 1, Figure 2A) and a reduction in the range of stool
consistency (-0.9 ± 1.3 (p=0.08)) (Table 1, Table S2, Figure S1 Supplemental Appendix).

There were significant reductions in the mean PAC-SYM and ROME III scores of -0.5 ± 1.1 (p=0.03), -1.0 ± 1.8 (p<0.01) (Table 1). A significant reduction in the proportion of responses meeting ROME III criteria (p<0.01) (Figure 2B) and in severity in all three subcategories of the PAC-SYM, abdominal (p=0.03), rectal (p=0.03) and stool (p<0.01) (Figure 3) were observed. Based on the changes observed in PAC-SYM and ROME III we calculated the sample sizes required to power future trials investigating the efficacy of a LRD (Figure S6). Using Cramér’s V as the effect size (EF) we estimated the total sample size required to achieve 80% power with an alpha of 0.05 in a randomised controlled trial. Based on the changes observed in stool consistency we would require 85 patients to detect an ES of 0.3, for the total PAC-SYM score an ES of 0.2 would require 183 patients, for individual sections of the PAC-SYM data, 173 patients would be needed for the abdomen section (ES 0.2), 210 patients for the rectal section (ES 0.2), and 117 for the stool section (ES 0.3). For the ROME III criteria data, 169 patients would be required (ES 0.2). Four different laxatives were routinely used by patients (Table 1). Radiological evidence of delayed GI transit (>20% of ROM retained in GI tract) was observed in 19 patients at baseline and 20 patients at 12-weeks (See Figure S2 Supplemental Appendix). No significant changes were observed in CTT (p=0.40) or total stool output (p=0.49) following the LRD (Table 1). The number of patients and daily use of osmotic and stimulant laxatives were reduced following the LRD intervention (Table 1), however, no statistical tests were conducted due to the small sample size.

There was a direct relationship between the NBD score and the total NMDAS at baseline (p<0.01, r=0.61). An inverse association was observed between the worst score for stool consistency and ROME III (p=0.02, r=-0.46) and PAC-SYM (p=0.03, r=-0.45) at baseline,
and the worst score for stool consistency and ROME III at 12-weeks (p<0·01, r=0·71) (scores of 1 and 2 were deemed the worst and second worst score, respectively, based on patients dysmotility). A direct relationship was observed between PAC-SYM and ROME III at baseline (p<0·01, r=0·72) and 12-weeks (p=0·04, r=0·42).

**Gut Metabolites and Blood Biochemistry**

No differences were observed in clinical blood biochemistry (Table 1). Among all the GI hormones and peptides assessed, only the GLP-1 level was significantly increased post-LRD (p=0·01) (Table S3 Supplemental Appendix and Figure 4B). The three main short-chain fatty acids (SCFA), acetic acid (-3·7 ± 14·0mmol/L) (p=0·08), propionic acid (-0·5 ± 2·4mmol/L) (p=0·61), and butyric acid (-0·9 ± 1·7mmol/L) (p=0·06) and total SCFA concentrations (-5·2 ± 7·5mmol/L) (p=0·18) were all reduced in patients following the LRD, although these changes were not statistically significant (Table S3 Supplemental Appendix and Figure 4A).

**Gut Microbiome Diversity of Patients vs. Control Subjects**

In terms species alpha diversity, no significant difference in the species estimates of Chao-1 (Mann-Whitney U, p=0·05) or Shannon indices (Mann-Whitney U, p=0·85) were observed between patients and controls subjects (Figure S8A). Similarly, no significant differences in UniFrac Weighted (p=0·19) or Unweighted (p=0·08) beta diversity (Figure S8B) were observed between patients and control subjects were observed by permutational analysis of variance (PERMANOVA) adjusting for age and BMI (the mean age and BMI were significantly different between patients and control subjects 52 (± 14) vs. 60 (± 10) years (p=0·01) and 25 (± 6) vs. 27 (± 3) kg/m² (p=0·05) respectively).

**Taxonomic Profiles of the Gut Microbiome**
Taxonomic profiles of all samples reflected a composition expected for human gut microbiome samples (Figure S7, Supplemental Appendix). No significant differences in taxa at any level were observed between male and female patients. Taxa at all levels of classification were tested for differential abundance between pre-LRD intervention patients and control subjects. At species level, the mean relative abundance of *Escherichia coli* (*E. coli*) (3.7 ± 4.1 vs. 0.6 ± 1.2) and *Bifidobacterium bifidum* (*B. bifidum*) (2.2 ± 1.9 vs. 1.6 ± 1.7) were significantly higher (ANCOM W>0.6) in patients when compared with control subjects (Figure 5A). Conversely, the abundances of *Faecalibacterium prausnitizi* (*F. prausnitizi*) (5.0 ± 3.4 vs. 2.0 ± 3.1) and *Roseburia intestinalis* (*R. intestinalis*) (2.0 ± 2.1 vs. 0.5 ± 0.8), established butyrate-producing species, were significantly higher (ANCOM W>0.6) in control subjects when compared with patients (Figure 5A).

**Functional Profiles of the Gut Microbiome**

Numerous functional pathways were identified that were significantly different (ANCOM W>0.6) between patients and control subjects (Figure S7C) (full pathway names in Supplementary Appendix Table S3). Amongst these, the greatest difference was a lower relative abundance of genes associated with starch degradation in patients compared to control subjects. Patients additionally had a significantly lower relative abundance of several other metabolic pathways (Supplementary Appendix), suggestive of a divergent metabolic repertoire in patients’ gut microbiota. Indeed, the three significant pathways most enriched in patients were those associated with simple sugar metabolism (glycolysis pathways I, II and IV), with this shift towards glycolysis pathways predominantly driven by the higher *E. coli* abundance observed in (Figure S9, Supplemental Appendix).

**Gut Microbiome Changes Pre and Post LRD in patients with mtDNA-related disease**
The LRD intervention had no significant effect on alpha or beta diversity (Figure S11A and S11B, Supplemental Appendix Results). When considering taxonomic comparisons, two species: *Ruminococcus bromii* (*R. bromii*) and *Alistipes putredinis* (*A. putredinis*), had a significantly (ANCOM W>0.6) higher relative abundance post LRD intervention (Figure S12, Supplemental Appendix). No significant differences in the abundances of functional pathways were observed in the gut microbiome following the LRD intervention.

We explicitly compared species that were significantly different in the comparison between control subjects and patients in the patient stool before and after implementation of a LRD, to assess the impact of the intervention on these taxa, but observed no changes in their relative abundance post-LRD intervention (Figure S10C, Supplemental Appendix). Additionally, we observed no significant difference in the beta diversity distances between the pre- and post-LRD intervention stool samples when compared to control subjects (Figure S12, Supplemental Appendix). ROME III at baseline was positivity associated with *R. intestinalis*, but no other associations were observed between bacterial abundances and bowel movements, GI symptoms and ROME III criteria (Figure S14, Supplemental Appendix).
Discussion

Our findings demonstrate that a LRD is a safe and effective treatment for severe constipation symptoms such as abdominal distension, pain, bowel urgency and diarrhoea in a well-characterised cohort of patients with mtDNA-related disease. The LRD was well tolerated, as evidenced by reductions in dietary fibre and SCFA concentrations. Stool consistency improved and translated into a reduced use of laxatives, with good compliance, no reported adverse events and no negative impact upon blood biochemistry or nutritional intake over the timespan of the intervention. These findings corroborate previous studies in other disease states, where low residue (fibre) diets have shown promise in alleviating abdominal urgency, pain, distension 16 and constipation. 17

GI dysmotility symptoms in mtDNA-related diseases are a common debilitating clinical manifestation in a disease were treatment strategies remain largely symptomatic. 24 Alleviation of GI mechanical irritation and symptoms associated with GI dysmotility, 15 in the absence of a change in the number of ROM in our study provides insight into the potential mechanism of action of a LRD in mitochondrial disease. In our study, we observed reductions in dietary fibre and SCFA concentrations, indicative of fibre fermentation and gas production. In healthy subjects gas production and retention has been shown to inhibit GI transit, decrease bolus propulsion and elicit GI symptoms such as abdominal distension and pain. 25 We propose that the short term-rescue of GI mechanical irritation and symptoms, bloating, abdominal pain and stomach cramps associated with GI dysmotility is directly attributable to the reduced fibre intake, gas production, and potentially a reduction in GI workload. 15 Moreover, we suggest that our findings could better inform revision of expert opinion guidelines (e.g. “https://www.newcastle-mitochondria.com/wp-content/cache/all/clinical-professional-home-page/clinical-publications/clinical-
where a LRD may form part of a multidisciplinary approach to treat GI dysmotility and debilitating GI symptoms in this patient population and other disease states manifesting mitochondrial dysfunction.  

Although the LRD provides short term rescue of GI symptoms, the pathological mechanisms responsible for GI dysmotility in mtDNA-related disease remains elusive, in part due to complex disease heterogeneity.  

Mitochondrial dysfunction in GI smooth muscle has been proposed, suggesting that enteric myopathy may underlie severe GI dysmotility, akin to cases of chronic intestinal pseudo-obstruction due to other aetiologies. The use of CTT as an objective measure of GI motility identified significant GI dysmotility in mtDNA-related disease patients and the retention of ROM may have important implications and guide clinicians in directing treatment approaches. However, the use of CTT is limited when differentiating between different forms of GI disorders and provides limited information relating to the pathophysiology of GI dysmotility. Moreover, it is important to acknowledge that GI symptoms, such as constipation, diarrhoea and bloating are not always associated with gut dysmotility, suggesting that the GI symptoms experienced by patients in this study may not be specific to the lower GI tract or to GI dysmotility. A number of patients had persistent loose stool throughout the study. Whilst this could simply relate to chronic laxative use, small intestinal bacterial overgrowth and deleterious changes in the gut microbiome as observed here, should also be considered. Moving forward, alternative techniques to assess whole GI transit, potentially involving several modalities, inclusive of the upper and lower GI tract, to distinguish between dys-synergic defecation, colonic inertia and proximal colon emptying may further our understanding regarding pathological mechanisms and guide clinical management.
Our study provides a novel insight into the gut microbiome of patients with mtDNA-related disease, implicating a higher relative abundance of *E. coli* as responsible for modulating the metabolic capabilities of the microbiome. We observed a significant preferential switch from starch and complex carbohydrate degradation pathways to simple sugar pathways in patients with mtDNA-related disease. The gut microbiome profiles of patients observed here are unlikely to be transient, driven by patient’s dietary intake and/or clinical phenotype and could be shaping the gut microbiome profile toward one that resembles inflammatory GI conditions. Similar increased *E. coli* abundance has also been observed in Parkinson’s disease where it was associated with increased gut permeability, serum markers of endotoxins and increased alpha-synuclein. It is not clear whether such microbiome alterations are causative or a result of changes in GI dymotility. However, pre-clinical models of primary mitochondrial disease hint at a pathological role, where *E. coli* virulence determinants contributed to myenteric neuropathy and inhibition of neuronal activity associated with GI transit. These potential microbiome-related aetiopathologies warrant further exploration in mtDNA-related disease.

Diet, a key feature that has been shown to rapidly and reproducibly modulate the gut microbiome, could also contribute to microbiome-related aetiopathologies. Although modest the LRD was able to moderately increase the relative abundance of *R. Bromii* and *A. putredinis* in patients, two species that have been shown to be involved in carbohydrate metabolism. The increase in *R. Bromii* and *A. putredini*, is likely to be due to baseline bacterial composition and/or due to the ability of *R. Bromii* and *A. putredini* to outcompete other strains of bacteria for what little dietary fibre remained in patients diets, colonising and therefore increasing their relative abundance. Our study provides novel findings that stimulate the need for a more focussed approach for the clinical management of GI
dysmotility in mtDNA-related disease patients. Potentially utilising dietary interventions that can increase the abundance of bacteria involved in SCFA production, such as *Faecalibacterium prausnitzii* (*F. Prausnitzii*) and *Roseburia intestinalis* (*R. Intestinalis*), whilst outcompeting species such as *E.coli* to improve patient’s GI dysmotility and clinical outcomes.

Early satiety, commonly reported in mitochondrial disease¹ may also impact upon GI motility. Low concentrations of SCFA may impair neurotransmitter release in the GI tract, such as serotonin,³⁶ exacerbating GI dysmotility through increased GLP-1 levels, evident in pre-clinical models, corroborating our findings.³⁷ Secondly, prolonged low intake of fibre may impair the GI tracts ability to process fibre, further reducing the relative abundance of bacteria involved in fermentation (*F. prausnitzii* and *R. intestinalis*). This in turn may add bulk to the digesta, potentially increasing GI workload, mechanical irritation and GI symptoms.¹⁵ Thirdly, chronic fibre deficiency may impair GI integrity, where the gut microbiome switches its preferred energy source to glycoproteins secreted by the mucus barrier, evident in germ free mice.³⁸ This may be exacerbated further due to low concentrations of SCFA, important for their anti-inflammatory properties and ability to modulate potentially pathogenic bacteria such as *E.coli*,³⁹ which has been linked with disease onset and progression in IBS.⁴⁰ Our data provide further evidence that the pursuit of microbiome-targeted therapeutics, although challenging, may benefit patients with neurological disorders manifesting with debilitating GI dysmotility by providing the potential beneficial effects of fibre whilst maintaining the improvements in GI symptoms observed here.

Limitations
There are several limitations in this study. Firstly, the small sample size and multiple outcome measures explored were due to the largely unknown effect sizes of the chosen outcomes in mtDNA-related disease. Secondly, no control arm was included in the intervention part of the study, meaning that a placebo effect cannot be excluded. However, the use of objective measures (CTT, gut microbiome and metabolites) reduced subjective interpretation and recall bias. Further, while we observed a positive association between NBD and NMDAS scores suggesting that the severity of GI dysmotility was proportional to the overall disease burden, the exploratory nature of this study precluded robust assessment of NBD and/or NMDAS as predictors of favourable response to LRD intervention. Due to the exploratory nature of this study NBD was only performed prior to the LRD, therefore, if the relationship between overall disease burden of mtDNA and NBD was present following the LRD, cannot be concluded. Assessing NBD pre and post any intervention will be included moving forward. Careful selection for suitable control subjects was considered to mitigate environmental factors; however, a more suitable control group such as Parkinson disease patients may provide greater insight into pathological mechanisms responsible for GI dysmotility and associated symptoms. Finally, the small change we observed in the gut microbiome in patients following the LRD may be in part due to the already low levels of dietary fibre being consumed at baseline, which are lower than the UK based recommendations of of >30g/day. However, the question remains if a -34% fold decrease in dietary fibre observed here would have affected the gut microbiome in other pathologies, or if the lack of change in the gut microbiome following the LRD is just a reflection of the complex phenotype and lifestyle of mtDNA patients. Although we observed significant differences in the gut microbiome between mtDNA patients and controls these are relative abundances, and moving forward qPCR would be useful to assess taxonomic abundances.
Conclusion

In summary, our findings show significant promise for the use of a LRD to improve GI symptoms in patients with mtDNA-related disease and chronic constipation. Early intervention with LRD for bowel urgency, diarrhoea, pain and distension and strategies to mitigate their progression might improve GI outcomes in patients with mtDNA-related disease.
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References


Table 1: Data are mean (± SD) or n (%) characteristics at baseline and following 12-weeks of the Low Residue Diet for mtDNA-related disease patients.

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<td>Gender (female/male)</td>
<td>16/8</td>
<td>···</td>
<td>···</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167 (± 12)</td>
<td>···</td>
<td>···</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69 (± 17)</td>
<td>70 (± 19)</td>
<td>0·10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 (± 6)</td>
<td>26 (± 6)</td>
<td>0·13</td>
</tr>
<tr>
<td>Waist / Hip Ratio</td>
<td>0·96 (± 0·09)</td>
<td>0·94 (± 0·09)</td>
<td>0·21</td>
</tr>
<tr>
<td>Physical activity/24hours (mg)</td>
<td>30 (± 12)</td>
<td>28 (± 8)</td>
<td>0·73</td>
</tr>
<tr>
<td>Sleep duration/24hours</td>
<td>453 (± 88)</td>
<td>432 (± 93)</td>
<td>0·51</td>
</tr>
<tr>
<td>Neurological Bowel Dysfunction:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Minor</td>
<td>6/24 (25%)</td>
<td>···</td>
<td>···</td>
</tr>
<tr>
<td>Minor</td>
<td>4/24 (17%)</td>
<td>···</td>
<td>···</td>
</tr>
<tr>
<td>Moderate</td>
<td>6/24 (25%)</td>
<td>···</td>
<td>···</td>
</tr>
<tr>
<td>Severe</td>
<td>6/24 (25%)</td>
<td>···</td>
<td>···</td>
</tr>
<tr>
<td>DNC</td>
<td>2/24 (8%)</td>
<td>···</td>
<td>···</td>
</tr>
<tr>
<td><strong>Blood Biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-Peptide (nmol/mmol)</td>
<td>0·90 (± 0·50)</td>
<td>0·90 (± 0·40)</td>
<td>0·50</td>
</tr>
<tr>
<td>estimated-Glomerular Filtration Rate (ml/min/1.73 m²)</td>
<td>···</td>
<td>···</td>
<td>···</td>
</tr>
<tr>
<td>&lt;60</td>
<td>3 (13%)</td>
<td>2 (8%)</td>
<td>···</td>
</tr>
<tr>
<td>&gt;60-75</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>···</td>
</tr>
<tr>
<td>&gt;75</td>
<td>3 (12%)</td>
<td>21 (88%)</td>
<td>···</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>49 (± 8)</td>
<td>46 (± 12)</td>
<td>0·10</td>
</tr>
<tr>
<td>Alkaline Phosphate (U/L)</td>
<td>69 (± 18)</td>
<td>68 (± 34)</td>
<td>0·33</td>
</tr>
</tbody>
</table>
Alanine Aminotransferase (U/L⁻¹) 21 (± 11) 23 (± 9) 0.47
Low-Density Lipoprotein (mmol/L) 3.4 (± 1.10) 3.5 (± 1.2) 0.21
High-Density Lipoprotein (mmol/L) 1.4 (± 0.30) 1.4 (± 0.4) 0.20
Triglycerides (mmol/L) 2.2 (± 0.90) 2.1 (± 0.8) 0.20

Clinical features associated with mitochondrial disease
Disease burden measured by total NMDAS score 30 (± 15) .. ..
Swallowing 7/24 (29%) .. ..
Gastrointestinal 18/24 (75%) .. ..
Seizures 3/24 (13%) .. ..
Stroke-like episodes 3/24 (13%) .. ..
Diabetes 15/24 (63%) .. ..

Questionnaires
PAC-SYM 1.7 (± 0.8) 1.1 (± 0.9) 0.03*
Rome III Criteria (>3 criteria) 3.7 (± 1.9) 2.7 (± 1.6) 0.01*

Bowel Movement Measures
Stool Frequency Mean 3.5 (± 1.4) 3.5 (± 1.5) 1.00
Stool Frequency Total 92 80 0.40
Total stool output over 5 days (grams) 294 (± 204) 266 (± 209) 0.49
Colonic Transit:
Normal <20% 3/24 (13%) 2/24 (8%) ..
Delayed ≥20% 19/24 (79%) 20/24 (84%) ..
DNC 2/24 (8%) 2/24 (8%) ..
Colonic Transit (ROM retained) 12.4 (± 6.2) 11.3 (± 7.4) 0.40
Bristol Stool Consistency Score (Frequency):
Hard/Constipated 1-2 35 32 ..
Normal 3-5 33 39 ..
Loose Stool 6-7 24 9 ..
Bristol Stool Score Range

2.8 (± 1.7) 2.1 (± 1.5) 0.08

**Laxatives**

**Osmotic Total Daily Use**

Macrogol (Laxido®/Movicol®) (sachets) 31 (14) 18 (10) ..
Lactulose (ml) 75 (2) 60 (2) ..

**Stimulant Total Daily Use**

Senna (mg) 180 (2) 105 (1) ..
Docusate (mg) 1200 (4) 1700 (7) ..
Dulcolax Pico (ml) 55 (6) 10 (1) ..
Sodium Picosulfate (mg) 0 (0) 10 (1) ..

**Serotonin 5HT4-receptor agonist Total Daily Use**

Prucalopride (mg) 1 (1) 1 (1) ..

**Bicyclic Fatty Acid Derived from Prostaglandin E1 Total**

Daily Use

Lubiprostone (mcg) 24 (1) 24 (1) ..

Laxatives presented as total daily usage (number of patients using laxatives) cm, centimetres; kg, kilograms; mg, millig; NMDAS, Newcastle Mitochondrial Disease Scale for Adults; DNC, Did Not Complete; PAC-SYM, Patient Assessment of Constipation Symptoms; ROM, Radiopaque Markers; Bristol Stool Score Grouping is based on ROME III criteria and is frequency of each score; cm, centimetre; kg, kilogram; mg, micrograms; g, grams; ml, millilitre; mcg, millicentigram; mmol, millimoles; pg, pictograms. * and # denotes a significant differences at <0.01 and 0.05, respectively. a and b denotes 13.125 and 13.8g sachets. The threshold for individual clinical features to be interpreted as binary trait, previously described 3.
Figures

Figure 1: Consort diagram of trial profile.

Figure 2: A – Pre and post LRD cumulative frequency of stool consistency based on the (A) Bristol Stool Score ROME III (1-2: constipation, 3-5: normal stool and 6-7: loose stools) and (B) Proportional changes in ROME III criteria pre and post LRD. BM: bowel movement (n=24).

Figure 3. Pre and post proportional changes in Patient Assessment of Constipation Symptoms (PAC-SYM): A – Abdominal (p=0.03), Rectal (p=0.03) and Stool (p<0.01) (n=24).

Figure 4: Violin dot plot of A) Changes in SCFA concentrations and B) Changes in Ghrelin and Glucagon-like peptide-1 (GLP-1) (n=24)

Figure 5. Microbiome profile for controls (n=10) and mtDNA related disease patients (n=10).
Excluded (n=6)
• Patients did not meet ROME III criteria for constipation (n=4)
• Not on stable GI drug regime (n=2)

Assessed for Eligibility (n=46)
• patients (n=36)
• control subjects (n=10)

Enrolled (n=40)
• patients (n=30)
• control subjects (n=10)

Drop Out (n=2)
• Underwent medical procedures within study time frame

Drop Out (n=4)
• Health related problem (n=2) (anxiety (n=1) and muscle pain (n=1))
• Did not wish to continue diet (n=2)

Enrollment

Visit 1
• mtDNA disease patient: BS, BSS, questionnaires, food diary, PA and demographics.
• healthy controls: anthropometrics

Visit 2
• mtDNA disease patient: stool collection and transit time.
• Control subjects: stool collection

12 week intervention
• mtDNA disease patient: bi-weekly support via phone calls.

Visit 3
• mtDNA disease patient: repeat of visit 1.

Visit 4
• mtDNA disease patient: repeat of visit 2

End of Study
• patients (n=24)
Figure 1

Screening

Enrollment

Visit 1
- mtDNA disease patient: BS, BSS, questionnaires, food diary, PA and demographics.
- Healthy controls: anthropometrics

Visit 2
- mtDNA disease patient: stool collection and transit time.
- Control subjects: stool collection

12 week intervention
- mtDNA disease patient: bi-weekly support via phone calls.

Visit 3
- mtDNA disease patient: repeat of visit 1.

Visit 4
- mtDNA disease patient: repeat of visit 2

Assessed for Eligibility (n=46)
- Patients (n=36)
- Control subjects (n=10)

Excluded (n=6)
- Patients did not meet ROME III criteria for constipation (n=4)
- Not on stable GI drug regime (n=2)

Enrolled (n=40)
- Patients (n=30)
- Control subjects (n=10)

Drop Out (n=2)
- Underwent medical procedures within study time frame

1. patients (n=28)
2. control subjects (n=10)

Drop Out (n=4)
- Health related problem (n=2)
  (anxiety (n=1) and muscle pain (n=1))
- Did not wish to continue diet (n=2)

3. patients (n=28)
4. control subjects (n=10)

End of Study
- Patients (n=24)
Figure 2.
The diagrams illustrate the percentage distribution of various gastrointestinal symptoms before and after treatment. The symptoms include:

- Abdominal Bloating
- Abdominal Discomfort
- Abdominal Pain
- Stomach Cramps
- Incomplete Bowel Movements
- Painful Bowel Movements
- Rectal Burning – During or After
- Too Hard Bowel Movements
- Too Small Bowel Movements
- Straining During Bowel Movements
- False Alarm Bowel Movements

The x-axis represents different symptoms, and the y-axis shows the percentage range from 0% to 100%. The bars are color-coded to indicate severity levels: Absent, Mild, Moderate, Severe, and Very Severe.
Figure 3.

Figure 4.
Figure 5.