Effect of lactoferrin treatment on symptoms and physical performance in long COVID patients: a randomised, double-blind, placebo-controlled trial

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Shareable abstract (@ERSpublications)
The randomised, double-blind, placebo-controlled LARGO trial investigated the effect of lactoferrin on long COVID symptoms. In both long COVID arms, clinical outcomes improved after 6 weeks without benefit for lactoferrin compared to placebo. https://bit.ly/3TvXHFC

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Abstract

Background Long COVID is a heterogeneous condition with a variety of symptoms that persist at least 3 months after SARS-CoV-2 infection, often with a profound impact on quality of life. Lactoferrin is an iron-binding glycoprotein with anti-inflammatory and antiviral properties. Current hypotheses regarding long COVID aetiology include ongoing immune activation, viral persistence and auto-immune dysregulation. Therefore, we hypothesised that long COVID patients may potentially benefit from lactoferrin treatment. The aims of the present study were to investigate the effect of lactoferrin on various long COVID domains: fatigue, anxiety, depression, cognitive failure and muscle strength.

Methods We performed a randomised, double-blind, placebo-controlled trial in long COVID patients aged 18–70 years within 12 months after proven SARS-CoV-2 infection. Patients were randomised (1:1) to 6 weeks of lactoferrin (1200 mg daily) or placebo. At three hospital visits (T0, T6 and T12 weeks), patient-reported outcome measures were collected, physical performance tests were performed and blood was drawn. The difference in fatigue at T6 was the primary outcome.

Results 72 participants were randomised to lactoferrin (n=36) or placebo (n=36). We found a significant decrease in fatigue, as measured with the Fatigue Assessment Scale, between T0 and T6 in both study arms, but without significant difference between the study arms (lactoferrin: 3.9, 95% CI 2.3–5.5, p=0.007; placebo: 4.1, 95% CI 2.3–5.9, p=0.013). No significant differences were found in any of the other outcomes in favour of the lactoferrin arm at T6 or T12.

Conclusion Although both long COVID arms showed improved clinical outcomes at T6, the improvement did not continue until T12. Lactoferrin provided no benefit in terms of fatigue, other patient-reported outcome measures or physical functioning.

Introduction

Many patients experience prolonged symptoms after acute COVID-19 infection [1]. The synonyms "post-acute COVID-19 syndrome" or "long COVID" are defined as the presence of symptoms for usually 3 months from symptom onset, with symptoms that last for at least 2 months without explanation by alternative diagnoses [2]. Patients may experience a wide range of symptoms such as fatigue, dyspnoea, chest pain, headache, cognitive disturbances (brain fog), depressive symptoms, anxiety and muscle pain, with often profound effects on the quality of life [3–5].

Although the literature about the pathophysiology of acute COVID-19 is expanding, the underlying causative mechanism of long COVID remains unclear [6]. Current hypotheses regarding the

pathophysiology of long COVID include ongoing immune dysregulation, viral persistence or reactivation, auto-immunity and endothelial dysfunction [4, 7–9].

The current therapeutic interventions for long COVID involve physiotherapy and occupational therapy, mostly targeted at restoring the energy balance [10–12]. Nonetheless, patients recovering from COVID-19 still experience many limitations in their daily activities after 6 months [13].

Given the current insights regarding long COVID pathophysiology with a key role for ongoing immune activation and viral persistence or reactivation, we hypothesised that long COVID patients may potentially benefit from lactoferrin treatment. Lactoferrin is an iron-binding glycoprotein and is highly concentrated in colostrum and milk. Lactoferrin is secreted at mucosal surfaces of different organs to protect against pathogens [14, 15]. It also protects the cells from oxidative stress by inhibiting the free ferric ions [16]. Several studies have indicated that lactoferrin protects the host cell in different pathways such as inhibition of the attachment of viruses to the host cell, reducing viral replication and stimulating the immune response [15, 17]. Bovine lactoferrin blocks virus–host cell attachment *in vitro* for different coronavirus subtypes and shortens the conversion from positive to negative PCR during acute COVID-19 *in vivo* [18–20]. Considering hypotheses about the pathophysiology of long COVID, lactoferrin might reduce viral replication and/or inhibit the (ongoing) immune activation. Therefore, we aimed to investigate the effect of lactoferrin on the recovery of long COVID patients in a randomised clinical trial, assessing multiple long COVID domains: fatigue, anxiety, depression, cognitive failure and muscle strength.

Methods

Study design and population

The lactoferrin in the treatment of long COVID study (LARGO study) is a single-centre, randomised, double-blind, placebo-controlled study, conducted at the Franciscus Gasthuis & Vlietland hospital. Patients were included between January and July 2022 and randomised into the intervention arm (600 mg lactoferrin capsules twice a day for 6 weeks) or the placebo arm (placebo capsules twice a day for 6 weeks) (information on the investigational product can be found in the supplementary methods). The placebo and lactoferrin capsules were identical in appearance. Patients who were already receiving usual care, such as physiotherapy, occupational therapy and/or psychobiological support, continued the usual care during the study period.

Study procedures

We collected all measurements at T0, T6 and T12 weeks of follow-up. At baseline, patients were randomised to the lactoferrin or placebo arm. From T6 to T12, patients continued the study without using the study product.

All long COVID patients who were referred to the hospital were first assessed for eligibility by performing diagnostic tests to rule out other pathologies, *i.e.* chest radiography, pulmonary function tests (spirometry and diffusion), ECG and laboratory findings (total blood count, creatinine, potassium, C-reactive protein, creatinine kinase, ferritin and transferrin saturation).

Recruitment and inclusion

Patients were recruited through referral from primary care or secondary care. The following inclusion criteria were applied: between 18 and 70 years of age, PCR-confirmed SARS-CoV-2 infection and at least two long COVID symptoms according to World Health Organization consensus criteria lasting <1 year [21]. Exclusion criteria were prior admission to the intensive care unit due to COVID-19, abnormal or changed chest radiography or pulmonary function test results, current acute COVID-19 infection or active systemic immunological disorders, current psychiatric disorders as diagnosed by a psychiatrist, use of oral/inhaled corticosteroids or other immune-modulatory medication, current pregnancy or lactation, and allergy to milk or components of milk. The study was approved by the Medical Ethical Committee and the local board of the Franciscus Gasthuis & Vlietland hospital. The trial is registered at Dutch Trial Register (NL9742) and conducted in accordance with the principles of the Declaration of Helsinki. Each patient provided written informed consent before any study procedures.

Randomisation

The participants were randomised using the web-based program Sealed Envelope (www.sealedenvelope.com/randomisation) using block randomisation without stratification. During the study, all investigators and patients were blinded to allocation. The randomisation list was secured in a safe at the clinical hospital pharmacy and only the pharmacy assistant of the hospital had access. The pharmacy assistant allocated the

bottle based on the randomisation list and secured the concealment by removing the identity label and attaching the study label.

Primary and secondary outcome measures

The primary outcome of this study was the difference in fatigue between T0 and T6, as measured with the Fatigue Assessment Scale (FAS). The FAS score includes a physical and mental subscale defined by a selection of specific questions. The validated minimal clinically important difference (MCID) for this questionnaire is a ⩾4-point difference (supplementary methods) [22].

The secondary outcomes included other patient-reported outcome measures (PROMs) (cognitive failure assessed using the Cognitive Failure Questionnaire (CFQ) and anxiety and depression assessed using the Hospital Anxiety and Depression Scale (HADS); supplementary methods) and measures of physical functioning (handgrip strength for muscle strength and the 30-s sit-to-stand test for cardiorespiratory fitness) [23–27].

During the three hospital visits, venepunctures were performed to evaluate different blood parameters. Complete blood counts and haemoglobin were measured by a haematology analyser (XN-9100, Sysmex). Ferritin and transferrin saturation were measured by an immunoanalyser (Architect i2000, Abbott). Creatinine kinase was measured by a photometric chemical analyser (Architect C8000, Abbott). Serum samples were collected and stored at –80°C.

Data collection

The following information was collected at baseline: demographics (age, sex, weight, height and body mass index), medical history and medication use, date of SARS-CoV-2 positive PCR test, symptoms and course during acute infection, persistent long COVID symptoms, vaccination status (classified as not vaccinated, fully vaccinated without booster (one Janssen vaccine or two doses Pfizer or AstraZeneca vaccines) or partly vaccinated), smoking status (classified as former, active and nonsmoker), prior treatment for long COVID (physiotherapy, occupational therapy and/or psychobiological support) and adverse events.

Statistical analysis

Normally distributed variables are reported as mean±SD or otherwise as median (interquartile range) and categorical variables are reported as n (%). Statistical tests were not performed for every baseline characteristic owing to the study design.

The study was powered on the expected increased improvement in fatigue symptoms with the use of lactoferrin combined with usual care compared to usual care alone. We expected to find a 20% difference in reaching the MCID of fatigue (FAS scale) after 6 weeks of treatment in favour of the lactoferrin treatment arm. To have an 80% chance of detecting the 20% difference in the primary outcome measure at a 5% significance level (assuming that participants had an average FAS score of 30±8.5 points), 2×32 participants were required for this study. Considering a possible drop-out of 10% we included a total of 72 participants (2×36 participants).

Analyses of all primary and secondary outcomes were performed for the difference between T0 and T6 according to the intention-to-treat principle. To evaluate if differences between T0 and T6 persisted after stopping treatment, differences between T6 and T12 were analysed. Participants who continued lactoferrin treatment after 6 weeks were excluded from the analyses between T6 and T12 (*i.e.* noncompliance with study protocol). The number of participants reaching the FAS MCID during the study period was analysed and compared between the two groups.

To assess between-group differences, an independent samples t-test was used. For non-normally distributed data, a Mann–Whitney U-test was used. To test the difference in time within the same group, a paired samples t-test was performed. For non-normally distributed data, Wilcoxon signed rank test was used to assess paired scores. To correct for multiple testing, the Holm's procedure was performed for all primary and secondary outcome parameters with a maximum of 1.0. A p-value <0.05 was considered significant. All analyses were performed using SPSS version 28 (IBM Corp.).

Results

Baseline characteristics

A total of 72 patients with long COVID were enrolled, of whom 36 participants were randomised to the treatment arm with lactoferrin and 36 to the placebo arm, both in addition to usual care. Seven participants were lost to follow-up; four in the lactoferrin arm and three in the placebo arm (supplementary figure S1).

Table 1 contains the baseline demographics for both study arms. Most participants in both arms were female (62.5%). The median age of the participants was 48.0 years (38.3–55.0 years) and the median long COVID disease duration at inclusion was 230.5 days (129.8–333.0 days). Participants had a positive PCR between February 2022 and March 2023.

Most participants had COVID-19 infection once (81.9%), and most of the participants were vaccinated at inclusion (91.7%). The timing of the COVID infection varied around COVID vaccinations (table 1).

The most common symptoms among the patients with long COVID in this study were fatigue (91.7% in lactoferrin arm *versus* 97.2% in control arm), cognitive disturbances (94.4% in lactoferrin arm *versus* 91.7% in control arm) and dyspnoea (55.6% in lactoferrin arm *versus* 69.4% in control arm). Furthermore, 36% of the participants subjectively reported muscle weakness/pain, while only 3% spontaneously reported anxiety or depressive symptoms at baseline (table 1).

TABLE 1 Baseline characteristics

	Lactoferrin	Placebo	p-value
Subjects (n)	36	36	
Age (years)	48.5 (38.0–54.8)	48.0 (39.5–55.0)	0.92
BMI (kg·m^{-2})	25.5 (22.9–27.9)	24.9 (22.2–27.0)	0.53
Female sex	21 (58.3)	24 (66.7)	0.47
Hospital admission during acute infection	5 (13.9)	2 (2.8)	0.63
Comorbidities	14 (38.9)	12 (33.3)	0.62
Cardiovascular	3 (8.3)	4 (11.1)	
Pulmonary	1 (2.3)	1 (2.3)	
Orthopaedic	5 (13.9)	3 (8.3)	
Former psychiatric disorder	4 (11.1)	1 (2.3)	
Other comorbidities	5 (13.9)	5 (13.9)	
Vaccinated	31 (86.1)	35 (97.2)	0.20
Disease duration at inclusion (days)	204.5 (121.3–320.8)	288.0 (134.3–337.0)	0.18
Smoking status			
Active smoker	1 (2.8)	2 (5.6)	1.0
Nonsmoker	35 (97.3)	34 (94.4)	
Former smoker	11 (30.6)	9 (25)	0.79
Never-smoker	24 (66.7)	25 (69.4)	0.80
Symptoms reported at baseline			
Fatigue (FAS)	33 (91.7)	35 (97.2)	0.30
Cognitive disturbance (CFQ)	34 (94.4)	33 (91.7)	0.64
Dyspnoea	20 (55.6)	25 (69.4)	0.22
Muscle weakness/pain	16 (44.4)	10 (27.8)	0.14
Headache	10 (27.8)	10 (27.8)	1.00
Chest pain	6 (16.7)	10 (27.8)	0.26
Sore throat	3 (8.3)	1 (2.8)	0.30
Depressive or anxiety symptoms (HADS)	2 (5.6)	0 (0)	0.15
Low-grade fever	1 (2.8)	0 (0)	0.31
Frequency of COVID infections			
One	28 (77.8)	31 (86.1)	–
Two	7 (19.4)	4 (11.1)	–
Three	0 (0)	1 (2.8)	–
Unknown	1 (2.8)	0 (0)	–
Timing of COVID infection and vaccination			
Before vaccination	14 (38.9)	19 (52.7)	–
Between first and second dose	3 (8.3)	2 (5.6)	–
After complete vaccination[#]	14 (38.9)	12 (33.3)	–
After vaccination booster dose	0	2 (5.6)	–
No vaccination	5 (13.9)	1 (2.8)	–

Data are presented as median (interquartile range) or n (%), unless otherwise indicated. No statistical tests were performed for COVID infection and vaccination status. BMI: body mass index; FAS: fatigue assessment scale; CFQ: Cognitive Failure Questionnaire; HADS: Hospital Anxiety and Depression Scale. [#]: complete vaccination defined as one dose of Janssen vaccine or two doses of Pfizer or AstraZeneca vaccine.

Primary outcome

The FAS score decreased significantly at T6 compared to T0 but did not show an apparent difference between arms (p=1.0) (figure 1). The FAS score in the placebo arm improved from a median 33.5 points (30.0–37.0 points) at T0 to 28.0 points (25.0–34.0 points) at T6 (p=0.013), while the FAS score for the lactoferrin users decreased from 33.5 points (29.3–36.8 points) at T0 to 27.0 points (25.0–35.0 points) at T6 (p=0.007) (figure 1 and supplementary table S1).

The mental and the physical subscales of the FAS score did not improve during the first 6 weeks of treatment in the lactoferrin arm or placebo arm (both p=1.0). A total number of 40 participants (19 (54.3%) and 21 (62.8%) from the lactoferrin and placebo arms, respectively) experienced a minimum of a 4-point improvement (MCID) in the FAS questionnaire 6 weeks after treatment (p=1.0).

Furthermore, the distribution of the symptom duration was analysed and the two peaks reflected two peaks of COVID-19 incidences in the Netherlands, around April 2021 and January 2022. There was no significant difference in FAS improvement at T6 between participants who were infected in the first or second wave (p=1.0).

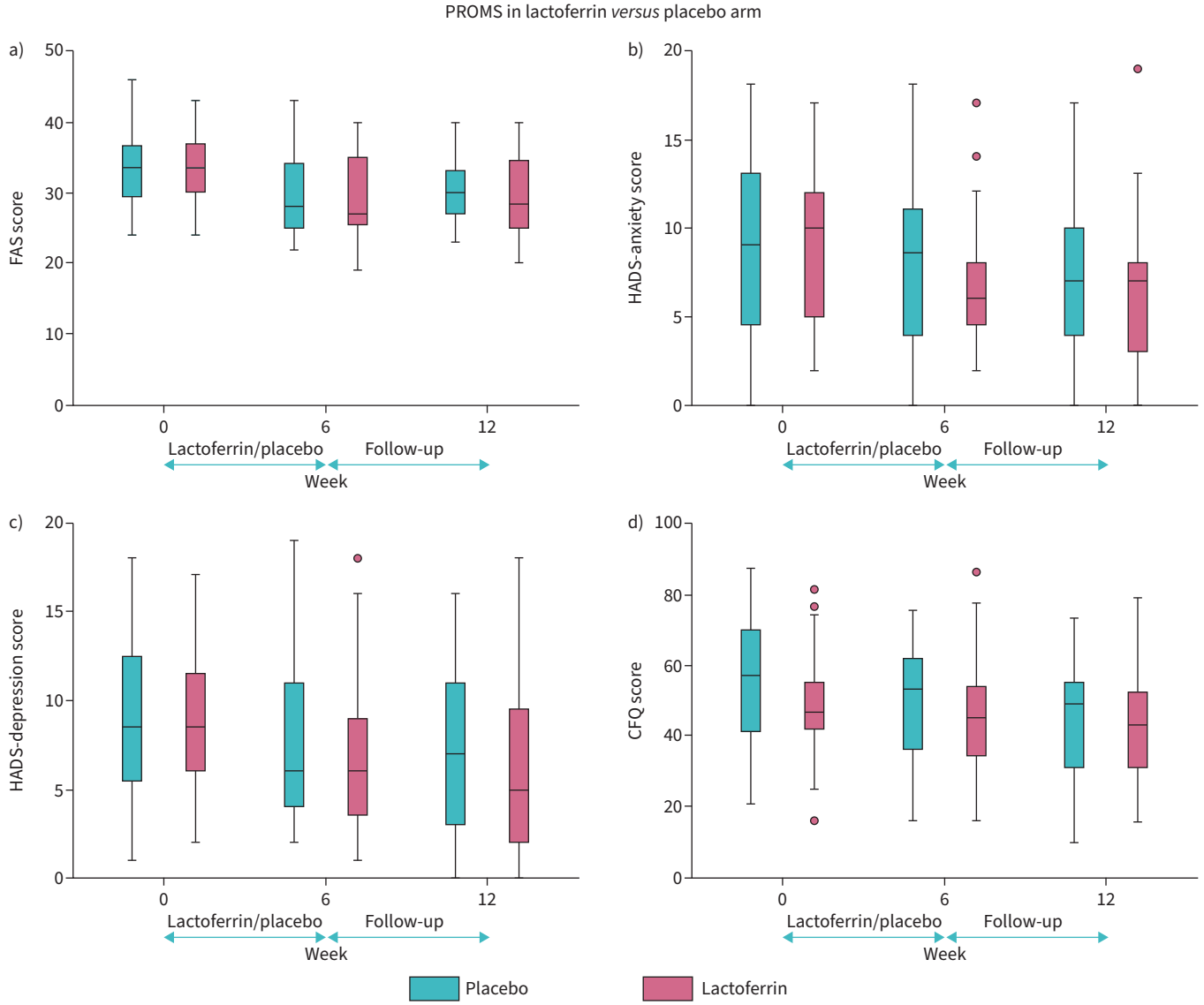

FIGURE 1 Patient-reported outcome measures (PROMs) assessed during the LARGO study at T0, T6 and T12 weeks, presented for the placebo arm and lactoferrin arm separately. a) Fatigue Assessment Scale (FAS), b) Hospital Anxiety and Depression Scale (HADS)-anxiety, c) HADS-depression, d) Cognitive Failure Questionnaire (CFQ). Variables are presented as boxplots, including median and interquartile range.

Secondary outcomes

PROMs

At T6, there was a significant improvement in the HADS-anxiety domain in the lactoferrin arm and the HADS-depression domain in the placebo arm, without significant differences between the groups (both p=1.0) (figure 1 and supplementary table S1).

There was no improvement for the CFQ score in either study arm between baseline and 6 weeks (lactoferrin p=1.0 and placebo p=0.19) and there was no significant difference between the two groups (figure 1 and supplementary table S1).

Physical functioning

The score for the 30-s sit-to-stand test only improved significantly after the use of placebo at T6 (p=0.001), without a significant difference between the two study groups.

At baseline, eight participants (11.1%) in the study scored below the reference value, adjusted for age and gender, for the handgrip strength test. The difference in number of participants who scored below the reference value was nonsignificant between the two groups (p=1.0). Overall, the handgrip strength test did not improve in the lactoferrin or placebo arm (figure 2 and supplementary table S1).

Laboratory parameters

Various general laboratory values were measured and there were no significant differences between T0 and T6 (supplementary table S2).

Follow-up outcomes 6–12 weeks

At T12, there was no significant difference between the study groups regarding the primary or any of the secondary outcomes. Regarding the trajectory T6–T12, there were no significant differences found within the lactoferrin arm for any of the outcomes. In the placebo arm, the CFQ score decreased between 6 and 12 weeks (p=0.013) (figure 2 and supplementary table S1).

Side effects

Both study arms reported possible side effects such as diarrhoea, nausea and rash. Eight lactoferrin users (22.2%) and nine placebo users (25.0%) reported possible side effects without significant differences between the arms (p=1.0).

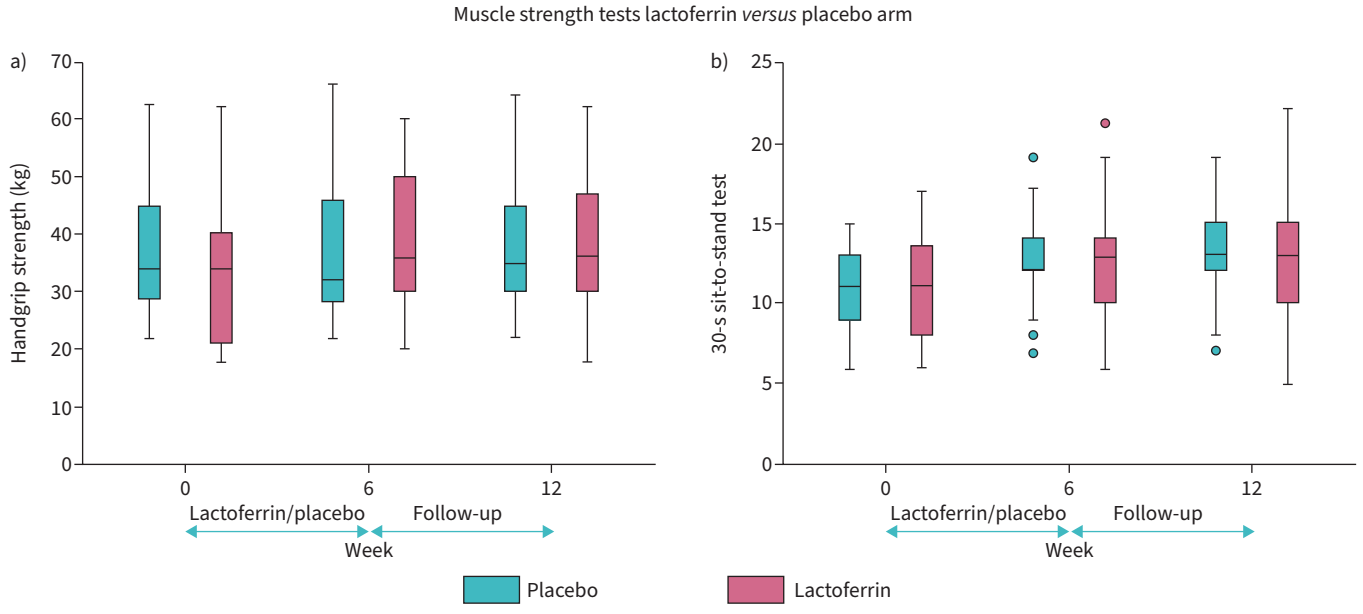

FIGURE 2 Muscle strength tests assessed during the LARGO study at T0, T6 and T12 weeks, presented for the placebo arm and lactoferrin arm separately. a) Handgrip strength and b) 30-s sit-to-stand test. Variables are presented as boxplots, including median and interquartile range.

Discussion

This is the first randomised, double-blind, placebo-controlled trial investigating the effect of lactoferrin on long COVID symptoms. The results showed an improvement in the first 6 weeks for fatigue in both study arms, for anxiety in the lactoferrin arm, and for depression and cognitive failure in the placebo arm. However, there were no significant differences in primary outcome or secondary outcomes between the placebo and lactoferrin arm, indicating no efficacy of lactoferrin on long COVID symptoms.

There is an unmet need among long COVID patients for effective treatment options, so many patients resort to trying out various treatments themselves. Many patients rely on supplements with promising health claims but unknown efficacy and significant overall costs. This study is relevant in this context, because we demonstrate the lack of efficacy of lactoferrin supplementation in long COVID.

Fatigue is the most frequently described long COVID symptom among our patients and in the literature [28]. A recent longitudinal study showed a natural decrease in fatigue symptoms between 6 weeks and 6 months after acute infection [29]. The FAS score in our study improved in both groups during the period of the study without a significant difference between the study arms at T6. Interestingly, the FAS score did not further improve after stopping the study medication and was unrelated to the time since COVID infection. This suggests that the initial decrease in fatigue symptoms during the first 6 weeks of active treatment may be due to a considerable placebo effect. This is relevant to consider when studying interventions in long COVID with such PROMs, and again stresses the need for controlled studies, even if this concerns a nonpharmaceutical intervention study.

Several other intervention studies, also using supplements, have been performed to improve the fatigue symptoms in long COVID patients. In a double-blind randomised controlled trial (RCT), patients with persisting symptoms for 4–87 days after acute COVID-19 infection were randomised to systemic probiotics or placebo. The recovery of fatigue symptoms was significantly greater in the intervention arm compared to the placebo arm. However, the duration of the symptoms at baseline was not similar to the duration of the long COVID definition in general and long COVID symptoms in our study [30].

A significant decrease in fatigue symptoms was also reported in a prospective cohort study comparing coenzyme Q10+α-lipoic acid *versus* no treatment. After 60 days of treatment, 53.5% of the participants in the intervention arm reported a complete response *versus* 3.5% in the control arm [31]. A well performed RCT comparing the efficacy of coenzyme Q10 for mitochondrial functioning in long COVID with placebo demonstrated a lack of efficacy on symptom burden [32].

There are conflicting results about the effect of vitamin D in acute COVID-19 infection and long COVID. Vitamin D may have beneficial effects in the acute phase but the evidence for long COVID is scarce [33].

In our study, we analysed the effect of symptom duration and the improvement of FAS score, because it is known that most natural recovery occurs in the first 6 months, slowing down thereafter [34]. Different SARS-CoV-2 variants were dominant during those periods, the Alpha variant in April 2021 and the Omicron variant in January 2022 [35]. There was no significant difference in the FAS score between the two peaks of positive PCR tests.

Overall, our results indicate improvement of several clinical outcomes during the 6 weeks of intervention (placebo or lactoferrin), but this improvement did not continue in the following 6 weeks. Participating in a study, undergoing additional diagnostic tests or receiving extra support during the study could have influenced the participants' symptoms (Hawthorne effect) [36]. A review indicated that the expectation of the patient is an important factor for placebo effect [37]. Both groups in our study might have had higher expectations for the first six intervention weeks and therefore experienced less fatigue during the intervention period.

The most important strength of this study is the double-blind RCT design. This study was the first to date to analyse the effect of lactoferrin in long COVID patients, adding to the current limited body of literature regarding efficacy of interventions for long COVID. Regarding limitations, participants subsequently or simultaneously underwent several other treatments, such as physiotherapy. We cannot rule out that any of these treatments could have influenced the results. Also, we do not have any measurements before the participants developed long COVID and so we do not know whether any previous complaints were already present and perhaps long lasting. Finally, we are not certain that the dose, frequency and duration of the treatment, although based on the available literature, would be sufficient to induce a clinical effect on long COVID several months after the initial infection. Small variations between the study arms might be

significant in a study with a greater sample size. Furthermore, differences might be significant in a RCT design with a longer intervention and follow-up duration including more frequent measurements.

Conclusion

Fatigue improved in long COVID patients during the first 6 weeks of this study, without a significant difference between treatment with placebo and lactoferrin treatment. This suggests that the observed improvement could be due to Hawthorne or placebo effect. Because most patients with long COVID have a strong unmet urge for medical treatment, future trials must have a robust study design including randomisation and blinding of treatment because this is pivotal to prevent patients from undertaking expensive and unnecessary treatments.

Provenance: Submitted article, peer reviewed.

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This study is registered at https://www.onderzoekmetmensen.nl with identifier number NL9742. Individual participant data will be available (including data dictionary) after deidentification, as well as the study protocol, informed consent form and study report, immediately following publication, with no end date, for anyone who wishes to access the data, for any purpose, through https://onderzoekmetmensen.nl/nl/trial/21473.

Ethics statement: The approval of an ethics committee was required due to the study design (intervention with a study product) and study procedures (performing questionnaires, muscle strength tests and blood draw).

Conflict of interest: A-L. Redel and F. Miry have nothing to disclose. M.E. Hellemons reports honoraria for lectures and consultancy from Boehringer Ingelheim, Pfizer and Takeda, unrelated to this study; and is an associate editor of this journal. L.M.A. Oswald reports honoraria for lectures from Stichting Medische Opleidingen. G.J. Braunstahl reports support for the present manuscript from Bonusan B.V.; honoraria for lectures and consultancy from GSK, AstraZeneca, Novartis and Sanofi Genzyme; and research grants from Sanofi Genzyme, GSK and AstraZeneca, not related to this study; and is Chairman of the NVALT Asthma Section, Secretary of the ERS Task Force on Allergy and Immunology and on the Scientific Advisory Board of Longfonds.

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