Adjunctive albuterol enhances the response to enzyme replacement therapy in late-onset Pompe disease

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ABSTRACT **Effective dosages for enzyme replacement therapy (ERT) in Pompe disease are much higher than for other lysosomal storage disorders, which has been attributed to low cation-independent mannose-6-phosphate receptor (CI-MPR) in skeletal muscle. We have previously demonstrated the benefit of increased CI-MPR-mediated uptake of recombinant human acid-α-glucosidase during ERT in mice with Pompe disease following addition of albuterol therapy. Currently we have completed a pilot study of albuterol in patients with late-onset Pompe disease already on ERT for >2 yr, who were not improving further. The 6-min walk test (6MWT) distance increased in all 7 subjects at wk 6 (30±13 m; *P*=0.002), wk 12 (34±14 m; *P*=0.004), and wk 24 (42±37 m; *P*=0.02), in comparison with baseline. Grip strength was improved significantly for both hands at wk 12. Furthermore, individual subjects reported benefits; *e.g.*, a female patient could stand up from sitting on the floor much more easily (time for supine to standing position decreased from 30 to 11 s), and a male patient could readily swing his legs out of his van seat (hip abduction increased from 1 to 2+ on manual muscle testing). Finally, analysis of the quadriceps biopsies suggested increased CI-MPR at wk 12 (*P*=0.08), compared with baseline. With the exception of 1 patient who succumbed to respiratory complications of Pompe disease in the first week, only mild adverse events have been reported, including tremor, transient difficulty falling asleep, and mild urinary retention (requiring early morning voiding). Therefore, this pilot study revealed initial safety and efficacy in an open label study of adjunctive albuterol therapy in patients with late-onset Pompe disease who had been stable on ERT with no improvements noted over the previous several years.—Koeberl, D. D., Austin, S., Case, L. E., Smith, E. C., Buckley, A. F., Young, S. P., Bali, D., Kishnani, P. S. Adjunctive albuterol enhances the response to enzyme replacement therapy in late-onset Pompe disease. *FASEB J.* 28, 2171–2176 (2014). www.fasebj.org**

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EFFECTIVE DOSAGES FOR enzyme replacement therapy (ERT) in Pompe disease are up to 100-fold higher than those in other lysosomal storage disorders. This high-dose requirement has been attributed to several factors, including the low abundance of cation-independent mannose-6-phosphate receptor (CI-MPR) in skeletal muscle (1, 2) and the very large muscle mass (comprising ~40% of body mass). It has also been established that type II muscles are resistant to ERT in association with low CI-MPR expression (1, 2). The effect of CI-MPR-mediated uptake of recombinant human (rh) acid-α-glucosidase (GAA) on ERT in GAA-knockout (KO) mice with Pompe disease has been demonstrated by administering selective β_2 agonists, thereby enhancing CI-MPR expression and increasing efficacy from ERT (3, 4). The clearance of stored glycogen was increased by β_2 agonist treatment during ERT, as demonstrated by lower glycogen content in skeletal muscle following the addition of clenbuterol or albuterol treatment (4). The skeletal muscles comprised primarily of type II myofibers responded more efficaciously to ERT when clenbuterol or albuterol therapy was added, including the tibialis anterior muscle (3). Albuterol treatment has been associated with increased muscle mass and strength in normal individuals and in patients with muscular dystrophy (5, 6); moreover, a pilot study of albuterol in individuals with Pompe disease demonstrated beneficial effects on muscle function and no serious adverse events prior to the availability of ERT (7).

In the current pilot study, the safety and bioactivity of adjunctive albuterol was evaluated in adult patients with Pompe disease. Secondary endpoints included muscle

Abbreviations: 6MWT, 6-min walk test; AE, adverse event; BID, *bis in die*; CI-MRP, cation-independent mannose-6-phosphate receptor; CK, creatine kinase; ER, extended release; ERT, enzyme replacement therapy; GAA, acid-α-glucosidase; Glc4, glucose tetrasaccharide; H&E, hematoxylin and eosin; KO, knockout; rh, recombinant human

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function and respiratory function testing, similar to the endpoints used in clinical trials of ERT in Pompe disease. The effect of albuterol on skeletal muscle was investigated with regard to CI-MPR expression, histology, and glycogen clearance.

MATERIALS AND METHODS

Study design

This was a 24-wk open-label study of albuterol in adult patients with Pompe disease, during which subjects underwent an evaluation including blood testing, physical examination, safety testing, muscle function and strength testing, and 6-min walk test (6MWT) at baseline and wk 6, 12, and 24. Electrocardiogram, pulmonary function testing, and muscle biopsy were performed at baseline and wk 12. Telephone visits at wk 1, 7, and 13 were performed to inquire regarding any adverse event (AE). Patients initiated albuterol at baseline and continued taking it until the wk 24 visit. The initial dose for albuterol extended release (ER) was 4 mg *bis in die* (BID). At wk 6, the dose of albuterol was increased to 8 mg BID, barring dose-limiting AEs. This study was approved by the Duke University Institutional Review Board, and written consent was obtained at study entry.

Patients

All eligible patients were adults >18 yr old, had a confirmed diagnosis of Pompe disease (GAA deficiency), and were treated stably with Lumizyme (alglucosidase alfa) for >2 yr at the standard dose (20 mg/kg every other week). Exclusion criteria included continuous invasive ventilation, chronic heart disease, history of seizure disorder, hyperthyroidism, pregnancy, hypersensitivity to albuterol, or taking contraindicated medications. In addition, patients with continued clinical improvement on ERT alone were excluded from the study. Eight subjects enrolled (4 male and 4 female), and 6 of 8 subjects completed the study at wk 24. One subject delayed the final visit until 40 wk, remaining on albuterol throughout (subject 2). One male subject with significant pulmonary compromise died at home during wk 2. Five additional patients were screened for the study but were excluded based on study criteria.

Muscle biopsy evaluation

Flash-frozen and fixed samples (10% formalin) were collected by needle muscle biopsy of the quadriceps. Western blotting was performed as described using the hGAA monoclonal antibody (courtesy of Genzyme Corp., Framingham, MA, USA), the CI-MPR antibody (catalog no. GTX28093; Gene Tex, Irvine, CA, USA), LAMP-2 rabbit polyclonal antibody (Abcam, Cambridge, MA, USA), and GAPDH monoclonal antibody (Abcam) (8). Glycogen content was analyzed as described previously (9).

Biomarker analysis

Urinary glucose tetrasaccharide was measured as the total hexose tetrasaccharide fraction (Hex_4) using stable isotope dilution ultra-performance liquid chromatography-tandem mass spectrometry as described previously (10, 11) at baseline, wk 12, and wk 24.

Statistical analyses

Comparisons were assessed using Prism software (GraphPad, La Jolla, CA). Single comparisons were analyzed by a paired *t* test, and multiple comparisons were analyzed by repeated measures ANOVA. A value of $P < 0.05$ was considered to be statistically significant.

Histological analyses

Paraffin sections stained with hematoxylin and eosin (H&E), Trichrome, PAS, and PASD were prepared from skeletal muscle biopsy tissue at baseline and 12 wk, using standard techniques. These sections were examined microscopically by a pathologist (A.F.B.) blinded to sample identity. Frozen sections stained with H&E, Trichrome, PAS, and PASD that had been prepared for clinical diagnosis prior to the study (subjects 1–6) were also examined. The histological features assessed included vacuolation/glycogen deposition, myophagocytosis, fiber atrophy and hypertrophy as measured by an ocular micrometer, evidence of regeneration as determined by the presence of internal nuclei, and increased interstitial stroma/fibrosis. Features were scored as absent (none), minimal, mild, moderate, or marked according to their extent and/or severity. Representative photographs of the histopathology of each biopsy were taken while the pathologist was still blinded to the clinical outcomes.

RESULTS

Subjects were followed at research visits for safety and efficacy and contacted by telephone to inquire regarding incidence of any AE during this 24-wk study of adjunctive albuterol (**Fig. 1*A***). Distance for the 6MWT increased in 7 subjects following initiation of albuterol (Fig. 1*B*), from an average of 305 m at baseline to 346 m at wk 24. 6MWT distance increased in all 7 subjects at wk 6 (30±13 m; $P=0.002$), wk 12 (34±14 m; $P=0.004$), and wk 24 (42±37 m; $P=0.02$), in comparison with baseline. Hand grip strength was significantly increased on the right from average of 79 pounds at baseline to 84 pounds at wk 12 (Fig. 1*C*). Likewise, left hand grip strength increased significantly from average 76 pounds at baseline to 86 pounds at wk 12 (Fig. 1*D*). In addition, anecdotal reports indicated benefits that were associated with improved responses in muscle function testing: a female patient could stand up from sitting on the floor much more easily (time for supine to standing position decreased from 30 to 11 s), and a male patient could readily swing his legs out of his van seat (hip abduction increased from 1 to 2+ on manual muscle testing).

Bioactivity was assessed by Western blot analysis of CI-MPR expression in the muscle biopsy from quadriceps (**Fig. 2*A***). CI-MPR trended higher at wk 12 in comparison with baseline (Fig. 2*B*), when normalized to GAPDH ($P=0.08$). GAA was increased for 5 of 6 biopsies analyzed (Fig. 2*C*), and LAMP2 was decreased, (Fig. 2*D*), although these changes did not achieve statistical significance.

Subjects in the pilot study of albuterol have been monitored for safety at wk 6, 12, and 24 visits, and with

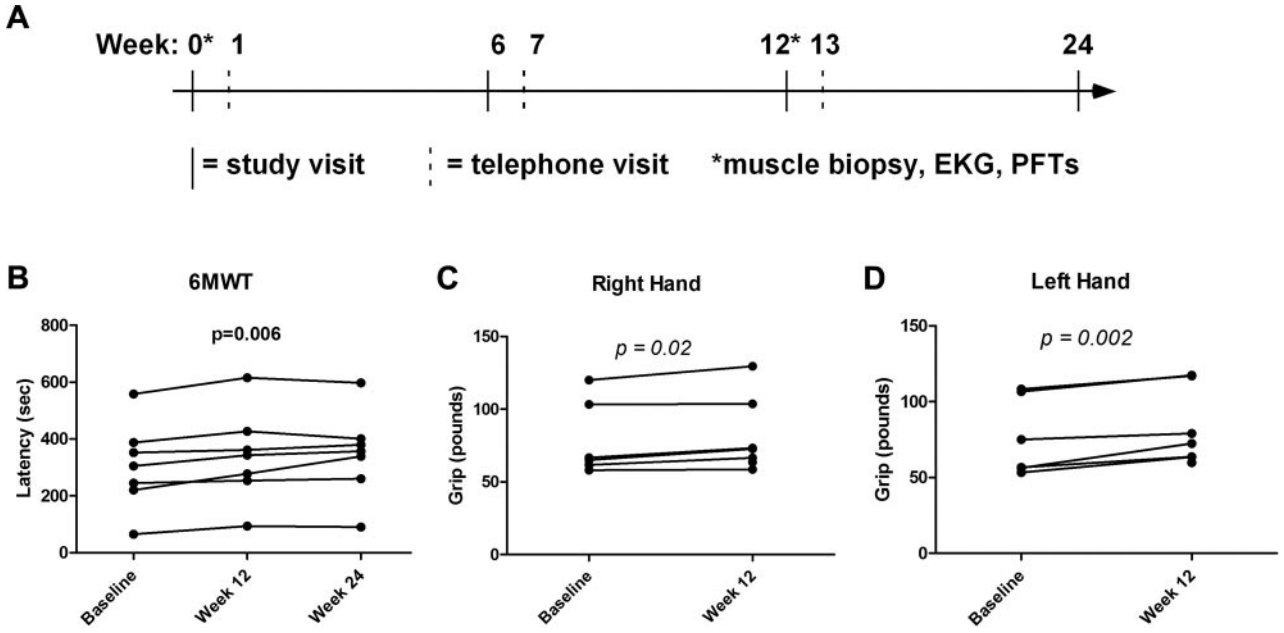

Figure 1. Study design and efficacy. *A*) Study design, indicating timing for study visits when patients were seen, telephone visits, electrocardiograms (EKGs), and pulmonary function tests (PFTs). *B*) 6MWT distance at the indicated study visits. Each line connects the data points for 1 research subject. *C*, *D*) Right (*C*) and left (*D*) hand grip strength tested by dynamometry.

phone visits at wk 1 and 7. The albuterol dose was gradually increased to minimize AEs, starting with 4 mg each morning *per os* for the first week. If no AEs greater than mild were reported at the wk 1 phone visit, the dose was increased to 4 mg BID *per os*. If the albuterol dose was similarly well tolerated at the wk 6 visit, the dose was increased to 8 mg in the morning, and remained at 4 mg in the evening for the next week. Finally, if the wk 7 phone visit revealed no more than mild AEs, the evening dose was increased to 8 mg. This dose titration has prevented attrition related to the effects of albuterol, and all 5 subjects who completed the wk 6 visit have tolerated the 8 mg BID dose.

One patient succumbed to complications of Pompe disease, which was deemed unrelated to the study. That subject had severe respiratory compromise [supine

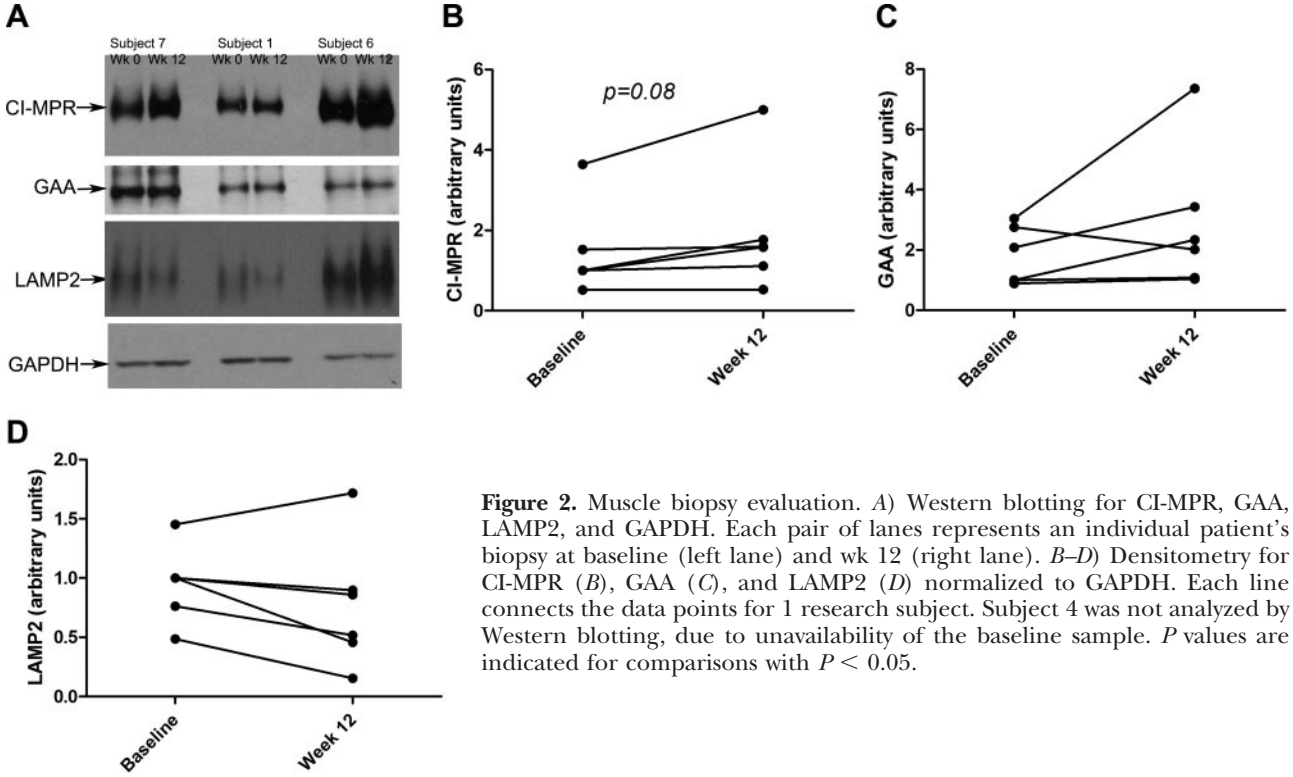

Figure 2. Muscle biopsy evaluation. *A*) Western blotting for CI-MPR, GAA, LAMP2, and GAPDH. Each pair of lanes represents an individual patient's biopsy at baseline (left lane) and wk 12 (right lane). *B–D*) Densitometry for CI-MPR (*B*), GAA (*C*), and LAMP2 (*D*) normalized to GAPDH. Each line connects the data points for 1 research subject. Subject 4 was not analyzed by Western blotting, due to unavailability of the baseline sample. *P* values are indicated for comparisons with $P < 0.05$.

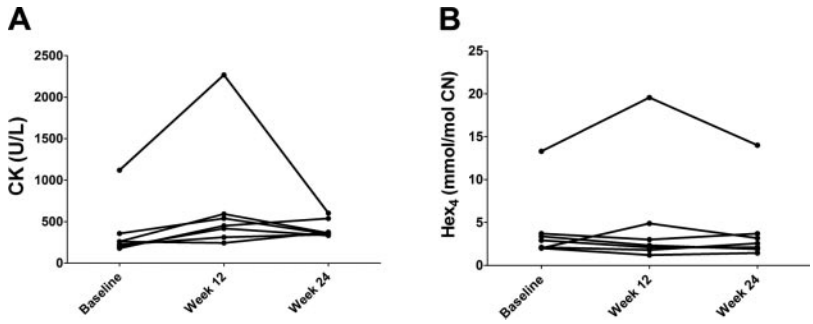

Figure 3. Safety testing. *A*, *B*) Serum CK (*A*) and urinary glucose tetrasaccharide (Glc4; *B*) at baseline, wk 12, and wk 24. Each line connects the data points for 1 research subject. Subject 2 had increased CK and Glc4 at wk 12, which were decreased on returning late for the final visit at wk 40 (shown with other visit data from wk 24).

forced vital capacity (FVC) 10% of expected] and died at home during wk 2, apparently related to respiratory complications of Pompe disease. That subject lived alone and had a history of being unable to lie supine without assisted ventilation. Other mild study-related AEs were reported, including tremor, transient difficulty falling asleep, and mild urinary retention (requiring early morning voiding). One patient preferred a dose reduction to 4 mg BID after 12 wk due to difficulty falling asleep. Creatine kinase (CK) remained stable with the exception of 1 patient for whom CK was elevated at wk 12, and subsequently the CK for that patient returned to the concentration observed at baseline by the last visit (wk 40 for that subject; **Fig. 3*A***). The urinary biomarker, glucose tetrasaccharide (Glc4), was elevated in correlation with serum CK for the same patient at wk 12 (Fig. 3*B*). The above-mentioned patient was the only subject to have increased LAMP2 in the muscle biopsy at wk 12 (Fig. 2*D*).

Histology from the muscle biopsy revealed features relevant to the response to therapy in some subjects (**Table 1**); this was not consistent, but sampling error applies in these small biopsies. Hypertrophy has been associated with clenbuterol treatment in preclinical studies (3, 12), and 3 of 7 subjects had increased hypertrophy at wk 12, in comparison with baseline (Table 1). Of note, subject 1 had a marked improvement in the 6MWT of 119 m over the course of the study, which correlated with an increase in both muscle fiber hypertrophy and evidence of regeneration between baseline and wk 12; this subject otherwise had only mild histopathologic features overall on both biopsies (**Fig. 4**, top panels; and Table 1). Also noteworthy, subject 2 had an increase in both muscle fiber hypertrophy and evidence of regeneration between baseline and wk 12 (Fig. 4, middle panels; and Table 1). Subject 2 otherwise had moderate histopathologic features at baseline and showed increased fiber atrophy and fibrosis at wk 12, with increased serum CK at that time point (Fig. 3*A*). Finally, subject 4, who had higher 6MWT than other subjects, had only mild histopathologic features, which were even less evident on the 12-wk biopsy; this subject showed no fiber hypertrophy at wk 12 (Fig. 4, bottom panels; and Table 1).

DISCUSSION

This pilot study of oral albuterol in patients with late-onset Pompe disease while treated with ERT revealed acceptable safety, initial efficacy, and bioactivity. AEs were mild and transient, consistent with previous studies of albuterol in patients with neuromuscular disorders, including Pompe disease (6, 7). The observed increase in 6MWT over the first 6 wk was is equivalent to the increased time in the 6MWT observed

TABLE 1. *Histopathologic features of muscle biopsies*

Subject	Visit	Vacuolation	Myophag	Atrophy	Hypertrophy	Regeneration	Interstitial stroma
1	Baseline	Mild	None	Mild	Mild	None	Minimal
	wk 12	Mild	None	Mild	Moderate	Mild	Minimal
2	Baseline	Moderate	Mild	Moderate	None	Mild	Mild
	wk 12	Moderate	Mild	Marked	Marked	Marked	Moderate
3	Baseline	Moderate	Mild	Moderate	Moderate	Marked	Moderate
	wk 12	Moderate	None	Moderate	Moderate	Marked	Moderate
4	Baseline	Mild	None	Minimal	None	None	None
	wk 12	Minimal	None	Minimal	None	None	None
5	Baseline	Mild	None	Moderate	Mild	Moderate	Minimal
	wk 12	Mild	Mild	Mild	Moderate	Moderate	Mild
6	Baseline	Marked	Mild	Moderate	Mild	Moderate	Mild
	wk 12	Mild	None	Mild	Mild	Moderate	None
7	Baseline	Minimal	None	Marked	Marked	Marked	Marked
	wk 12	Mild	Mild	Moderate	Moderate	Moderate	Mild

Vacuolation: muscle fiber vacuolation and/or deposition of glycogen. Myophag: myophagocytosis. Atrophy: fibers with a diameter < 40 μm, or subsarcolemmal nuclear aggregates indicating terminal fiber atrophy. Hypertrophy: fibers with a diameter > 100 μm. Regeneration: fibers with internal nuclei. Intersitital stroma: increased interstitial stromal area on PASD or fibrosis on Trichrome.

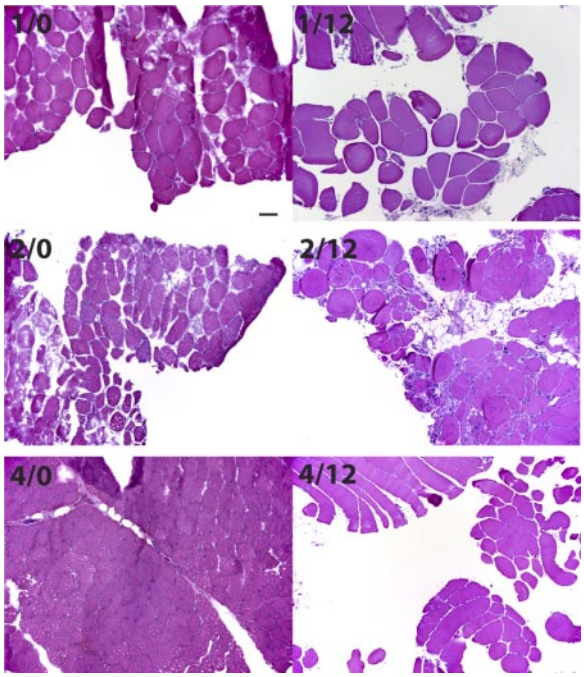

Figure 4. Histopathologic images. H&E-stained skeletal muscle tissue sections photographed in representative fields, using a ×10 objective. Scale bar=100 μm. Baseline biopsies are frozen sections; 12-wk biopsies are paraffin sections. 1/0: subject 1 at baseline; 1/12: subject 1 at 12 wk of treatment; 2/0: subject 2 at baseline; 2/12: subject 2 at 12 wk of treatment; 4/0: subject 4 at baseline; 4/12: subject 4 at 12 wk of treatment.

after 24 wk in the initial double-blinded study of ERT in late-onset Pompe disease (13). Of note, all of the current subjects had been stably treated with ERT for >2 yr, and none had shown clinical improvement after the initial year of ERT. As anticipated, CI-MPR trended higher in muscle following initiation of albuterol, as described previously for mice with GAA-KO when treated with β2-agonists (3, 4, 14).

The paucity of CI-MPR in mammalian adult muscle has underscored the concept that CI-MPR is limiting for ERT in Pompe disease (1, 2); moreover, we have been the first to directly address this problem (3, 4). Previously, low levels of CI-MPR were demonstrated in skeletal muscle of GAA-KO mice, specifically in muscles comprising primarily type II myofibers (1, 2). The importance of CI-MPR expression to ERT in Pompe disease was demonstrated by the enhanced efficacy of modified rhGAA engineered to increase the number of mannose-6-phosphate moieties (15, 16). However, increasing mannose-6-phosphate residues on rhGAA cannot overcome the extremely low CI-MPR on muscle, and modifications of a commercially approved ERT product will prove to be expensive, and likely immunogenic. Consistent with our central hypothesis that modulation of CI-MPR will increase the uptake and lysosomal targeting of GAA, fibroblasts from patients with Pompe disease were found to be deficient in CI-MPR recycling, and uptake of rhGAA was impaired (17). To understand the influence of CI-MPR expression on therapy in Pompe disease, we have demonstrated the effect of increased CI-MPR expression on the efficacy of ERT in GAA-KO mice (3). Currently we have demonstrated that CI-MPR up-regulation can be achieved by albuterol treatment in human patients with Pompe disease, which might improve the efficacy from standard-of-care ERT in this condition.

Albuterol was generally well tolerated in this pilot study, and secondary endpoints were improved, including 6MWT and hand strength. One subject demonstrated transient, mild toxicity, as evidenced by transiently increased CK and increased Glc4 at wk 12 (Fig. 3), which correlated with increased LAMP2, reflecting accumulated lysosomal glycogen at wk 12 (Fig. 2*D*). That subject reported much greater physical activity prior to the wk 12 visit, including taking up bow hunting and traveling unaccompanied for the first time to the wk 12 visit. One subject died at home early during study participation after taking albuterol ER at a dose of 4 mg BID for 5 d, which was attributed to compromise of respiratory function and the lack of adequate assistance in the home. Subsequently, we have encouraged adult subjects with Pompe disease to have a live-in caretaker or an electronic paging device if they were deemed susceptible to falling or unable to stand up independently from the supine position.

Caution has been urged due to the relatively high dosages of β2 agonists administered to rodent models (12), because of the potential for dose-related adverse effects from these drugs. Our preclinical study with albuterol administered ~210 μg/d to mice with Pompe disease, given an albuterol concentration of 30 μg/ml in drinking water and estimated water consumption of 7 ml/d (4). A dose conversion based on body surface area would recommend a human dose of 48 mg/d (18). The current study administered a 3-fold lower dose of albuterol to patients with late-onset Pompe disease based on studies in normal individuals and patients with facioscapulohumerol muscular dystrophy that demonstrated muscle effects (5, 6). Thus, our preclinical experiment suggested that the daily dose of 16 mg administered to patients might be too low to achieve the muscle effects observed in albuterol-treated mice. However, a 5-fold dose reduction for another β2 agonist, clenbuterol, retained muscle effects in mice with Pompe disease (4). Furthermore, albuterol treatment at the same dose increased muscle strength or muscle mass within 12 wk in boys with Duchenne muscular dystrophy, accompanied by mild adverse events (19, 20). Therefore, we suspect that the effective dose for albuterol might be lower than that predicted by our preclinical experiments, not yet having performed a dose reduction experiment with albuterol in mice.

This pilot study has several limitations, including lack of placebo controls; performance of only a single evaluation at baseline, which prevented calculation of the baseline change in 6MWT prior to initiating the study drug; and monitoring for only 24 wk to detect efficacy. Nonetheless, the observed trends support fur-

ther evaluation of albuterol in patients with Pompe disease, while treated with ERT, to enhance the receptor-mediated uptake of rhGAA in skeletal muscle. FJ

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