

Cognitive impairment in coeliac disease improves on a gluten-free diet and correlates with histological and serological indices of disease severity

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SUMMARY

Background

Mild impairments of cognition or 'Brain fog' are often reported by patients with coeliac disease but the nature of these impairments has not been systematically investigated.

Aim

This longitudinal pilot study investigated relationships between cognitive function and mucosal healing in people with newly diagnosed coeliac disease commencing a gluten-free diet.

Methods

Eleven patients (8 females, 3 males), mean age 30 (range 22–39) years, were tested with a battery of cognitive tests at weeks 0, 12 and 52. Information processing efficacy, memory, visuospatial ability, motoric function and attention were tested. Small bowel biopsies were collected via routine gastroscopy at weeks 12 and 52 and were compared to baseline Marsh scores. Cognitive performance was compared to serum concentrations of tissue transglutaminase antibodies, biopsy outcomes and other biological markers.

Results

All patients had excellent adherence to the diet. Marsh scores improved significantly ($P = 0.001$, Friedman's test) and tissue transglutaminase antibody concentrations decreased from a mean of 58.4 at baseline to 16.8 U/mL at week 52 ($P = 0.025$). Four of the cognitive tests assessing verbal fluency, attention and motoric function showed significant improvement over the 12 months and strongly correlated with the Marsh scores and tissue transglutaminase antibody levels ($r = 0.377$ – 0.735 ; all $P < 0.05$). However, no meaningful patterns of correlations were found for nutritional or biochemical markers, or markers of intestinal permeability.

Conclusions

In newly diagnosed coeliac disease, cognitive performance improves with adherence to the gluten-free diet in parallel to mucosal healing. Suboptimal levels of cognition in untreated coeliac disease may affect the performance of everyday tasks.

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INTRODUCTION

Coeliac disease (CD) is an inflammatory autoimmune disorder that affects at least 1% of the adult population in many countries, and a strict, lifelong gluten-free diet (GFD) is the only recommended treatment.^{1, 2} While the disease is primarily an intestinal disorder that is histologically characterised by intraepithelial lymphocytosis, crypt hyperplasia and villous atrophy, there is increasing support for a broader concept of a systemic inflammatory disease. Support for this view comes from clinical observations of extra-intestinal manifestations such as dermatologic, hepatic, osteologic, endocrine and neurological signs. Reported neurological manifestations include amnesia, ataxia, acalculia, epilepsy, chronic neuropathies, confusion and personality changes.^{3–6} Some of these severe neurological symptoms can improve upon treatment with a GFD.^{3, 7, 8}

A milder form of cognitive impairment frequently reported by people with CD has been colloquially termed 'brain fog'. Brain fog can include difficulty concentrating, problems with attentiveness, lapses in short-term memory, word-finding difficulties, temporary loss in mental acuity and creativity, and confusion or disorientation.⁹ In our experience, patients often report that brain fog dissipates after treatment on a GFD or returns after inadvertent gluten exposure.

Few studies have investigated such subtle cognitive deficits. In a group of eight 'cognitively normal' participants with CD and idiopathic cerebellar ataxia significant impairments were found in immediate recall from episodic and semantic memory, and there was a trend towards deficits in phonemic verbal fluency and executive function.¹⁰ Further, a study of 15 elders with cognitive decline that began within 2 years of the onset of CD symptoms suggested a link might exist between cognitive impairment and CD.³ The possibility of such a link has been strengthened by the findings of a retrospective study of elderly patients in which CD was diagnosed after the age of 60 years.¹¹ Of the seven patients identified, two presented with cognitive decline that had been attributed to Alzheimer's disease, but was ameliorated after the initiation of a GFD, while a third patient had peripheral neuropathy that completely resolved after the initiation of a GFD.

The present pilot study investigated relationships between levels of cognitive functioning and mucosal healing in people commencing on a GFD after being diagnosed with CD. It was hypothesised that the level of cognitive function would improve concomitantly with mucosal healing.

MATERIALS AND METHODS

Participants

Participants were recruited via the Box Hill Hospital Coeliac Clinic (Melbourne, Australia), advertisements in local Coeliac Society publications, and from a local private dietetic practice. Patients were eligible for the study if they were aged between 18 and 40 years, had been diagnosed with CD and had not commenced a GFD more than 4 weeks prior to enrolment. The diagnosis of CD was made on the basis of duodenal biopsies showing villous atrophy (at least Marsh 3a) or Marsh 1 or 2 with positive CD-specific antibodies. All participants were required to express the HLA-DQ2 and/or DQ8 haplotype. Exclusion criteria comprised other significant gastrointestinal or inflammatory diseases, pregnancy, a history of neurological or psychiatric events or of brain injury resulting in unconsciousness for more than 30 min, existing neurodegenerative disease, and an inability to speak and write in English or to give written informed consent.

Protocol

The timeline for interventions in the study is summarised in Figure 1. During screening and at defined time points over the following 12 months, patients were assessed clinically by a gastroenterologist, had blood and intestinal permeability tests, underwent cognitive testing, and completed prospective 7-day food diaries to assess dietary compliance. Duodenal biopsies were performed at 12 and 52 weeks. The study protocol was approved by the Human Research and Ethics Committees at Monash University and Eastern Health in Melbourne, Australia. The longitudinal design alleviated issues associated with the small sample size. For instance, inter-participant variability was eliminated because the participants served as their own control through their baseline measures.

Compliance measures

At baseline screening and at weeks 12 and 52, participants completed a food diary for seven consecutive days, recording all food and drink consumed as well as ingredients for home-prepared meals. The diaries were assessed by an experienced dietitian, with face-to-face consultations provided if concerns were identified by the participant, clinician or dietitian. Adherence was judged by an arbitrary scale comprising 'excellent' when no sources of gluten ingestion were identified, 'good' if

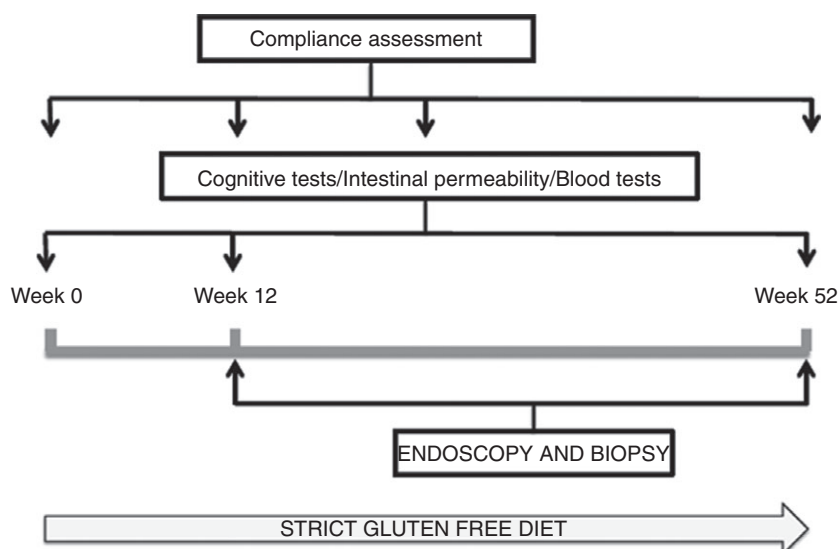


Figure 1 | Timeline of the study protocol. Compliance of the 15 participants to the GFD was assessed via 7-day food diaries and interview by a dietitian. Intestinal permeability was assessed using a dual sugar methodology. Blood tests examined biochemical, nutritional and serological markers.

minor gluten intake (estimated <0.3 g gluten per episode) occurred no more than six times in the 12 months, 'fair' if minor gluten intake (<0.3 g) occurred up to one episode per week, and 'poor' if gluten intake was more frequent than once a week.

Biological measures

Duodenal biopsies were obtained during routine upper gastrointestinal endoscopy at weeks 12 and 52 of the study. Two biopsies were taken from the first part of the duodenum and at least four from the second part. Histological assessment was performed by an experienced pathologist (PH) who was blinded to the baseline histology. Marsh scores were assigned using the most severe lesion found in the biopsies. The index biopsies were reviewed by the same (blinded) pathologist and scored retrospectively in a similar way.

Intestinal permeability (IP) was assessed by a dual sugar permeability test. After an overnight fast, the bladder was emptied, and 1 g L-rhamnose and 5 g lactulose dissolved in 120 mL water was ingested. Urine was collected for 5 h after ingestion. Concentrations of both sugars were measured by high performance liquid chromatography and the lactulose:rhamnose (L:R) ratio was calculated.

Venous blood samples were collected at baseline (screening) and at weeks 12 and 52. Sera were assessed for tissue transglutaminase (tTG) IgA antibodies (QUANTA Lite ELISA, Inova Diagnostics, Inc., San Diego, CA, USA), and haemoglobin (Hb), vitamins D and B₁₂, iron studies and liver function tests were performed as routine pathology tests. Thyroid function tests were assessed at baseline and weeks 26 and 52.

Cognitive measures

Cognitive tests are outlined in detail in Table 1. They were administered in a consistent order to keep the testing time to a minimum while ensuring that there was adherence to the temporal requirements of tests with multiple components (e.g., delayed memory components). The Subtle Cognitive Impairment Test (SCIT) was administered, followed by Trail Making Test A & B, Controlled Oral Word Association Task (COWAT), Rey-Osterrieth Complex Figure (ROCF) immediate recall, Rey Auditory Verbal Learning Task (RAVLT) (trials 1–5, foil trial and immediate recall trial), Anxiety and Depression subscales of the State-Trait Personality Inventory (STPI), Grooved Pegboard Task, RAVLT delayed recall trial, and finally the ROCF delayed recall trial. All cognitive testing was conducted during morning visits. Cognitive test performances were scored according to their respective published manuals.

Data analysis

Most datasets violated the assumption of normality (Shapiro–Wilkes analyses) and, since Marsh scores are an ordinal measure, the Friedman test, a nonparametric alternative to the repeated measures ANOVA, was used to investigate whether significant changes in any of the variables had occurred over the duration of the study. Wilcoxon signed rank tests were used as *post hoc* tests to determine when significant changes may have occurred. Given the exploratory nature of this study and to avoid rejecting potential true effects that could be followed up in a larger study, $P < 0.1$ was considered to represent statistical significance. To investigate whether significant changes had occurred for tTG, IP and serum ferritin

Table 1 | Description of the psychological tests used in this study

Test	Purpose	Procedure	Read-out
Subtle Cognitive Impairment Test (SCIT) ¹⁵	Measures the speed and effectiveness (efficacy) of information processing from iconic memory to short-term memory	Participants respond to a simple asymmetrical stimulus appearing on the screen for varying duration from 16 to 133 ms by pressing the left or right mouse button corresponding to the shorter side of the stimulus. Each of the two versions of the stimulus is presented six times and response time and error rate averages are calculated over 96 trials. Data for the four shortest exposure durations (16–66 ms) are pooled to provide two scores from the head of their respective curves: response time (SCIT-RT _H) and error rate (SCIT-E _H). Data from the four longer presentation durations (83–133 ms) are pooled to provide two scores from the tail of the data curve for response time (SCIT-RT _T) and error rate (SCIT-E _T)	Response time (SCIT-RT _H and SCIT-RT _T) Error rate (SCIT-ER _H and SCIT-ER _T)
Trail Making Test A & B ³⁷	Measures speed of processing, attention, sequencing, flexibility, visual search and fine motoric function	The participant connects letters and numbers in sequential order from letters and numbers randomly dispersed on a page	The time needed to complete the task in seconds
Rey-Osterrieth Complex Figure (ROCF) ³⁸	Evaluates visuospatial ability and visuospatial memory	A copy of a complex figure is drawn by the participant and is then repeated from memory 3 and 30 min later. Maximum obtainable score is 36	Presence and correct placement of the each element of the drawings
Controlled Oral Word Association Task (COWAT) ³⁹	Measures word fluency as an indicator of frontal lobe functioning	The participant is asked to produce as many words as possible in 1 min, starting with a given letter. Each word produced is scored	Sum of the number of words in three trials, each with a different letter
Rey Auditory Verbal Learning Task (RAVLT) ⁴⁰	Evaluates verbal learning and memory	The participant is asked to recall a 15-word list several times: first directly after it has been read out loud, then after a 3-min and a 30 min delay	Number of correctly recalled words per trial
Grooved Pegboard Task ⁴¹	Evaluates manual dexterity	Participant inserts small pegs with little keys on one side into a pegboard where the holes have randomly placed slots in which to fit the keys. This is a timed activity and each hand is tested separately (dominant and nondominant hands)	Sum of time to completion in seconds, pegs inserted and number of dropped pegs
Anxiety and Depression subscales of the State-Trait Personality Inventory ⁴²	Evaluates state (at the moment) and trait (in general) anxiety and depression	Self-report questionnaire in which participant is asked to rate forty statements on a four-point scale, ranging from 'not at all' to 'very much so'	Four separate scores each for state and trait
Wechsler's Test of Adult Reading (WTAR) ⁴³	Estimates pre-morbid IQ	Participant reads aloud 50 words that are irregular in their grapheme-to-phoneme translation	Number of correctly pronounced words

over the duration of the study, a one-way repeated measures ANOVA was performed. Correlations between measures across time points were calculated using Spearman's rho, and the False Discovery Rate was used to adjust for type 1 errors.^{12, 13} The significance levels for the correlations were kept at $P < 0.05$ to provide a manageable and meaningful number of correlations.

RESULTS

Characterisation of the participants

Sixteen participants enrolled for the study. Five withdrew: three due to an inability to meet the time commitments, one due to pregnancy before the week 12 endoscopy and one due to a clinical diagnosis of depression at week 4 based on analysis of the HADS from the screening visit (symptoms that predated the diagnosis of CD). Thus, 11 participants with a mean age of 30 (range 22–39) years completed the 12-month study. Demographical, clinical, laboratory and histopathological characteristics of the patients at baseline are summarised in Table 2. Participants commenced a GFD 4–26 days (mean 14 days) before study enrolment. Based on dietary history and food diary analysis, adherence to the GFD was considered to be excellent in all participants across all time points in the study. One participant with Marsh 3c histology and DQ2 haplotype was antibody-negative.

Table 2 | Characteristics at baseline of the 11 patients studied. Data are shown as (median) where applicable

Age (years)	22–39 (33)
Female: gender	8 (73%)
Body mass index (kg/m ²)	23.0–28.6 (25.3)
Marsh score of duodenal biopsy	
1	2
2	0
3a	3
3b	2
3c	4
Anti-tissue transglutaminase IgA (<20 U/mL)*	4–203 (49)
Haemoglobin (120–150 g/L)*	112–156 (142)
Ferritin (30–400 µg/L)*	18–399 (71)
B ₁₂ (156–698 pmol/L)*	215–708 (297)
Vitamin D (>75 nmol/L)*	35–165 (71)
Thyroid stimulating hormone (0.27–4.2 mIU/L)*	0.75–2.58 (1.33)
Intestinal permeability: lactulose:rhamnose ratio (<0.20)*	0.04–0.91 (0.06)
Intelligence Quotient (IQ)	90–112 (114)

* Normal reference range in parenthesis.

Both participants with Marsh 1 lesions had an elevated tTG and were HLA-DQ2 positive.

Changes in biological measures over time

At week 0, the median Marsh score was 3b (range 1–3c), at 12 weeks it was 3a (0–3a) and by week 52 it had decreased to 1 (0–3a). Marsh scores improved across the three time points [$\chi^2_{F(2)} = 15.70$, $P = 0.001$; (Friedman's test), Figure 2]. Histological improvement was marked in the first 12 weeks ($T = 1.15$, $P = 0.01$), but improvement between weeks 12 and 52 was not significant ($P = 0.371$). At 52 weeks, mucosal remission was achieved in four and mucosal response (Marsh 1) in five, while one stayed at Marsh 3a and biopsy data was not obtained for the last participant.

Serum tTG levels significantly decreased in all patients from a mean of 58.4 at baseline to 32.5 at 12 weeks and 16.8 U/mL at 52 weeks [$F(1.14, 10.26) = 6.8$, $P < 0.023$, Figure 2]. Further, differences between weeks 0 and 12, as well as between weeks 12 and 52, were also significant [$F(1, 9) = 6.15$, $P = 0.03$ and $F(1, 9) = 5.42$, $P = 0.04$ respectively]. tTG had normalised in two patients by 12 weeks and in seven patients at 52 weeks. Normalisation of serum tTG concentrations was associated with mucosal healing in the two patients with normalised tTG levels at 12 weeks, and for five patients at 52 weeks. For the remaining two patients in whom tTG concentrations fell over time, the duodenal mucosa either did not improve from the diagnostic biopsy at Marsh 3a or deteriorated from Marsh 0 to 1 by 52 weeks. Furthermore, Marsh scores improved in two of the four patients who did not achieve normal tTG levels.

One participant had abnormally high vitamin B₁₂ levels as a result of supplementation. Mild iron deficiency anaemia (Hb concentration 112 g/L) was present in one participant who remained mildly anaemic throughout the study. Two other participants were found to be iron deficient at baseline, but with normal Hb concentration. Only one of the three iron-deficient patients was given iron repletion therapy via a total dose iron infusion between weeks 12 and 26. The serum ferritin levels over the 12 months are (Figure 2d) did not differ between the three time points [$F(1.08, 8.67) = 0.64$, $P = 0.46$]. Vitamin D concentrations were low in six participants at baseline. These remained low in all but one participant despite oral vitamin D replacement therapy (administered to all six). In two further participants, the initially normal vitamin D levels fell below normal at 12 and 52 weeks. No patient had abnormal thyroid function and no changes were noted in thyroid function tests or liver

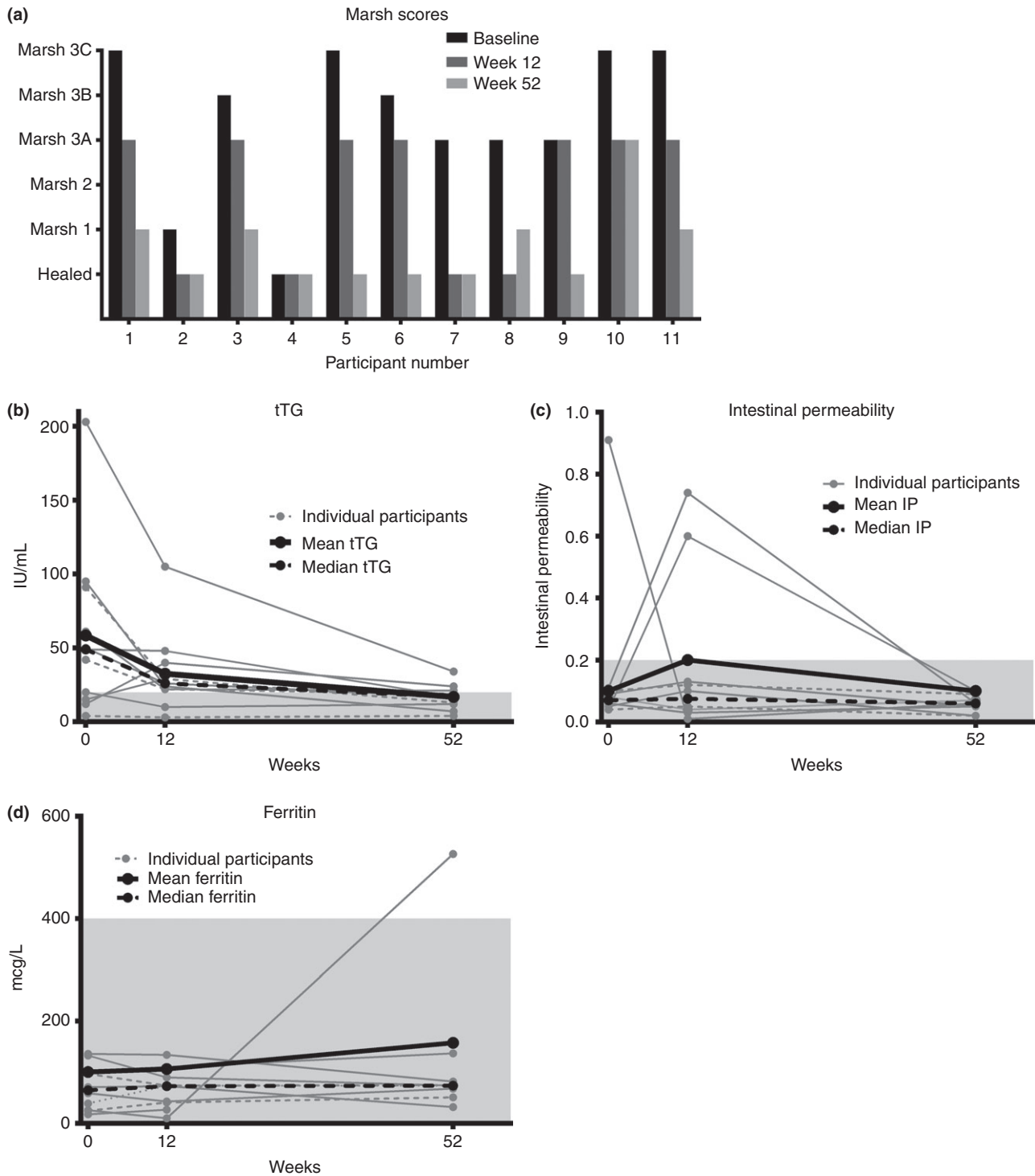


Figure 2 | Changes in selected physiological measures during 12 month on a gluten-free diet. (a) Median Marsh scores for each participant ($n = 11$) improved significantly across the three time points ($P = 0.001$; Friedman's test). (b) Tissue transglutaminase antibodies concentrations (shown as mean $\pm$ 1 SEM) ($n = 11$) fell significantly across the three time points ($P = 0.02$). (c, d) Intestinal permeability and serum ferritin are shown as mean $\pm$ 1 SEM ($n = 11$). Neither measure differed significantly between the time points.

function tests (data not shown). IP was elevated in three participants, one of these at baseline the other two at week 12. All IP levels were within the normal range at 52 weeks (Figure 2c). Changes in IP across the three time points were not statistically significant [$F(2,18) = 0.90$, $P = 0.42$].

Cognitive changes over time

Four of the eight cognitive tests demonstrated a significant improvement in performance between time 0 and 52 weeks (Figure 3). The time taken to perform the TRAILS A part of the Trail Making Task improved from 23.7 s in week 0–17.0 s in week 52 ($T = 6.50$; $P = 0.032$). Mean performance on SCIT-RT_H improved from 580 ms in week 0–538 ms in week 52 ($T = 7.00$;

$P = 0.066$). Similarly, the scores for accurately recalling the complex figure of the ROCF after 3 min improved from 21.1 to 27.7 ($T = 52.00$; $P = 0.012$), and the scores for accurately recalling of the ROCF after 30 min improved from 20.5 to 26.5 ($T = 49.0$; $P = 0.028$). The changes in performance between weeks 0 and 52 on all other cognitive tests were not significant at $P < 0.1$ and are not included in Figure 3.

Correlation between cognitive outcomes, other indices and Marsh scores

Performance on the following cognitive tests and physiological measures did not correlate significantly with Marsh scores across the three time points: SCIT-E_H and -E_T, Trail making task parts B and B minus A, ROCF,

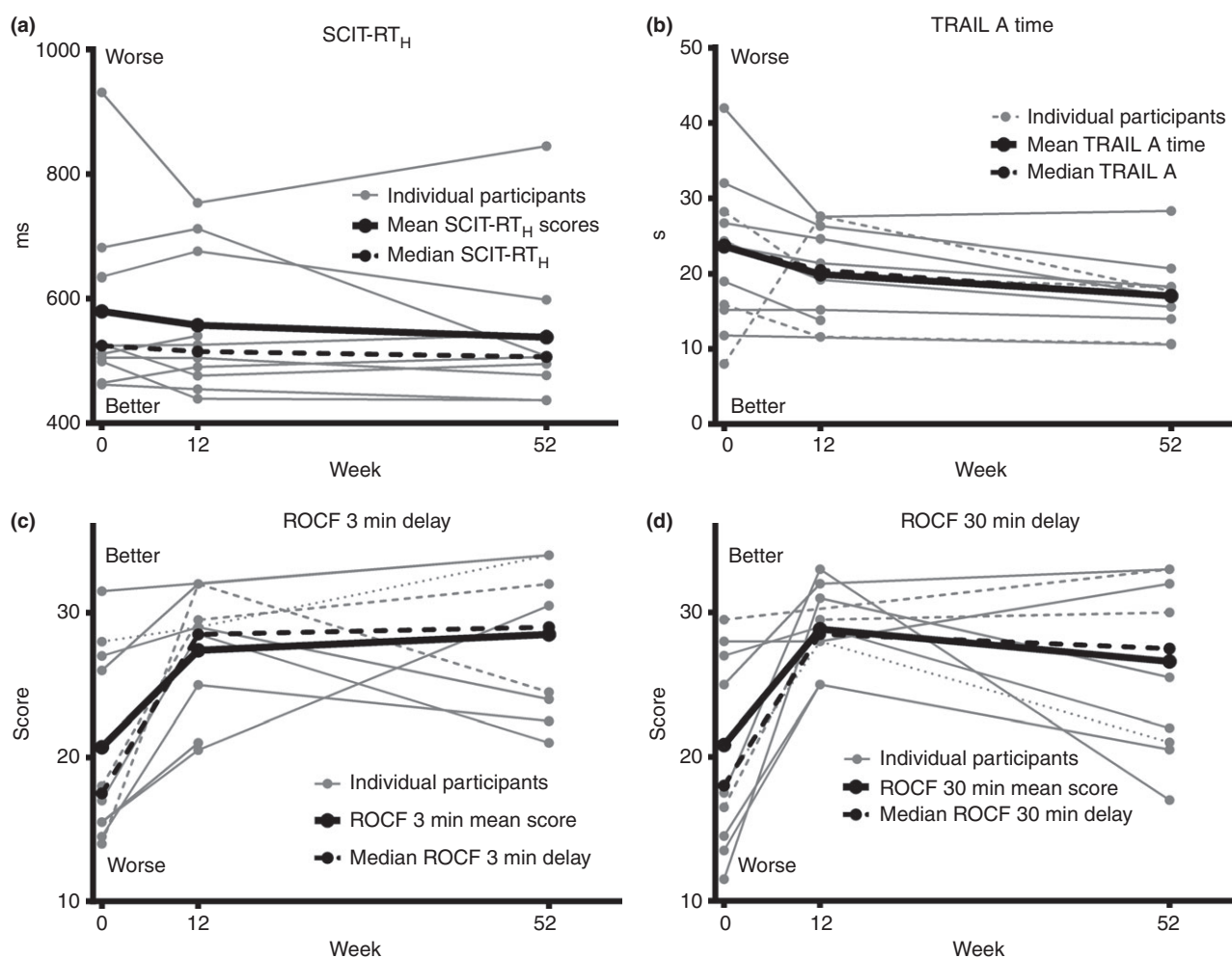


Figure 3 | Changes in selected cognitive measures during 12 month on a GFD. Results are shown as mean $\pm$ 1 SEM, ($n = 11$). Data for SCIT-RT, TRAILS A and tTG are plotted with improvement indicated by a downward trend. The difference in performance on SCIT-RT_H between weeks 0 and 52 was significant at $P = 0.06$, for TRAILS A at $P = 0.03$, ROCF 3 min $P = 0.01$ and ROCF 30 min $P = 0.028$. COWAT differences were not significant at $P = 0.184$.

RAVLT immediate recall, and recall at 30 min, STPI, Hb, IP, and serum levels of ferritin and vitamins B₁₂ and D. Performance on the following tests correlated significantly with Marsh scores and tTG levels: TRAILS A and COWAT at $P < 0.01$, and SCIT-RT_H, SCIT-RT_T, RAVLT 3 min at $P < 0.05$. As shown in Figure 4, the correlations (Spearman's r) were large and ranged from 0.377 to 0.735.

Performance on the following cognitive tests and physiological measures did not correlate significantly with tTG serum titre across the three time points: SCIT-E_H and -E_T, all of the ROCF measures, RAVLT immediate recall and recall at 30 min, STPI, Hb and serum levels of ferritin and vitamins B₁₂ and D. Performance on the following tests correlated significantly with tTG levels: SCIT-RT_H, SCIT-RT_T, TRAILS A, B and COWAT (all at $P < 0.01$) as well as TRAILS B minus A, RAVLT1-5, grooved pegboard (dominant and non-dominant hands) and IP (at $P < 0.05$). No meaningful patterns could be found for the relationships between the cognitive test results and the serum concentrations of ferritin, Hb, vitamin B₁₂ and vitamin D, or between cognitive test outcomes and IP for the participants with elevated IP.

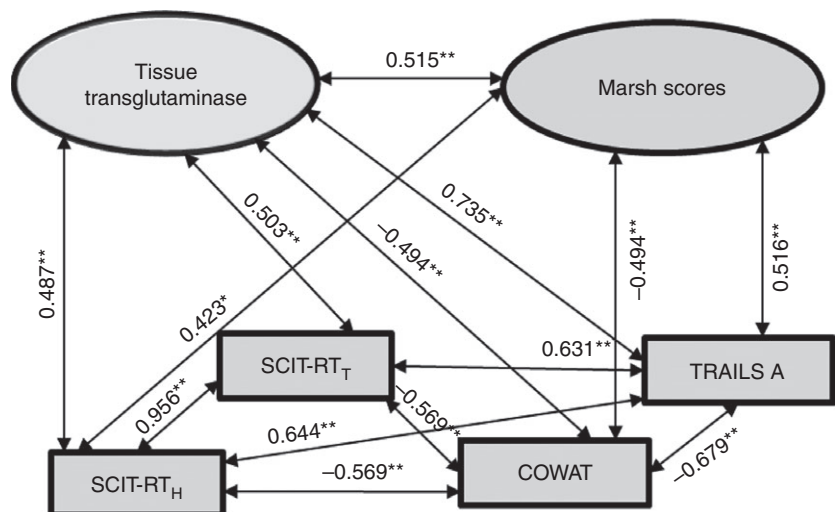
DISCUSSION

This pilot study examined the relationships between cognitive function and mucosal healing in patients who had been recently diagnosed with CD, and who adhered to a GFD over the first year of treatment. The study demonstrated the presence of cognitive impairment that improved with therapy and was correlated with histological evidence of mucosal recovery and/or healing.

This study provides the beginnings of an evidence-base for the ill-defined, yet frequently reported symptoms of brain fog in CD. While participants improved on all nine cognitive tests, only TRAILS A, ROCF and SCIT-RT_H demonstrated a significant improvement over the course of the study. Two of these tests are strongly dependent on processing speed: and include speed of response in the SCIT-RT_H, and visuomotor speed in the TRAILS A test. ROCF is mainly a test for visuospatial short-term memory. Although improved performance on the TRAILS A test might be due to practice effects, the published test-retest reliability over 3–12 months is high, suggesting that any practice effect would be minor.¹⁴ Alternative versions were used for the ROCF and COWAT, and the SCIT does not have learning or practice effects.^{15, 16}

It is notable that the level of impairment on the SCIT-RT_H at week 0 was similar to that in people with a blood alcohol level of 0.05 g/100 mL,¹⁶ which is the upper legal limit for driving in Australia. It is also equivalent to level of impairment in SCIT-RT_H observed in participants with severe jetlag (SR Robinson & GW Yelland, unpublished data). Further, the 8% relative change in SCIT performance in people with untreated CD to their paired test after being adherent to the GFD, was similar to that demonstrated between 0.05 and 0.02 blood alcohol levels and to the recovery from jetlag over a 24-h period. In people with undiagnosed CD, such cognitive deficits might result in impaired performance in driving and at work.¹⁷ If these findings are confirmed in a larger study, they may have important health and safety implications. When viewed together, the present results indicate that short-term memory, movement and processing speed are impaired in untreated CD and that

Figure 4 | Participants' ($n = 11$) performance on four cognitive tests showed significant correlations with each other and with histological scores and tTG levels (* $P < 0.05$, ** $P < 0.001$; Spearman's Rho).



they improve during adherence to a GFD. These impairments, although subclinical, may contribute to the sensation of brain fog that is commonly reported by people with CD.

There are three reasons why cognition might be impaired in patients with untreated CD. First, nutrient deficiencies involving, for example, iron, vitamin D and folate have been associated with cognitive impairment.^{18–20} As part of the malabsorption that might occur in patients with CD, such micronutrient deficiencies do occur. In this study however, changes in iron levels were not associated with cognitive performance, and the persisting low levels of vitamin D in six participants argues against a link to the cognitive improvement found over the duration of the study.

Second, cognitive impairment in patients with untreated CD may be due to the high levels of circulating cytokines associated with systemic inflammation.^{21, 22} Elevated concentrations of circulating cytokines have been associated with changes in behaviour, mood and cognition.^{23–29} The brain possesses receptors for circulating cytokines, whilst cytokine activation of neurones and subsequent central signalling may also be important.²⁶

Third, the cognitive improvement may be related to reduced exposure to gluten *per se*. Animal studies have shown dietary gluten may reduce brain tryptophan concentrations. Since tryptophan is the precursor of the neurotransmitter, serotonin, it has been speculated that gluten may impair cognitive function by lowering brain serotonin levels.³⁰ Alternatively, opioid peptides derived from partially digested gluten, so-called 'exorphins', can have several effects on higher brain function in rodents,³¹ although no studies have been reported in humans. Furthermore, changes to the diet can alter the gut microbiota, and this has been found to strongly influence behaviour in rats.^{32–35} Humans might be similarly affected, since a recent brain imaging study reported that a 4-week intake of probiotic fermented milk by healthy women affected the activity of brain regions that control central processing of emotion and sensation.³⁶

This study demonstrated that improvements in cognition in CD are significantly correlated with the extent of intestinal healing, as indicated by Marsh scores. Indeed, the strength of these correlations was as good as those obtained for serum tTG. The fact that significant correlations between disease activity measures and cognitive function were obtained with a sample of just 11 participants, underscores the magnitude

of the effect sizes. The strength of these correlations raises the interesting possibility that a small battery of cognitive tests might offer a noninvasive means to regularly screen CD patients for intestinal healing. Currently, there is need for an accurate biomarker of healing that can lessen the requirement for repeated intestinal biopsies. A larger prospective study will be needed to determine whether cognitive tests have sufficient reliability to serve as a proxy of intestinal healing.

In conclusion, this pilot study indicates that cognition is impaired in people with untreated CD. Cognitive function improves after commencement of a strict GFD, and this improvement is correlated with a normalisation of histopathological markers of disease severity. These results support patient reports of brain fog in untreated CD, and demonstrate that impairments in cognitive performance are an additional extra-intestinal or systematic manifestation of CD. Our findings introduce the possibility that cognitive tests have the potential to provide a noninvasive, cost-efficient marker of intestinal healing.

AUTHORSHIP

Guarantor of the article: G.W. Yelland.

Author contributions: Irene Lichtwark analysed and interpreted the psychological test data, wrote the manuscript and prepared the illustrations. Evan Newnham contributed to study design, recruited participants, administered psychological assessments, collected blood samples, collated the biological data and contributed to writing the clinical sections of the manuscript. Stephen Robinson contributed to study design, data interpretation and writing of the manuscript. Susan Shepherd performed dietary/compliance assessments, recruited participants and reviewed the manuscript. Patrick Hosking interpreted the histology from biopsies. Peter Gibson contributed to study design, data interpretation and writing of the manuscript. Gregory Yelland contributed to study design, administered psychological assessments, data interpretation and writing of the manuscript. All authors approved the final version of the manuscript.

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Declaration of personal interests: Susan Shepherd has published several books on coeliac disease and dietary management relevant to coeliac disease. Peter Gibson has published two books on dietary management relevant to coeliac disease. Stephen Robinson is a patent

holder for the Subtle Cognitive Impairment Test. Gregory Yelland is a patent holder for the Subtle Cognitive Impairment Test.

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REFERENCES

- Kang JY, Kang AHY, Green A, Gwee KA, Ho KY. Systematic review: worldwide variation in the frequency of coeliac disease and changes over time. *Aliment Pharmacol Ther* 2013; **38**: 226–45.
- Gibson PR, Shepherd SJ, Tye-Din JA. For celiac disease, diagnosis is not enough. *Clin Gastroenterol Hepatol* 2012; **8**: 900–1.
- Hu WT, Murray JA, Greenaway MC, Parisi JE, Josephs KA. Cognitive impairment and celiac disease. *Arch Neurol* 2006; **63**: 1440–6.
- Currie S, Hadjivassiliou M, Clark M, *et al*. Should we be 'nervous' about coeliac disease? Brain abnormalities in patients with coeliac disease referred for neurological opinion. *J Neurol Neurosurg Psychiatry* 2012; **83**: 1216–21.
- Zelnik N, Pacht A, Obeid R, Lerner A. Range of neurological disorders in patients with celiac disease. *Pediatrics* 2004; **113**: 1672–6.
- Sapone A, Bai JC, Ciacci C, *et al*. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Med* 2012; **10**: 13.
- Neto JIS, Costa ACLV, Magalhaes FG, Silva GS. Neurological manifestations of celiacs disease. *Arq Neuropsiquiatri* 2004; **62**: 969–72.
- Bardella MT, Velio P, Cesana BM, *et al*. Coeliac disease: a histological follow-up study. *Histopathology* 2007; **50**: 465–71.
- Anderson J. Celiac Disease and Gluten Sensitivity, 2013. Available at: <http://celiacdisease.about.com/od/symptomsofceliacdisease/a/Celiac-Disease-Brain-Fog.htm>. Accessed December 8, 2013.
- Brück K, Bösch S, Müller CA, *et al*. Sporadic cerebellar ataxia associated with gluten sensitivity. *Brain* 2001; **124**: 1013–9.
- Lurie Y, Landau DA, Pfeffer J, Oren R. Celiac disease diagnosed in the elderly. *J Clin Gastroenterol* 2008; **42**: 59–61.
- Howell D. *Statistical Methods for Psychology*. Belmont, CA: Wadsworth, 2013.
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Stat Soc Series B (Methodological)* 1995; **57**: 289–300.
- Sánchez-Cubillo I, Periañez JA, Adrover-Roig D, *et al*. Construct validity of the Trail Making Test: role of task-switching, working memory, inhibition/interference control, and visuomotor abilities. *J Int Neuropsychol Soc* 2009; **15**: 438–50.
- Yelland GW, Robinson SR, Friedman T, Hutchinson C. Assessment of cognitive impairment. Australian Official Journal of Patents. Australia: Monash University, 2004.
- Friedman TW, Robinson SR, Yelland GW. Impaired perceptual judgement at low blood alcohol concentrations. *Alcohol* 2011; **45**: 711–8.
- Lezak MD. *Neuropsychological Assessment*. Oxford: Oxford University Press, 2004.
- Murray-Kolb LE, Beard JL. Iron treatment normalizes cognitive functioning in young women. *Am J Clin Nutr* 2007; **85**: 778–87.
- Balton C, Griffith L, Striffler L, *et al*. Vitamin D, cognition, and dementia: a systematic review and meta-analysis. *Neurology* 2012; **79**: 1397–405.
- Ramos M, Allen L, Mungas D, *et al*. Low folate status is associated with impaired cognitive function and dementia in the Sacramento Area Latino Study on Aging. *Am J Clin Nutr* 2005; **82**: 1346–52.
- Forton DM, Allsop JM, Main J, Foster GR, Thomas HC, Taylor-Robinson SD. Evidence for a cerebral effect of the hepatitis C virus. *Lancet* 2001; **358**: 38–9.
- Senzolo M, Schiff S, D'Aloiso C, *et al*. Neuropsychological alterations in hepatitis C infection: the role of inflammation. *World J Gastroenterol* 2011; **17**: 3369–74.
- Maier SF. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychol Rev* 1998; **105**: 83.
- Turrin NP, Plata-Salamán CR. Cytokine–cytokine interactions and the brain. *Brain Res Bull* 2000; **51**: 3–9.
- Larson SJ, Dunn AJ. Behavioral effects of cytokines. *Brain Behav Immun* 2001; **15**: 371–87.
- Wilson CJ. Cytokines and cognition—the case for a head-to-toe inflammatory paradigm. *J Am Geriatr Soc* 2008; **50**: 2041.
- Kapadia M, Sakic B. Autoimmune and inflammatory mechanisms of CNS damage. *Prog Neurobiol* 2011; **95**: 301–33.
- O'Connor JJ. Actions of the pro-inflammatory cytokine IL-1 beta on central synaptic transmission. *Exp Physiol* 1999; **84**: 601.
- Banks WA, Farr SA, Morley JE. Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. *Neuroimmunomodulation* 2002; **10**: 319.
- Choi S, DiSilvio B, Fernstrom MH, Fernstrom JD. Meal ingestion, amino acids and brain neurotransmitters: effects of dietary protein source on serotonin and catecholamine synthesis rats. *Physiol Behav* 2009; **98**: 156–62.
- Takahashi M, Fukunaga H, Kaneto H, Fukudome S-I, Yoshikawa M. Behavioral and pharmacological studies on gluten exorphin A5, a newly isolated bioactive food protein fragment, in mice. *Jpn J Pharmacol* 2000; **84**: 259–65.
- Bercik P, Denou E, Collins J. The intestinal microbiota affect central levels of brain-derived neurotrophic factor and behavior in mice. *Gastroenterology* 2011; **141**: 599–609. e3.
- Heijtz RD, Wang S, Anuar F. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci* 2011; **108**: 3047–52.
- Neufeld K, Kang N, Bienenstock J, Foster J. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil* 2011; **23**: 255–e119.
- Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* 2012; **13**: 701–12.
- Tillisch K, Labus J, Kilpatrick L. Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology* 2013; **144**: 1394–401.
- Reitan RM. The relation of the trail making test to organic brain damage. *Consult Psychol* 1955; **19**: 393–4.
- Knight JA, Kaplan E. *The Handbook of Rey-Osterrieth Complex Figure Usage: Clinical and Research Applications*. Lutz, FL: Psychological Assessment Resources, Inc., 2003.

39. Spreen O, Strauss E. *A Compendium of Neuropsychological Tests*. New York, NY: Oxford University Press, 1991.
40. Rey A. *L'examen clinique en psychologie*. Paris, France: Presses Universitaires de France, 1964.
41. Lafayette Instrument Company. (2002) Grooved pegboard test user instructions. Available at: <http://www.si-instruments.com.au/product/show/146/grooved-pegboard-test/>. Accessed June 25, 2005.
42. Spielberger CD. *State-Trait Personality Inventory (STPI) Research and Manual Sampler Set*. Tampa, FL: Mind Garden, Inc, 1995.
43. Wechsler D. *Wechsler Test of Adult Reading*. San Antonio, TX: Psychological Corporation, 2001.