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# Oral Reduced L-Glutathione Improves Growth in Pediatric Cystic Fibrosis Patients

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## ABSTRACT

**Background and Objective:** Consensus nutritional guidelines for patients with cystic fibrosis (CF) recommend aggressive treatment of growth failure. Oral reduced glutathione (GSH) has been shown to improve cachexia and case reports have demonstrated improved growth in pediatric patients with CF. No controlled studies using oral GSH in CF have, however, been reported. The aim of the study was to determine whether oral GSH could improve growth in CF. Secondarily, to determine whether oral GSH could improve other systemic clinical markers.

**Methods:** We performed a placebo-controlled, randomized, double-blind, repeated-measures clinical trial in 44 pediatric patients with CF ages 18 months to 10 years. Primary outcomes were change in weight percentile, body mass index (BMI) percentile, height percentile, and fecal calprotectin. Secondary outcomes included liver function tests and measures of systemic inflammation. Each participant was studied for 6 months, with data obtained at baseline, 3 months, and 6 months. Blood samples were obtained on the baseline and 6-month visits. Subjects were treated with oral GSH or placebo (calcium citrate), each $65\,mg \cdot kg^{-1} \cdot day^{-1}$ divided into 3 doses per day at mealtimes, and administered daily for 6 months.

**Results:** The GSH treatment group gained an average of 0.67 standard deviation (SD) in weight-for-age-and sex $z$ score (wfaszs), (19.1 weight percentile points) during the course of 6 months, with no adverse effects (vs placebo with an increase of 0.1 SD in wfaszs [2.1 weight percentile points], $P < 0.0001$). Fecal calprotectin improved, GSH −52.0 vs placebo 0.5), also BMI for GSH improved 0.69 SD BMI-adjusted-for-age-and-sex $z$ score versus placebo 0.22 SD (BMI percentile 21.7 GSH vs 5.2 placebo), and height 0.2 SD in height-for-age-and-sex $z$ score (hfaszs) GSH versus −0.06 SD hfaszs placebo [height percentile 7.0 GSH vs −2.6 placebo], all $P < 0.0001$). Secondary outcomes improved significantly, as well.

**Conclusions:** Oral reduced L-GSH significantly improves measures of growth status and gut inflammation in CF.


**Key Words:** body mass index, cachexia, calprotectin, cystic fibrosis, glutathione, nutritional status

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*www.clinicaltrials.gov* registration number: NCT02029521. The trial was registered also in Italy with Federazione Italiana Medici Pediatri (Verbano Cusio Ossola) number FIMP/clin.stud/2010/1.

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### What Is Known

- Malnutrition affects a majority of children with cystic fibrosis (CF) who are pancreatic insufficient.
- Lung function scores in CF correlate strongly with nutritional status.
- CF causes significantly reduced efflux of glutathione (GSH) from most cells.
- GSH is a Food and Drug Administration–approved treatment for AIDS-related cachexia.

### What Is New

- A total of 44 CF children entered a randomized, double-blind, placebo-controlled, 6-month trial of oral GSH.
- GSH subjects increased on average 0.67 SD in weight-for-age $z$ score, whereas the placebo group increased on average 0.1 SD in weight-for-age $z$ score ($P < 0.0001$). Body mass index, fecal calprotectin, height, and secondary outcomes also improved significantly with treatment.

Cystic fibrosis (CF) is known principally for its pulmonary consequences. For most individuals with CF, the earliest manifestations are, however, not pulmonary, but gastrointestinal (GI). Many children experience growth failure. Chronic gut inflammation also develops (1). Research has also established that lung function scores are significantly correlated with body mass index (BMI) and weight percentile in CF (2–6). Therefore, interventions to improve the GI dimension of CF in early childhood have the potential to ameliorate the course of the disease during the life span of the patient. Both Cochrane Database reviews and a review commissioned by the Cystic Fibrosis Foundation found only fair evidence for present nutritional treatments (7–9). Therefore, there is a pressing need for a treatment for CF growth failure that is demonstrated to be effective and less invasive than present treatments.

The discovery that CF is associated with significantly diminished efflux of reduced glutathione (GSH) from most cells in the body (10–15) offers a new perspective on the pathophysiology of this disease. GSH plays several important roles; among the most important are the following: primary water-soluble antioxidant, mucolytic capable of cleaving disulfide bonds, and regulator of immune system function (12). The relation between redox ratio (GSH:GSSG) and total GSH (GSH+GSSG) and the initiation of inflammation is well established in the research literature (16–18).

GSH is also an important component of the epithelial lining fluid of the intestines, helping to keep intestinal mucus thin, serving to defend the intestinal system against reactive oxygen species, and keeping inflammation in check under normal circumstances (19–21). GSH is a Food and Drug Administration–approved treatment for acquired immunodeficiency syndrome (AIDS)–related cachexia (22). The growing recognition of GSH system dysfunction in CF, coupled with an established research literature on the role of GSH in GI function and weight gain in non-CF contexts, suggest that oral GSH may effectively treat CF growth failure in pediatric patients.

## METHODS

An age-stratified, placebo-controlled, double-blind, repeated-measures clinical trial was approved by the Comitato Etico of ASL TO3, Turin, Italy (November 18, 2010). To be included in the trial, pediatric patients needed to meet the following 3 criteria: have CF as measured by >60 sweat chloride test or paired deleterious DNA CFTR mutations (Ambry Genetics, Genentech, or ARUP), be pancreatic insufficient as defined by CF clinic prescription of pancreatic enzymes, and be between 18 months and 10 years of age at time of enrollment. Patients were not eligible for the trial if they had any 1 of the following 4 conditions: been hospitalized for bowel obstruction or surgery during the 6 months before enrollment, had a pulmonary exacerbation or oral steroid use or IV antibiotics within 1 month before enrollment, had been taking either GSH or *N*-acetyl cysteine in the 12 months before the trial, or were infected with *Burkholderia cepacia*. A screening visit for each subject was conducted 1 month before intervention to facilitate confirmation of the study eligibility.

Patients were recruited through an Internet CF group in Italy. Enrollment of patients began in November 2010 and continued until November 2011. Forty-seven patients were enrolled in the study: 24 in the treatment group and 23 in the placebo (Fig. 1). Patients were stratified by age group and then randomly assigned to the treatment and placebo groups. Placebo and GSH materials were encapsulated and identical in appearance. The containers were labeled "A" or "B" by the pharmaceutical supplier, thus blinding the study physician (principal investigator, PI), the clinic staff, and the research study team to their contents. There were equal numbers of patients within 2 age strata (1.5–3 years, 4–10 years) in each group. Patients were also blind to the treatment they were receiving. The blind was removed only after the trial had concluded and data analysis had begun. All of the parents signed informed consent and

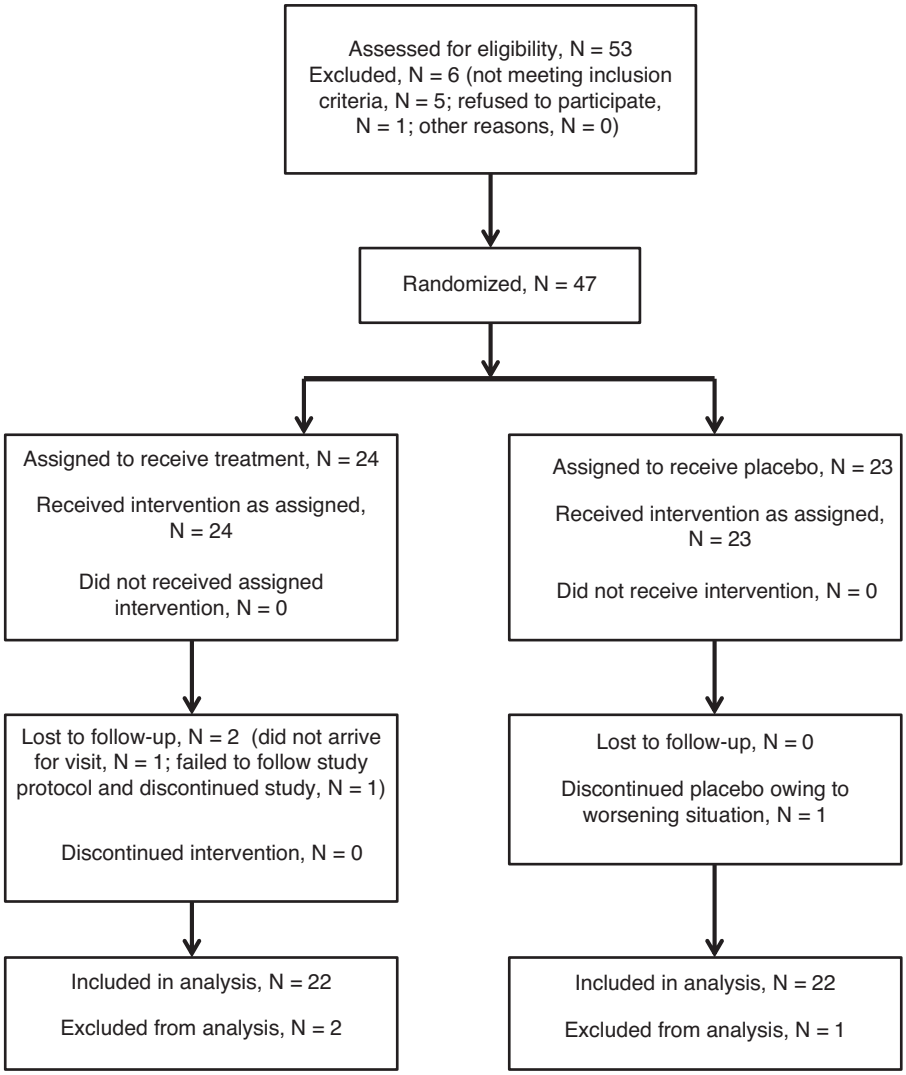


**FIGURE 1.** Patient flow Consolidated Standards of Reporting Trials chart.

in addition patients age ≥6 years provided oral assent after an age-appropriate version of the consent document was read to them.

## Specifics of the Research Design

Each patient was enrolled in the trial for 7 months. During the first month, patients were monitored for growth and health stability. Patients were considered stable if there was no deterioration in clinical status during this month as defined by the exclusion criteria of the study. Month 2 of the trial is considered baseline, that is, when initial outcome measures were taken, randomization occurred, and treatments (GSH or placebo) were started.

At baseline, basic demographic variables were taken (age and sex) as well as standard CF characteristics: sweat chloride test result, genetic mutations, present daily lipase units prescribed, and present daily treatments prescribed. All of the primary and secondary outcomes were also measured. Parents were given a journal in which they were instructed to keep a daily record of whether their child took their assigned dose, any change in their child's CF treatment made by their child's regular CF doctor, and any adverse effects their child experienced. Parents were instructed to contact the study physician (PI) if they experienced any adverse effects.

Patients were seen at the study clinic (center) by the study physician (PI) at 3 and 6 months after baseline. At the 3-month visit, patients' weight and height were measured. At the 6-month visit, all of the primary and secondary outcomes were measured, daily journal records were collected, and patients discontinued their treatment.

The study center was the professional office of Alfredo Visca, MD, in Turin, Italy, and the trial began in November 2010. All of the laboratory tests of blood and feces were performed at Azienda Ospedaliera O.I.R.M.-S.Anna, Turin, Italy, and weight and height were obtained at the study center.

## Instruments

Spirometric data were obtained using a micro loop pneumotachograph (Micro Medical Limited, Rhymney, UK), and predicted normal values for spirometric data were obtained from Peterson (2). For percentile values on weight, height, and BMI, *http://www.infantchart.com/child/*, accessed on June 2014, was used. We used World Health Organization growth charts for children age <2 years and Centers for Disease Control and Prevention growth charts for children age ≥2 years. Weights were obtained using Kern scales (model MPB300K100P) and Seca scales. Weights were taken by the same observer (A.V.) and rounded to the nearest 500 g using standard rounding techniques. Heights were measured to the nearest 0.01 m.

## Outcome Measures

The study has 4 primary growth outcomes: weight, height, and BMI converted to age- and sex-adjusted percentiles, and fecal calprotectin levels. Weight (kg) and height (m) were measured at every clinic visit, and a patient's BMI was calculated for patients age ≥2 years. Calprotectin reflects gut inflammation (23,24) and inflammation could impair intestinal function, and thus retards growth. Calprotectin levels (μg/dL) were assessed from patients' stool samples at baseline and 6 months. The study also has several secondary outcomes that are either related to GI symptoms or pertinent to CF pathophysiology.

The primary outcomes were weight-for-age-and-sex $z$ score (wfaszs) and weight percentile, height-for-age-and-sex $z$ score (hfaszs) and height percentile, BMI $z$ score adjusted for age and sex and BMI percentile for patients at least 2 years old, and calprotectin levels (fecal) (mg/dL). The secondary outcomes were white blood cell (WBC) count (1000/mm$^3$), alanine transaminase (ALT) (U/L), vitamin E level (mg/mL), and C-reactive protein (CRP) (mg/L).

## Dosage Protocol

A previous observational study showed that children with CF grew rapidly after starting to take GSH, 65 mg · kg$^{-1}$ · day$^{-1}$ (25). Thus, treatment for this study was pharmaceutical-grade reduced L-GSH with a daily dose of 65 mg/kg. The placebo was calcium citrate with a daily dose of 65 mg/kg. The daily dose of each substance was divided into 3 doses given at mealtime by the parents. For subjects too young to swallow capsules, the content of each capsule was sprinkled on food at mealtimes. Neither the protocol nor the methods/measures changed after the commencement of the trial.

## Sample Characteristics

### *Size*

Using data from a published study that included several patients who met our inclusion criteria (25), a power and sample size analysis indicated that a minimum of 22 patients in each group should be enrolled in the study. This sample size would accommodate a 10% attrition rate and allow for the detection of an effect size of 8 percentage points with 90% power. An effect size of 8% is substantially smaller than that observed in the relevant patients in the previously published study; however, the effect size in the proposed study could be smaller owing to the possibility of noncompliance and the unknown change in weight percentile in the control group during the 6-month period. This effect size would also be clinically relevant.

### *Age*

For each treatment group, we enrolled a minimum of 6 patients in the younger age group (ages 18 months to 3 years), and a minimum of 16 patients in the older age group (ages >3 years). This was done to ensure age comparability of the treatment and placebo groups.

## Interim Analysis

No interim analysis was conducted during the course of the trial.

## Statistical Analysis

All of the primary outcomes were analyzed using a repeated-measures mixed-model adjusted for both sex and age.

# RESULTS

## Baseline Characteristics

Table 1 provides a description of the baseline characteristics of the GSH and placebo groups. At baseline, the 2 groups are statistically similar on all characteristics except fecal calprotectin. Despite randomization, the GSH group had a significantly higher average level of fecal calprotectin than the placebo group ($P = 0.008$). Consistent with the overall clinical situation of patients with CF, mean weight and height percentiles for both groups are substantially <50th percentile.

The 2 groups' concomitant maintenance therapy profiles were similar at baseline. All of the patients were receiving typical CF maintenance therapy (eg, enzymes, hypertonic saline,

TABLE 1. Comparison of baseline characteristics of patients in the 2 treatment groups

| Characteristic | GSH (n = 22) mean (SD) | Placebo (n = 22) mean (SD) | *P* (2-sample *t* test) |
|---|---|---|---|
| Age, mo | 67.3 (29.8) | 66.9 (32.4) | 0.965 |
| Weight (*z* score) | −0.84 (0.51) | −0.58 (0.43) | 0.075 |
| Weight (percentile) | 24.1 (13.7) | 29.7 (13.2) | 0.172 |
| Height (*z* score) | −0.37 (0.40) | −0.30 (0.37) | 0.550 |
| Height (percentile) | 36.3 (14.4) | 39.1 (13.5) | 0.516 |
| BMI* (*z* score) | −0.76 (0.56) | −0.57 (0.54) | 0.259 |
| BMI* (percentile) | 25.4 (15.9) | 31.1 (14.1) | 0.242 |
| Fecal calprotectin, mL/dL | 113.2 (52.5) | 76.1 (32.2) | 0.008 |
| WBCs | 9.1 (2.0) | 8.9 (2.7) | 0.837 |
| ALT | 23.2 (9.3) | 22.6 (11.4) | 0.852 |
| Vitamin E | 7.7 (1.5) | 7.5 (1.6) | 0.703 |
| CRP | 13.3 (7.7) | 12.1 (6.6) | 0.599 |
| Sweat chloride | 87.7 (15.8) | 85.0 (15.6) | 0.574 |
| | Count (%) | Count (%) | |
| Female | 14 (64%) | 10 (45%) | 0.226 |
| *Pseudomonas aeruginosa* | 8 (36%) | 9 (41%) | 0.757 |

A *P* value for a 2-sample unpaired *t* test is included for a test of no difference in the groups. ALT = alanine transaminase; BMI = body mass index; CRP = C-reactive protein; GSH = glutathione; SD = standard deviation; WBC = white blood cell.
* n = 20 in both GSH and placebo groups. Excluded subjects were too young to calculate BMI.

bronchodilator, vitamins). In addition, 5 (23%) of the GSH patients and 8 (36%) of the placebo group were also taking antibiotics prophylactically. Full data are available at *clinicaltrials.gov* (#NCT02029521) and also at the link *http://uvicf.org/researchnewsite/glutathionenewsite/ViscaTrial_Data_and_SupplementaryMaterial.html* on these ancillary variables. The prevalence of positive *Pseudomonas aeruginosa* did not differ significantly at baseline between the 2 groups ($P = 0.757$, see Table 1).

## Treatment Effects

Both treatment and placebo were well tolerated, and no compliance issues surfaced. On the basis of the repeated-measures mixed-model analyses, patients in the GSH group showed significantly improved results ($P < 0.0001$) on a repeated-measures analysis of variance compared with the placebo group on all 4 primary outcome measures (Table 2).

TABLE 2. Results from repeated-measures mixed-models of primary outcomes

| Primary outcome | Baseline, mean (SD) | 3 mo, mean (SD) | 6 mo, mean (SD) | *P* (*F* test) |
|---|---|---|---|---|
| Weight *z* score | | | | |
| GSH (n = 22) | −0.84 (0.51) | −0.45 (0.39) | −0.17 (0.32) | 0.0001 |
| Placebo (n = 22) | −0.58 (0.43) | −0.51 (0.42) | −0.48 (0.40) | |
| Weight percentile | | | | |
| GSH (n = 22) | 24.1 (13.7) | 33.5 (14.9) | 43.2 (12.3) | 0.0001 |
| Placebo (n = 22) | 29.7 (13.2) | 31.0 (13.4) | 31.8 (14.0) | |
| Height *z* score | | | | |
| GSH (n = 22) | −0.37 (0.40) | −0.28 (0.31) | −0.17 (0.32) | 0.0001 |
| Placebo (n = 22) | −0.30 (0.37) | −0.32 (0.38) | −0.36 (0.36) | |
| Height percentile | | | | |
| GSH (n = 22) | 36.3 (14.4) | 39.3 (11.9) | 43.3 (12.5) | 0.0001 |
| Placebo (n = 22) | 39.1 (13.5) | 38.0 (13.8) | 36.5 (13.2) | |
| BMI *z* score* | | | | |
| GSH (n = 20) | −0.76 (0.56) | −0.39 (0.52) | −0.07 (0.38) | 0.0001 |
| Placebo (n = 20) | −0.57 (0.54) | −0.41 (0.40) | −0.35 (0.43) | |
| BMI percentile* | | | | |
| GSH (n = 20) | 25.4 (15.9) | 37.1 (18.4) | 47.5 (14.0) | 0.0001 |
| Placebo (n = 20) | 31.1 (14.1) | 33.6 (14.6) | 36.0 (17.0) | |
| Calprotectin, mL/dL | | | | |
| GSH (n = 22) | 113.2 (52.5) | Not collected | 61.2 (26.4) | 0.0001 |
| Placebo (n = 22) | 76.1 (32.2) | Not collected | 76.6 (30.7) | |

*F* test is from repeated-measures analyses comparing treatment groups over time adjusting for age and sex. BMI = body mass index; GSH = glutathione; SD = standard deviation.
* BMI could not be calculated for patients age <24 months.

### Weight Percentile

There was a statistically significant difference in growth as measured by $z$ score and percentile during the 6-month period between the GSH and placebo groups ($P < 0.0001$). At baseline, the GSH group had a mean wfaszs of −0.84 (weight percentile of 24.1), which is not statistically different from the mean wfaszs of −0.58 (weight percentile of 29.7) in the placebo group. At 3 months, the mean weight $z$ scores in the 2 groups were similar: mean wfaszs of −0.45 in the GSH group and −0.51 in the placebo group (weight percentile 33.5 in GSH and 31.0 in placebo.) At 6 months, however, the GSH mean wfaszs of −0.17 was significantly higher than the unchanged mean wfaszs of −0.48 in the placebo group. Weight percentiles after 6 months were 43.2 in the GSH group and 31.8 in the placebo group. For a 6-month period, the GSH subjects increased 0.67 standard deviation (SD) in wfaszs (19.1 weight percentile points), whereas the placebo group increased on average 0.1 SD in wfaszs (2.1 weight percentile points). SDs are provided in Table 2, and visualizations are provided in Figure 2.

### Height Percentile

There was a statistically significant difference in growth as measured by hfaszs and height percentiles during the 6-month period between the GSH and placebo groups ($P < 0.0001$). At baseline, the GSH group had a mean hfaszs of −0.37 (height percentile of 36.3), which was not statistically different from the mean hfaszs of −0.30 (height percentile of 39.1) in the placebo group. At 3 months, the mean hfaszs was −0.28 (height percentile 39.3) in the GSH group and the mean hfaszs was −0.32 (height percentile 38.0) in the placebo group. At 6 months, however, the GSH mean hfaszs was −0.17 (height percentile of 43.3), which was significantly higher than the mean hfaszs of −0.36 (height percentile of 36.5) in the placebo group. For a 6-month period, the GSH subjects increased on average 0.2 SD in hfaszs (7.0 height percentile points), whereas the placebo group decreased on average −0.06 SD in hfaszs (−2.6 percentile points.) SDs are provided in Table 2, and visualizations are provided in Figure 3.

### BMI Percentile

There was a statistically significant difference in growth as measured by BMI percentiles during the 6-month period between the GSH and placebo groups ($P < 0.0001$). At baseline, the GSH group had a mean BMI-for-age-and-sex $z$ score of −0.76 (percentile of 25.4), which was not statistically different from the average BMI-for-age-and-sex $z$ score of −0.57 (percentile of 31.1) in the placebo group. At 3 months, the mean BMI-adjusted-for-age-and-sex $z$ scores were −0.39 in the GSH group and −0.41 in the placebo group (BMI percentiles in the 2 groups were 36.6 in the GSH group and 34.2 in the placebo group). At 6 months, however, the GSH average BMI-adjusted-for-age-and-sex $z$ score was −0.07 (BMI percentile of 47.1), which was significantly higher than the mean BMI-for-age-and-sex $z$ score of −0.36 (BMI percentile of 36.3) in the placebo group. For a 6-month period, the GSH subjects increased on mean 0.69 SD in BMI age and sex-adjusted $z$ score (21.7 percentile points), whereas the placebo group increased on average 0.22 SD in adjusted BMI $z$ score (5.2 BMI percentile points). SDs are provided in Table 2, and visualizations are provided in Figure 4.

### Fecal Calprotectin Levels

There was a statistically significant difference in fecal calprotectin levels, a measure of gut inflammation, during the 6-month period between the GSH and placebo groups ($P < 0.0001$). The subjects in the GSH group dropped an average of 52 points, whereas the subjects in the placebo group remained essentially unchanged (0.5). SDs are provided in Table 2, and visualizations are provided in Figure 5. The concluding mean values of 61.2 GSH versus 76.6 placebo were not statistically significantly different ($P = 0.08$).

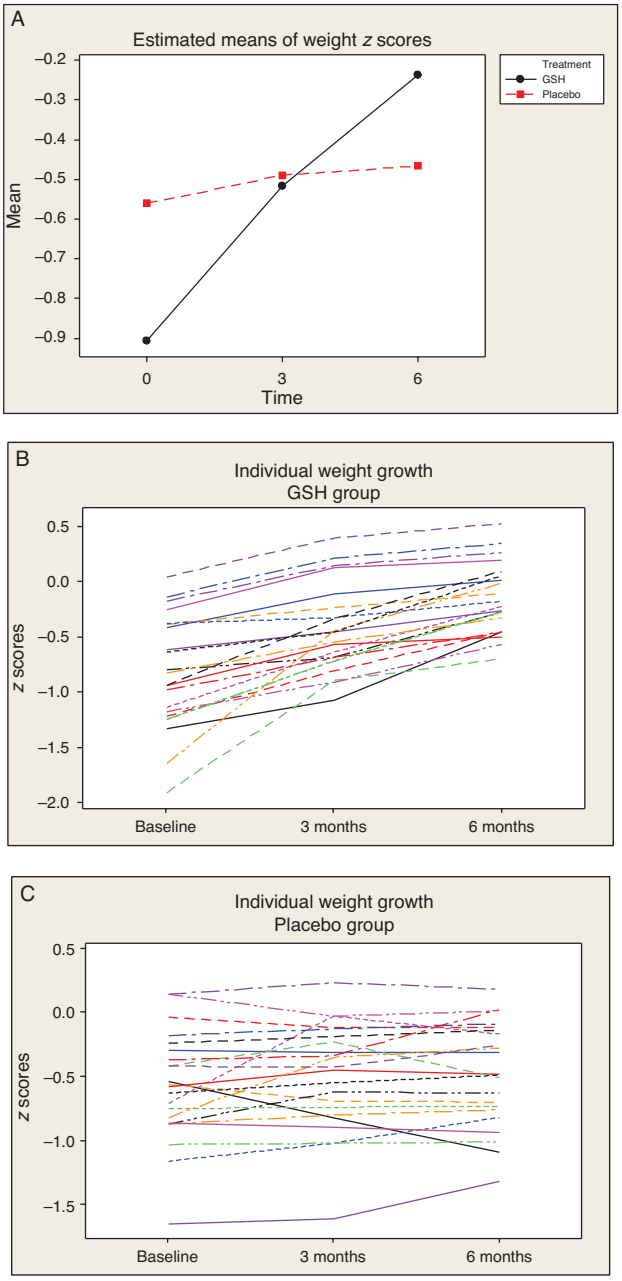


**FIGURE 2.** Change over time in weight $z$ score: (A) comparison means, (B) GSH treatment group patients, (C) placebo group patients. There is a significant difference ($P < 0.0001$) in weight $z$ scores between the GSH and placebo group during the 6 months. The GSH group had an average increase in their $z$ scores of 0.67, whereas the placebo group's average increase was 0.10. GSH = glutathione.

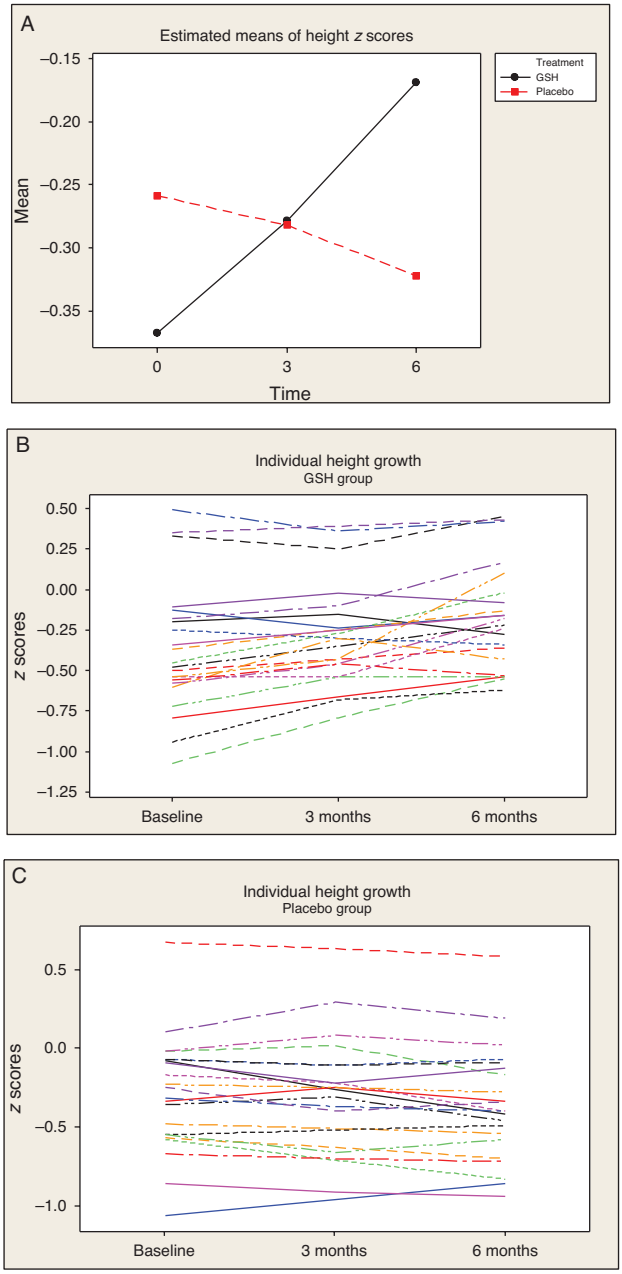


**FIGURE 3.** Change over time in height percentile: (A) comparison means, (B) GSH treatment group patients, (C) placebo group patients. There is a significant difference ($P < 0.0001$) in height *z* scores between the GSH and placebo group during the 6 months. The GSH group had an average increase in *z* score of 0.20, whereas the placebo group had an average decline of 0.06. GSH = glutathione.

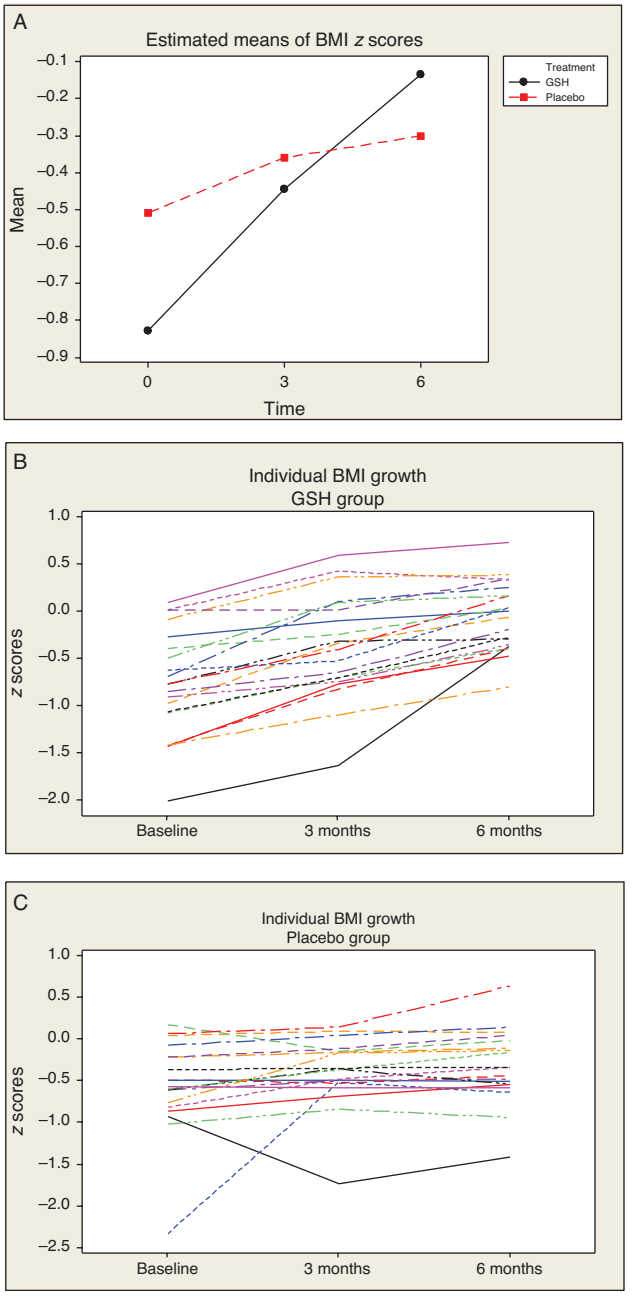


**FIGURE 4.** Change over time in BMI percentile: (A) comparison means, (B) GSH treatment group patients, (C) placebo group patients. There is a significant difference ($P < 0.0001$) in BMI *z* scores between the GSH and placebo group during the 6-month trial period. The GSH group had an average increase in BMI of 0.69, whereas the placebo group had an average increase of 0.22. BMI = body mass index; GSH = glutathione.

## *Secondary Outcomes*

Blood work was not performed at the 3-month time period. The average change (6 month − baseline) in secondary outcomes are reported in Table 3. The changes between the GSH and placebo groups are compared using 2-sample *t* tests. Corroborating the finding of a significant decrease in the primary outcome of fecal calprotectin level as a marker of inflammation, the results from WBC and CRP for the GSH treatment group demonstrated a statistically significant decrease over the placebo group (WBC: −0.7 GSH vs 0.6 placebo, CRP: −2.6 GSH vs 2.6 placebo, for both $P < 0.0001$). Ninety-five percent confidence intervals (CIs) for the difference in the change for each measure are also presented in Table 3.

Mean ALT levels decreased in the GSH treatment group by −5.1 units, whereas mean ALT levels in the placebo group increased by 3.2 units during the 6-month period of the trial

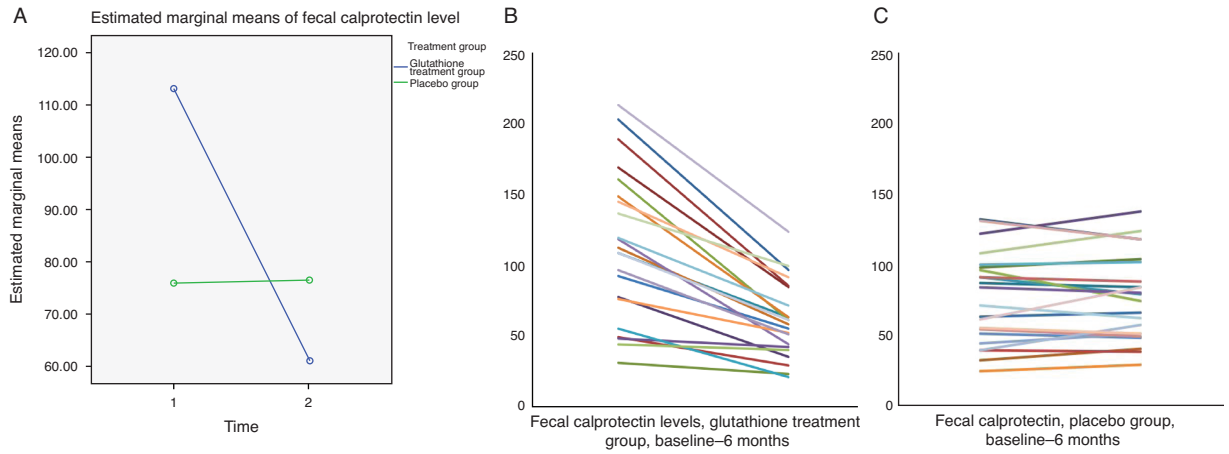


**FIGURE 5.** Change over time in fecal calprotectin levels: (A) comparison means, (B) GSH treatment group patients, (C) placebo group patients. GSH = glutathione.

($P < 0.0001$). Ninety-five percent CIs for the difference are presented in Table 3.

Mean vitamin E levels in the GSH treatment group increased by 0.9 units, whereas in the placebo group mean vitamin E levels dropped by −0.8 units ($P < 0.0001$). Ninety-five percent CIs for the difference are presented in Table 3.

## Adverse Effects

Although 1 patient in the placebo group chose to discontinue the study shortly after it began because of a pulmonary exacerbation requiring hospitalization, no other adverse events were noted in either the treatment or the placebo groups. Bacterial cultures were obtained from swab or sputum at the 3 time points; patients in the treatment group showed no worsening in the pathogenicity or number of bacterial species cultured. Full results on bacterial cultures are available at clinicaltrials.gov and also at the link *http://uvicf.org/researchnewsite/glutathionenewsite/ViscaTrial_Data_and_SupplementaryMaterial.html*.

No patient in the GSH group worsened on any of 11 subjective measures of GI symptoms during the course of the trial, according to the self-reported qualitative symptomatology assessment performed by each patient/parent, and there was a statistically significant trend toward the improvement in these symptoms in the GSH group over time compared with the placebo group, except for ''nausea, heart burn, and <2 bowel movements per week'' (Table 4).

# DISCUSSION

There have been mixed results from the use of inhaled reduced GSH in patients with CF (25–28). There has, however, never been a randomized clinical trial of oral reduced GSH in CF (although there has been 1 small observational study (25)). In addition, previous trials of inhaled GSH have not focused on changes in nutritional status.

The trial design contained some limitations: it was performed at a single center in 1 nation, subjects only between the ages of 18 months and 10 years were included, the study population was only 44 persons, and the trial concluded at 6 months while improvements were still being seen. The participants were presumed to be pancreatic insufficient based on the CF diagnosis and a physician prescription for enzymes. We, however, performed no independent testing to confirm the diagnosis. We do know that 41 of the 44 participants had weight percentiles <50th percentile. Furthermore, 41 of the 44 participants had symptoms such as gas, bloating, frequent stools, and poor appetite, suggestive of pancreatic insufficiency. Participants were given the option of sprinkling the contents of the capsule on their food at mealtime. We did not record how many participants sprinkled the capsule contents rather than swallowed the capsules. Although the GSH and calcium citrate smell differently, neither group knew which odor was associated with which substance. Opening capsules to sprinkle on food should thus have had minimal impact on blinding.

Despite randomization, the GSH group had significantly higher fecal calprotectin at baseline. At the end of 6 months, the fecal calprotectin levels were not significantly different between treatment and placebo groups ($P = 0.08$). Oral GSH may primarily be beneficial in those children with more severe inflammation of the gut, although further study would be necessary to confirm this.

Although differences in WBC, ALT, and vitamin E levels between treatment and placebo groups were statistically significantly different, it is not clear whether there is a meaningful clinical difference between these values because all mean values fell within

TABLE 3. Results from analysis of the secondary outcomes

| Secondary outcome | GSH group 6-mo change, mean (SD) | Placebo group 6-mo change, mean (SD) | *P* 2-sample *t* test | 95% CI for difference |
|---|---|---|---|---|
| WBCs | −0.66 (0.69) | 0.62 (0.71) | 0.0001 | −1.7 to −0.9 |
| ALT | −5.1 (3.6) | 3.2 (4.1) | 0.0001 | −10.6 to −6.0 |
| Vitamin E | 0.89 (0.5) | −0.75 (0.53) | 0.0001 | 1.3–1.9 |
| CRP | −2.6 (3.1) | 2.6 (2.9) | 0.0001 | −7.0 to −3.4 |

ALT = alanine transaminase; CI = confidence interval; CRP = C-reactive protein; GSH = glutathione; SD = standard deviation; WBC = white blood cell.

TABLE 4. Changes in self-reported GI symptomatology from baseline to 6 months

| Symptom | Patients who reported improved, % | Patients who reported no change, % | Patients who reported worsened, % | $P$ for $\chi^2$ test of association |
|---|---|---|---|---|
| Abdominal pain | | | | |
| GSH | 55 | 45 | 0 | 0.013 |
| Placebo | 18 | 64 | 18 | |
| Belching | | | | |
| GSH | 32 | 68 | 0 | 0.002 |
| Placebo | 0 | 77 | 23 | |
| Flatulence | | | | |
| GSH | 59 | 41 | 0 | 0.001 |
| Placebo | 9 | 77 | 14 | |
| Lack of appetite | | | | |
| GSH | 91 | 9 | 0 | 0.001 |
| Placebo | 18 | 59 | 23 | |
| Bloating | | | | |
| GSH | 55 | 45 | 0 | 0.001 |
| Placebo | 5 | 82 | 14 | |
| Nausea | | | | |
| GSH | 23 | 77 | 0 | 0.117 |
| Placebo | 9 | 77 | 14 | |
| Vomiting | | | | |
| GSH | 23 | 77 | 0 | 0.036 |
| Placebo | 5 | 77 | 18 | |
| Heartburn | | | | |
| GSH | 18 | 82 | 0 | 0.154 |
| Placebo | 5 | 91 | 5 | |
| Diarrhea | | | | |
| GSH | 59 | 41 | 0 | 0.007 |
| Placebo | 18 | 64 | 18 | |
| More than 2 BM daily | | | | |
| GSH | 73 | 27 | 0 | 0.001 |
| Placebo | 18 | 64 | 18 | |
| Less than 2 BM weekly | | | | |
| GSH | 18 | 82 | 0 | 0.38 |
| Placebo | 9 | 86 | 5 | |

BM = bowel movements; GI = gastrointestinal; GSH = glutathione.

a normal range. Some have suggested that GSH transport dysfunction in CF leads to a compensatory exhaustion of other antioxidant stores in the body (29). In this context, it is noteworthy that during the 6 months of the clinical trial, mean vitamin E levels increased in the treatment group, but fell in the placebo group ($P < 0.0001$).

Clearly, there are many questions that remain to be answered with additional and larger studies. We did not determine the effect plateau nor did we study different doses. Indeed the dose used in the present study was one that had been used in a previous observational study for which an effect had been seen in a small number of patients. The optimal dose has not been determined by this or any other study. In addition, a validated quality-of-life index should also be included in future studies.

The trial design did, however, control for sources of bias through randomization, placebo control, and double blinding, offering a high level of external validity and thus generalizability to the subpopulation of pediatric patients with CF. Previously noted differences in the treatment and placebo groups should not introduce bias because the placebo group showed little to no improvement, whereas the treatment group showed substantial improvement during the study period. Moreover, the GSH-treated group surpassed the placebo group in every measure with a high degree of statistical significance.

GSH (L-gamma-glutamyl-L-cysteinylglycine) is a tripeptide found in all eukaryotic cells. GSH is ubiquitous and a normal diet would provide approximately 100 to 250 mg/day (30). Oral GSH has no known toxicity (31,32), although the extant studies do not examine pediatric subpopulations. Furukawa et al note, ''Oral administration even of relatively high levels of GSH has been demonstrated to be safe and without adverse effects'' (33). As noted previously, oral GSH is a Food and Drug Administration–approved treatment for AIDS-related cachexia (22).

At the same time, there is a pressing need for safe, effective, and noninvasive techniques to augment weight gain in children with CF. The improvement in weight gain in pediatric patients using oral GSH, in this study, suggests a promising new approach to the treatment of CF growth failure. Early intervention in young children with CF with growth failure could forestall decline in pulmonary function in later years.

There also appear to be ancillary benefits from GSH to pediatric patients with CF, including a significant reduction in GI inflammation as measured by fecal calprotectin levels, CRP levels, WBC levels, vitamin E levels, ALT levels, and self-reported GI symptomatology. Although these data suggest that GSH helps pediatric CF children in gaining weight, it is not clear what mechanism is involved. The significant drop in fecal calprotectin suggests that ameliorating gut inflammation may improve nutrient

absorption regardless of disease etiology. This is another question for future study in CF and other causes of malnutrition in which oral GSH has demonstrated improvement, such as AIDS-related cachexia (22).

The results from this trial support further study of the role of GSH in both CF pathophysiology and therapy. Larger replication trials are warranted, including trials in additional populations such as adult patients with CF. Many other questions, such as optimal dose; proper formulation; length of therapy; other outcome variables such as spirometry and bacteriology; and other possible sequelae, including adjustment of enzyme requirements, also deserve further study. Because the trial concluded after 6 months, additional data would be needed to see whether these results persist over time with ongoing treatment.

Given the favorable safety profile of oral GSH, the ease and noninvasive character of administration, and the significant weight gain experienced by pediatric patients with CF taking the treatment in this trial, we recommend that clinicians, especially pediatric gastroenterologists and clinical dieticians affiliated with CF clinics, consider whether their patients may benefit from the addition of oral GSH to their treatment regimen.

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