

Adjunctive Albuterol Enhances the Response to Enzyme Replacement Therapy in Late-Onset Pompe Disease

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NONSTANDARD ABBREVIATIONS

acid α -glucosidase (GAA)

adverse event (AE)

cation-independent mannose-6-phosphate receptor (CI-MPR)

6 minute walk test (6MWT)

Recombinant human (rh)

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 (rhGAA) 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 more than two years, who were not improving further. The 6 minute walk test (6MWT) distance increased in all 7 subjects at Week 6 (30 +/- 13 meters; $p=0.002$), Week 12 (34 +/- 14 meters; $p=0.004$), and Week 24 (42 +/- 37 meters; $p=0.02$), in comparison with Baseline. Grip strength was improved significantly for both hands at Week 12. Furthermore, individual subjects reported benefits, including: 1) a female patient could stand up from sitting on the floor much more easily (time for supine to standing position decreased from 30 seconds to 11 seconds); 2) 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 Week 12 ($p=0.08$), compared with Baseline. With the exception of one 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.

Keywords: Mannose-6-phosphate receptor, acid alpha-glucosidase, acid maltase, glycogen storage disease type II.

Introduction

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 impact of CI-MPR-mediated uptake of recombinant human (rh) acid- α -glucosidase (GAA) upon 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 upon 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 function and respiratory function testing, similar to the endpoints used in clinical trials of ERT in Pompe disease. The effect of albuterol upon skeletal muscle was investigated with regard to CI-MPR expression, histology, and glycogen clearance.

Materials and Methods

Study design: This was a 24 week 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 minute walk test (6MWT) at Baseline, and Weeks 6, 12, and 24. Electrocardiogram, pulmonary function testing, and muscle biopsy were performed at Baseline and Week 12. Telephone visits at Weeks 1, 7, and 13 were performed to inquire regarding any adverse event (AE). Patients initiated albuterol at Baseline and continued taking it until the Week 24 visit. The initial dose for albuterol extended release (ER) was 4 mg BID. At Week 6 the dose of albuterol was increased to 8 mg BID, barring dose-limiting adverse events (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 years old, had a confirmed diagnosis of Pompe disease (GAA deficiency), and were treated stably with Lumizyme (alglucosidase alfa) for >2 years 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 was excluded from the study. Eight subjects enrolled (4 male and 4 female), and 6 of 8 subjects completed the study at Week 24. One subject delayed the final visit until 40 weeks, remaining on albuterol throughout (Subject 2). One male subject with significant pulmonary compromise died at home during Week Two. Five additional patients were screened for the study, but were excluded based upon 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), the CI-MPR antibody (catalog number GTX28093; Gene Tex, Irvine, CA), LAMP-2 rabbit polyclonal antibody (Abcam, Cambridge,

MA, USA), and GAPDH monoclonal antibody (Abcam) (8). Glycogen content was analyzed as described (9).

Biomarker analysis: Urinary glucose tetrasaccharide was measured as the total hexose tetrasaccharide fraction (Hex₄) using stable isotope dilution ultra performance liquid chromatography-tandem mass spectrometry as described (10, 11) at Baseline, Week 12, and week 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 p-value <0.05 was considered to be statistically significant.

Histologic analyses: Paraffin sections stained with H&E, Trichrome PAS, and PASD were prepared from skeletal muscle biopsy tissue at baseline and 12 weeks, using standard techniques. These sections were examined microscopically by a pathologist (AFB) 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 histologic 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 week study of adjunctive albuterol (Figure 1A). Distance for the 6MWT increased in 7 subjects following initiation of albuterol (Figure 1B), from an average of 305 m at Baseline to 346 m at Week 24. 6MWT distance increased in all 7 subjects at Week 6 (30 +/-

13 meters; $p=0.002$), Week 12 (34 +/- 14 meters; $p=0.004$), and Week 24 (42 +/- 37 meters; $p=0.02$), in comparison with Baseline. Hand grip strength was significantly increased on the right from average of 79 pounds at baseline to 84 pounds at Week 12 (Figure 1C). Likewise, left hand grip strength increased significantly from average 76 pounds at Baseline to 86 pounds at Week 12 (Figure 1D). Additionally, anecdotal reports indicated benefits that were associated with improved responses in muscle function testing: 1) a female patient could stand up from sitting on the floor much more easily (time for supine to standing position decreased from 30 seconds to 11 seconds); 2) 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 (Figure 2A). CI-MPR trended higher at Week 12 in comparison with Baseline (Figure 2B), when normalized to GAPDH ($p=0.08$). GAA was increased for 5 of 6 biopsies analyzed (Figure 2C), and LAMP2 was decreased, (Figure 2D), although these changes did not achieve statistical significance.

Subjects in the pilot study of albuterol have been monitored for safety at Week 6, 12, and 24 visits, and with phone visits at Week 1 and Week 7. The albuterol dose was gradually increased to minimize AEs, starting with 4 mg each morning per oral for the first week. If no AEs greater than mild were reported at the Week 1 phone visit, the dose was increased to 4 mg BID per oral. If the albuterol dose was similarly well-tolerated at the Week 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 Week 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 Week 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 FVC 10% of expected) and died at home during Week Two, 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 weeks due to difficulty falling asleep. Creatine kinase (CK) remained stable with the exception of one patient for whom CK was elevated at Week 12, and subsequently the CK for that patient returned to the concentration observed at Baseline by the last visit (Week 40 for that subject) (Figure 3A). The urinary biomarker, glucose tetrasaccharide (Glc₄), was elevated in correlation with serum CK for the same patient at Week 12 (Figure 3B). The above-mentioned patient was the only subject to have increased LAMP2 in the muscle biopsy at Week 12 (Figure 2D).

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 Week 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 Week 12; this subject otherwise had only mild histopathologic features overall on both biopsies (Figure 4, upper panels; Table 1). Also noteworthy, subject 2 had an increase in both muscle fiber hypertrophy and evidence of regeneration between Baseline and Week 12 (Figure 4, middle panels; Table 1). Subject 2 otherwise had moderate histopathologic features at baseline, and showed increased fiber atrophy and fibrosis at week 12, with increased serum CK at that timepoint (Figure 3A). Finally, subject 4, who had higher 6MWT than other subjects, had only mild histopathologic features which were even less evident on the 12-week biopsy; this subject showed no fiber hypertrophy at Week 12 (Figure 4, lower panels; 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 for over the first 6 weeks was is equivalent to the increased time in the 6MWT observed *after 24 weeks* in the initial double-blinded study of ERT in LOPD (13). Of note, all of the current subjects had been stably treated with ERT for > two years, and none had shown clinical improvement after the initial year of ERT. As anticipated, CI-MPR trended higher in patients' muscle following initiation of albuterol, as previously described for mice with GAA-KO when treated with β 2-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 comprised primarily of 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, Pompe disease patient fibroblasts were found to be deficient in CI-MPR recycling and uptake of rhGAA was impaired (17). In order to understand the influence of CI-MPR expression upon therapy in Pompe disease, we have demonstrated the impact of increased CI-MPR expression on the efficacy from 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 Week 12 (Figure 3), which correlated with increased LAMP2 reflecting accumulated lysosomal glycogen at Week 12 (Figure 2D). That subject reported much greater physical activity prior to the Week 12 visit, including taking up bowhunting and travelling unaccompanied for the first time to the Week 12 visit. One subject died at home early during study participation after taking albuterol ER at a dose of 4 mg BID for 5 days, 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 β 2 agonists administered to rodent models (12), due to the potential for dose-related adverse effects from these drugs. Our preclinical study with albuterol administered approximately 210 μ g /day to mice with Pompe disease, given an albuterol concentration of 30 mg/l in drinking water and estimated water consumption of 7 ml/day (4). A dose conversion based upon body surface area would recommend a human dose of 48 mg/day (18). The current study administered a 3-fold lower dose of albuterol to patients with late-onset Pompe disease based upon 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 β 2 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 weeks 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: 1) lack of placebo controls, 2) performing only a single evaluation at baseline, which prevented calculating the baseline change in 6MWT prior to initiating study drug, and 3) monitoring for only 24 weeks to detect efficacy. Nonetheless, the observed trends support further evaluation of albuterol in patients with Pompe disease, while treated with ERT, to enhance the receptor-mediated uptake of rhGAA in skeletal muscle.

Acknowledgements

This study was funded by a Bridge Funding grant from the Duke University Medical Center. We wish to express appreciation to Dr. Y.-T. Chen for discussions regarding study design and therapy in Pompe disease. Additionally, we deeply appreciate Ms. Songtao Li and Ms. Jian Dai for their outstanding technical support of this project, and Ms. Carla Johnson for logistical support.

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Figure Legends

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

Figure 2: Muscle biopsy evaluation. (A) Western blotting for CI-MPR, GAA, LAMP2, and GAPDH are shown. Each pair of lanes represents an individual patient's biopsy at Baseline (left lane) and Week 12 (right lane). Densitometry for CI-MPR (B), GAA (C), and LAMP2 (D) normalized to GAPDH. Each line connects the datapoints for one 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) Serum CK and (B) urinary Glc4 at Baseline, Week 12, and Week 24. Each line connects the datapoints for one research subject. Subject 2 had increased CK and Glc4 at Week 12, which were decreased upon returning late for the final visit at Week 40 (shown with other visit data from Week 24).

Figure 4: Histopathologic images H&E-stained **skeletal muscle** tissue sections photographed in representative fields, using a 10x objective (scale bar = 100 micrometers). Baseline biopsies are frozen sections; 12-week biopsies are paraffin sections. 1/0= subject 1 at baseline, 1/12= subject 1 at 12 weeks of treatment; 2/0= subject 2 at baseline, 2/12= subject 2 at 12 weeks of treatment; 4/0= subject 4 at baseline, 4/12= subject 4 at 12 weeks of treatment.

Table 1: Histopathologic features of muscle biopsies.

Subject	Visit	Vacuolation	Myophag	Atrophy	Hypertrophy	Regeneration	Interstitial stroma
1	Baseline	mild	none	mild	mild	none	minimal
	Week 12	mild	none	mild	moderate	mild	minimal
2	Baseline	moderate	mild	moderate	none	mild	mild
	Week 12	moderate	mild	marked	marked	marked	moderate
3	Baseline	moderate	mild	moderate	moderate	marked	moderate
	Week 12	moderate	none	moderate	moderate	marked	moderate
4	Baseline	mild	none	minimal	none	none	none
	Week 12	minimal	none	minimal	none	none	none
5	Baseline	mild	none	moderate	mild	moderate	minimal
	Week 12	mild	mild	mild	moderate	moderate	mild
6	Baseline	marked	mild	moderate	mild	moderate	mild
	Week 12	mild	none	mild	mild	moderate	none
7	Baseline	minimal	none	marked	marked	marked	marked
	Week 12	mild	mild	moderate	moderate	moderate	mild

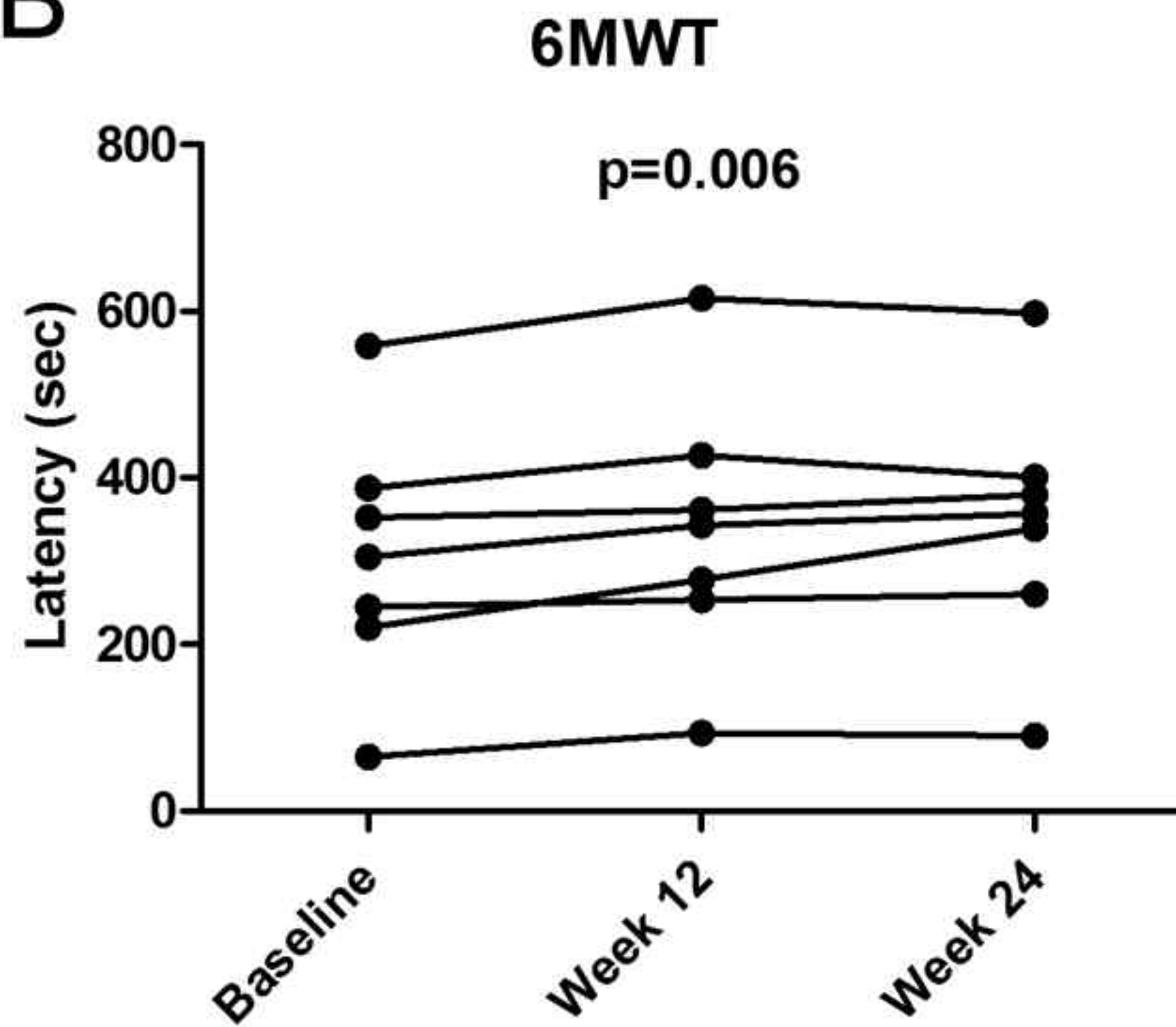
Vacuolation: muscle fiber vacuolation and/or deposition of glycogen; Myophag: myophagocytosis; Atrophy: fibers with a diameter less than 40 micrometers, or subsarcolemmal nuclear aggregates indicating terminal fiber atrophy; Hypertrophy: fibers with a diameter greater than 100 micrometers; Regeneration: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

Figure 1

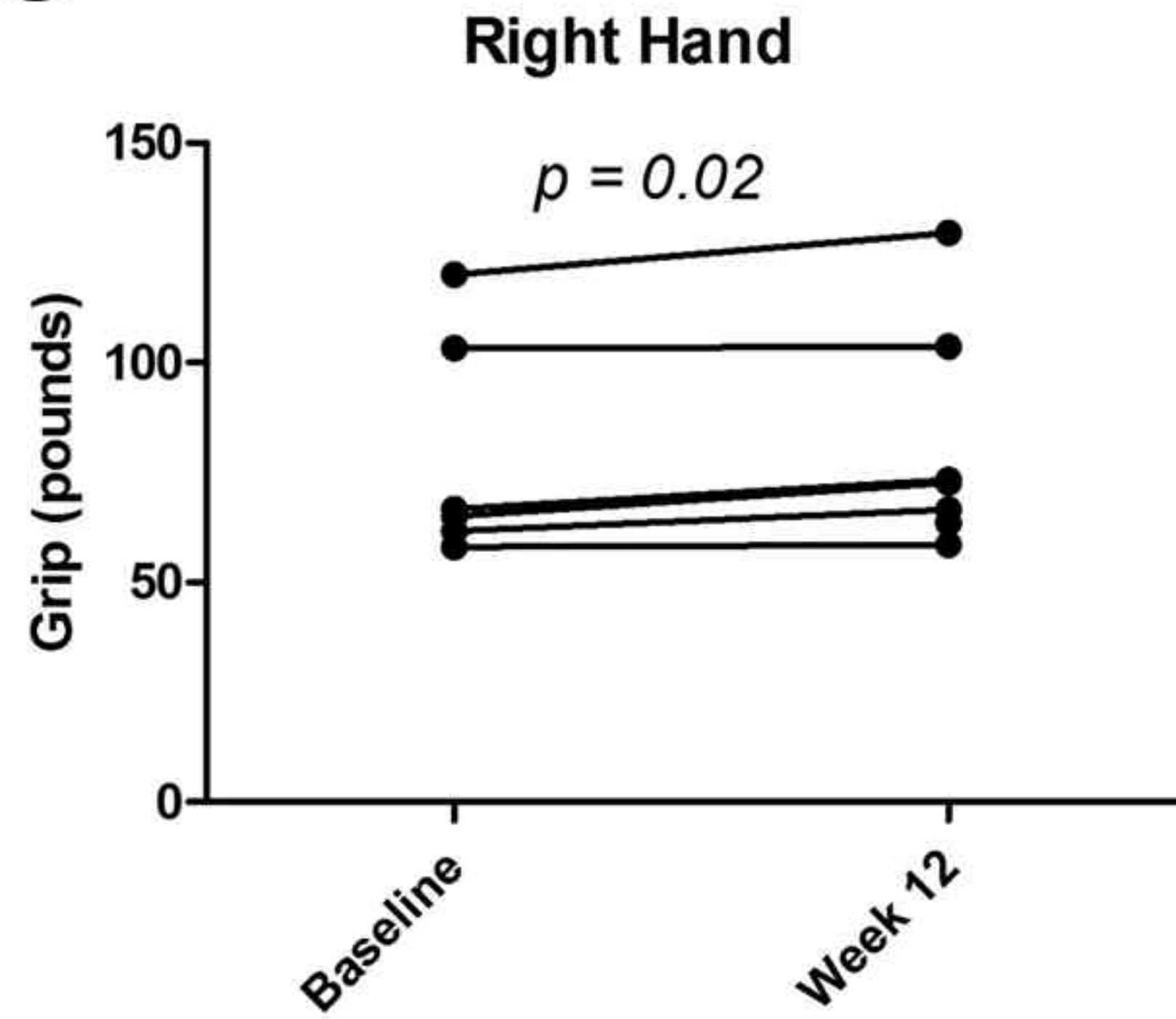
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B



C



D

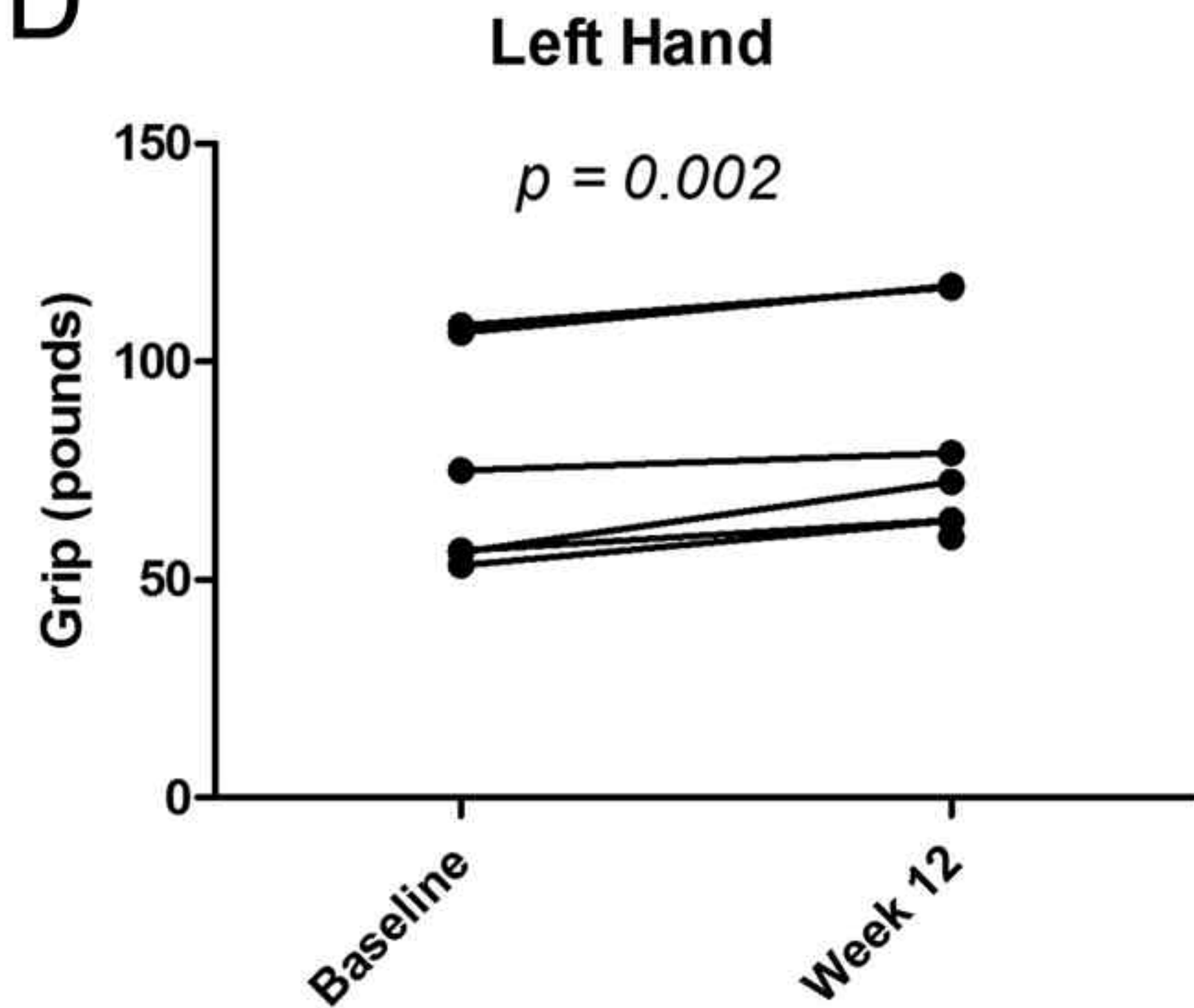


Figure 2

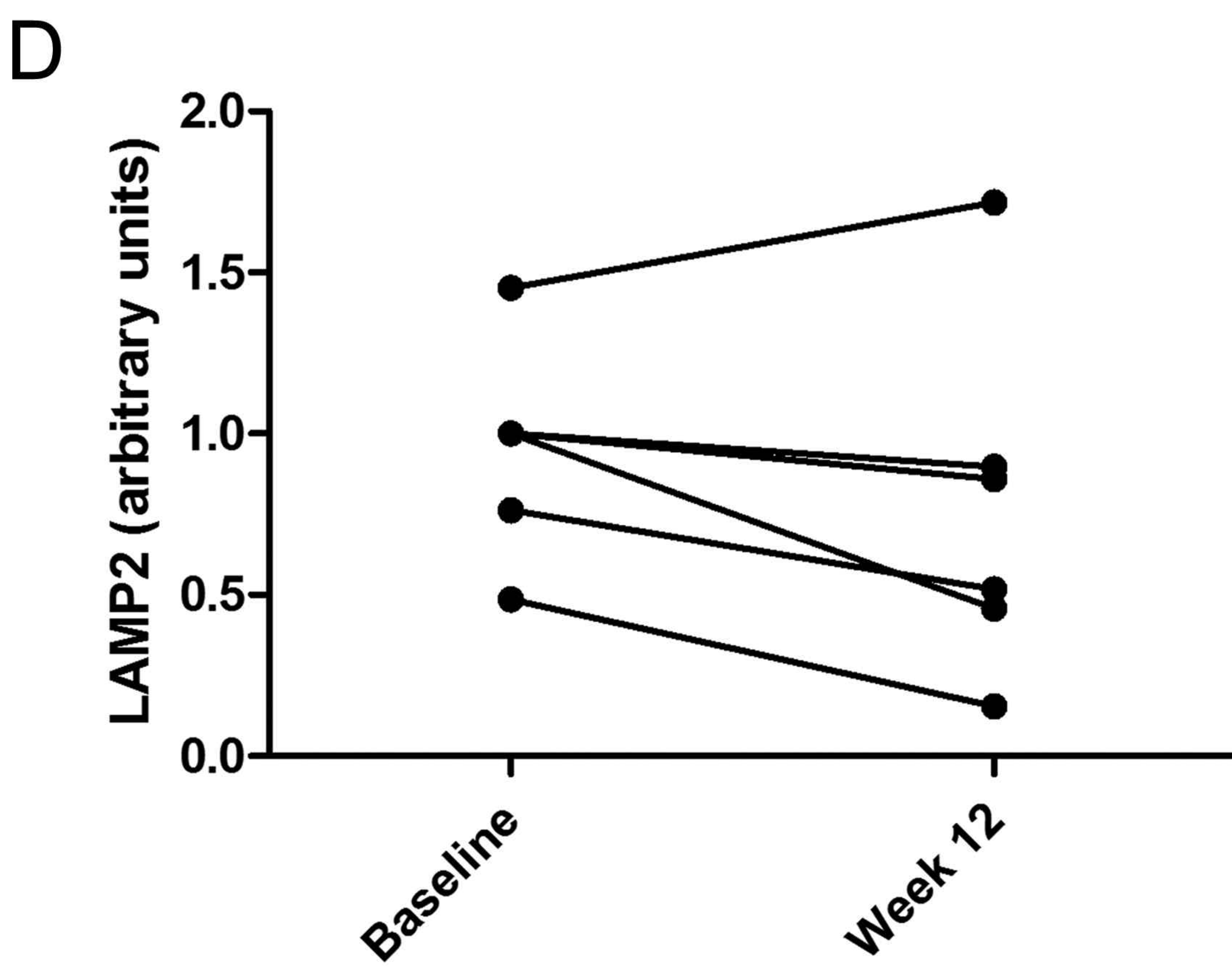
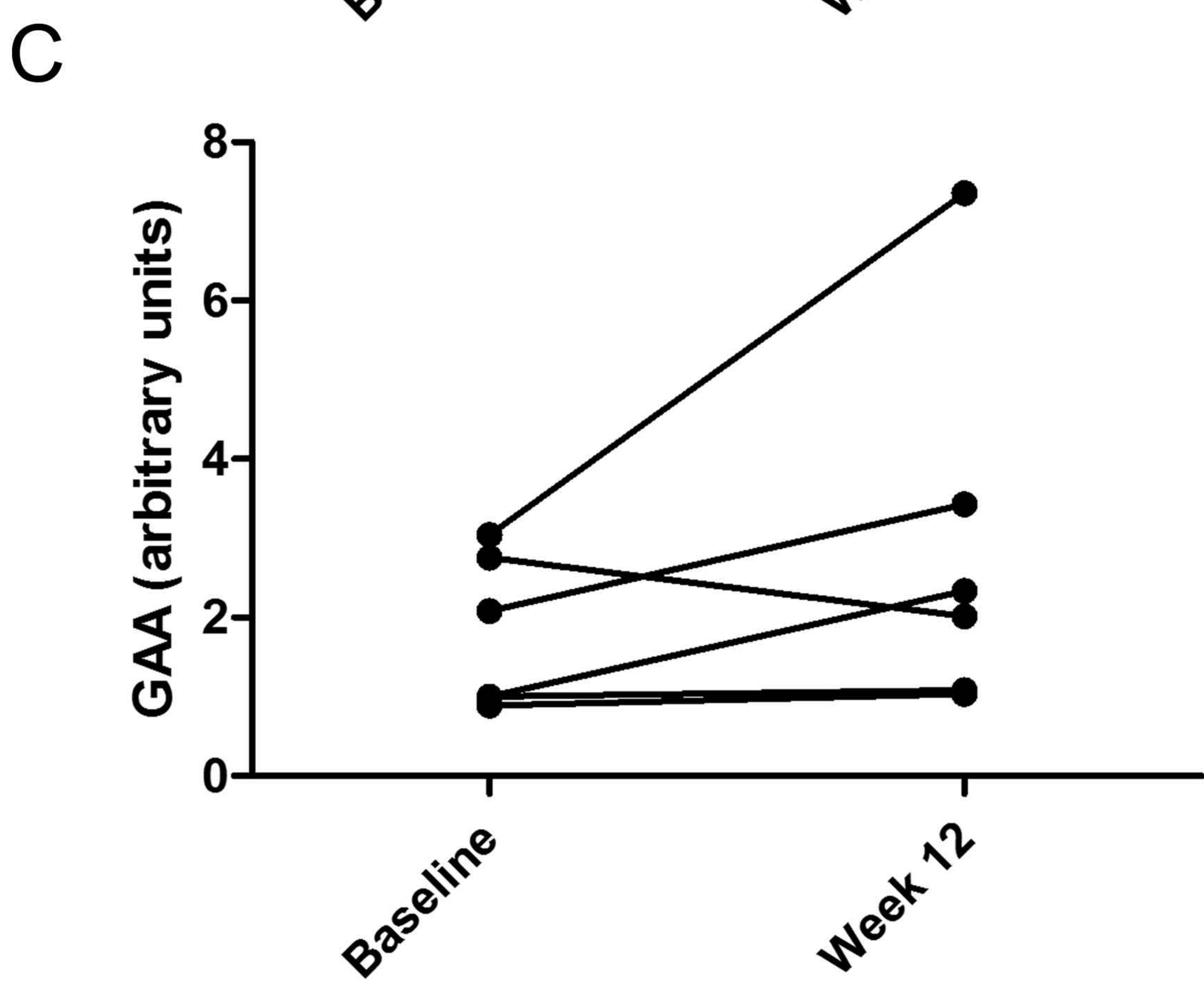
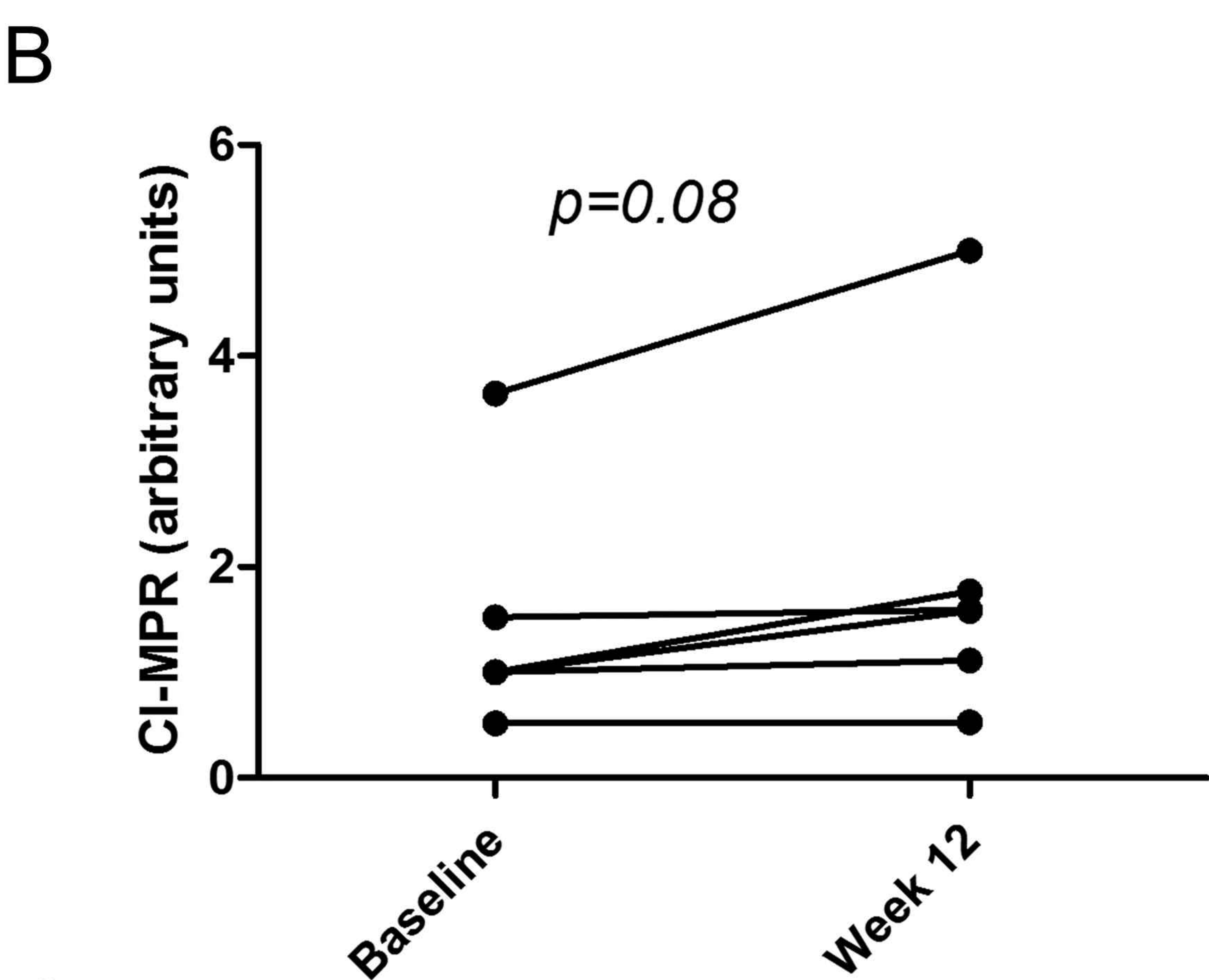
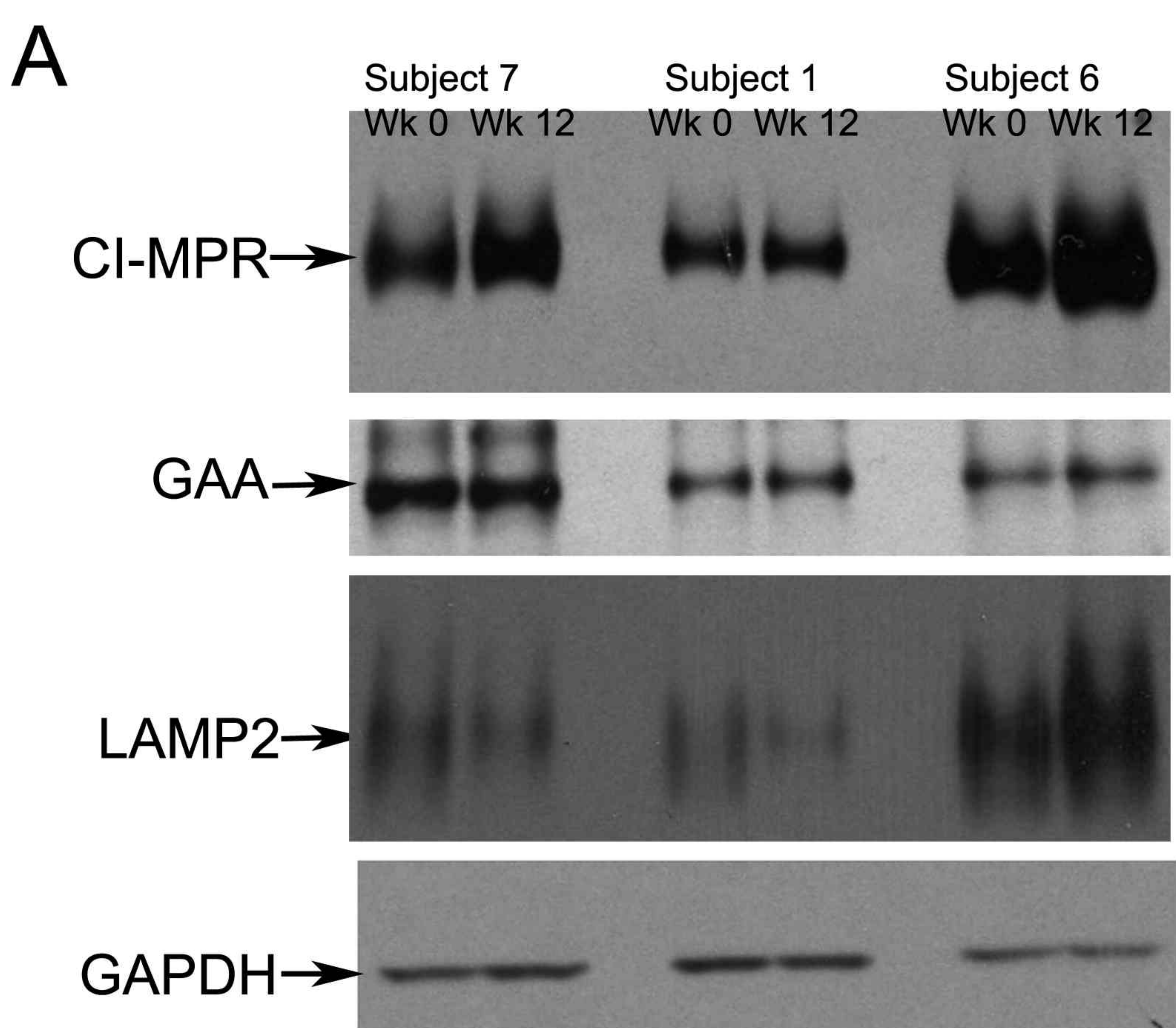
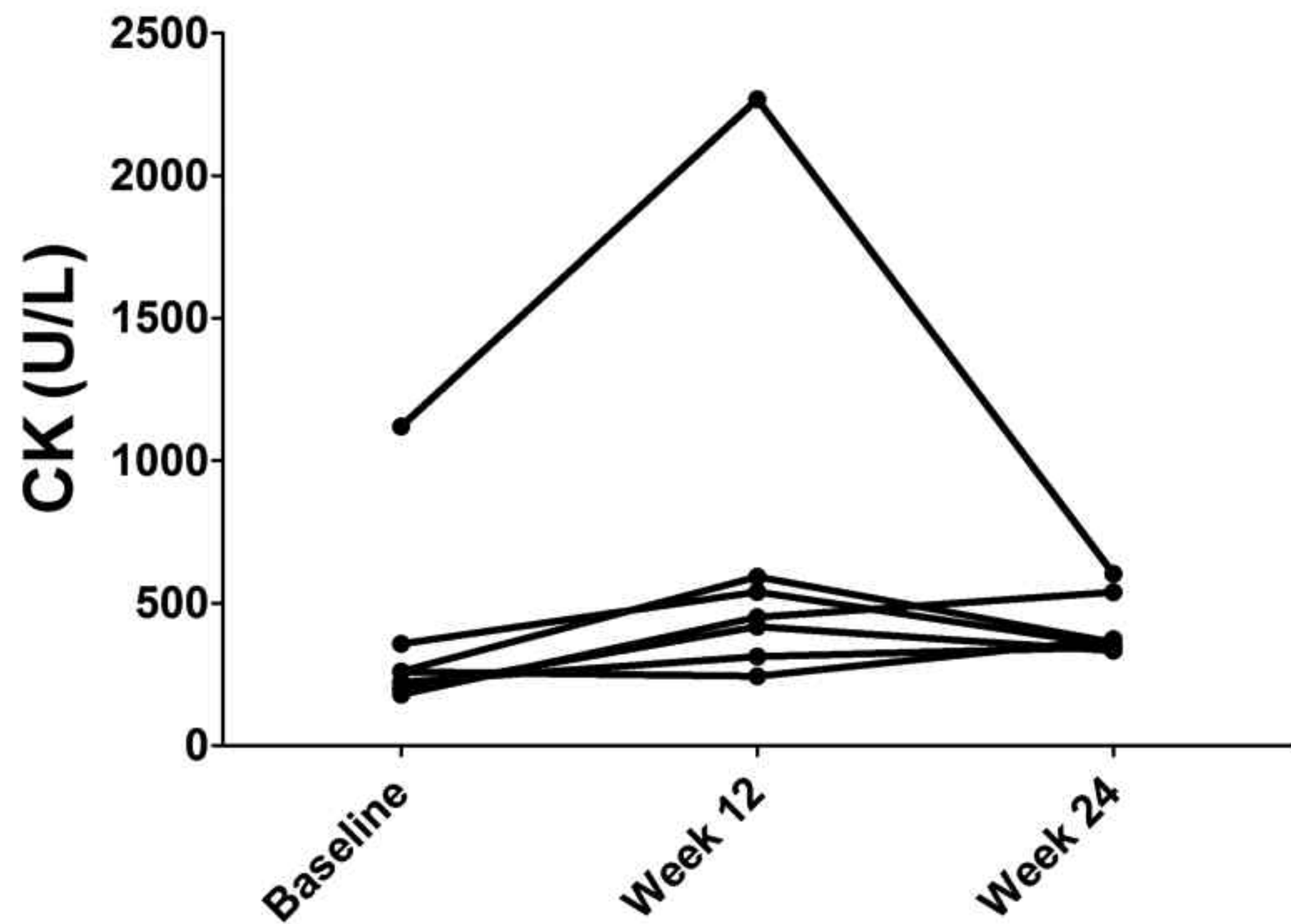


Figure 3

A



B

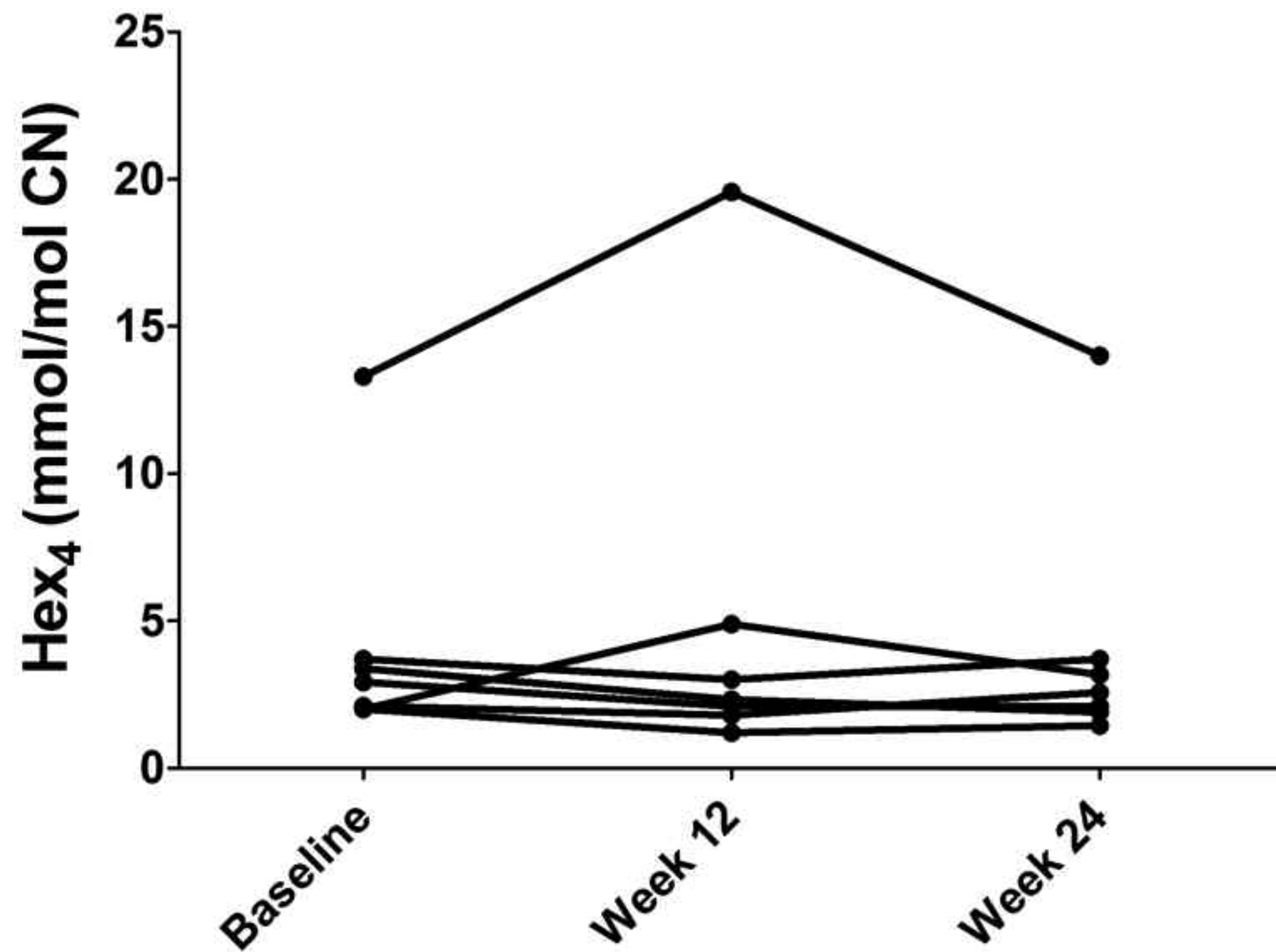


Figure 4

