

# Efficacy of photobiomodulation therapy on masseter thickness and oral health-related quality of life in children with spastic cerebral palsy

Maria Teresa Botti Rodrigues Santos<sup>1,2,3</sup> · Karla Santos Nascimento<sup>3</sup> · Simone Carazzato<sup>3</sup> · Alina Oliveira Barros<sup>1,4</sup> · Fausto Medeiros Mendes<sup>5</sup> · Michele Baffi Diniz<sup>1</sup>

Received: 17 November 2016 / Accepted: 16 May 2017 / Published online: 23 May 2017  
© Springer-Verlag London 2017

**Abstract** The study aimed to evaluate the efficacy of photobiomodulation therapy (PBMT) on bilateral masseter muscle thickness and amplitude of mouth opening in children with spastic cerebral palsy (CP), and the impact on their oral health-related quality of life (OHRQOL). Three groups were included: experimental CP group (EG:  $n = 26$  with oral complaints), positive control CP group (PCG:  $n = 26$  without complaints), and negative control group (NCG:  $n = 26$  without CP). In the EG, the masseter muscles on both sides were irradiated with an infrared low-level Ga-Al-As laser ( $\lambda = 808 \pm 3$  nm, 120 mW) using a 3 J/cm<sup>2</sup> energy dose per site, with a 20 s exposure time per site (spot area: 4 mm<sup>2</sup>; irradiance: 3 W/cm<sup>2</sup>; energy delivery per point: 2.4 J) six times over six consecutive weeks. Masseter thickness, assessed through ultrasonography, and the amplitude of mouth opening were measured in the EG before and after six

applications of PBMT and once in the PCG and NCG. The Parental-Caregiver Perception Questionnaire (P-CPQ) was used to evaluate OHRQOL. ANOVA, chi-square, *t* tests, and multilevel linear regression were used for statistical analysis. In the EG, the study results revealed average increments of 0.77 (0.08) millimeter in masseter thickness ( $P < 0.05$ ) and 7.39 (0.58) millimeter for mouth opening ( $P < 0.05$ ) and reduction in all P-CPQ domains ( $P < 0.001$ ), except for social well-being. The six applications of PBMT increased masseter thickness and mouth opening amplitude and reduced the impact of spastic CP on OHRQOL.

**Keywords** Cerebral palsy · Masseter muscle · Low-level laser therapy · Photobiomodulation therapy

## Introduction

Cerebral palsy (CP) is a group of prevalent, clinically important, and identifiable non-progressive permanent neuromotor disorders caused by damage to the immature or developing brain, with consequent activity limitations regarding movement and posture. It is the most common cause of severe physical disability during childhood [1]. The motor function impairment, excessive muscle tone, and fatigue disorders of CP are frequently accompanied by disturbances of cognition and behavior and late development of musculoskeletal problems, including oromotor and speech functions related to muscle spasticity [1].

Spasticity is the most frequent and prevalent type of movement disorder in CP patients (75% of cases) [2]. This condition results from motor neuron lesions responsible for hyperactivity of the alpha motor neurons in the spinal cord, resulting in muscle hypertonia [3]. The spastic muscles exhibit

✉ Maria Teresa Botti Rodrigues Santos  
drsantosmt@yahoo.com.br

<sup>1</sup> Graduate Program in Dentistry, Cruzeiro do Sul University, Rua Galvão Bueno 868, Liberdade, São Paulo SP CEP 01506-000, Brazil

<sup>2</sup> Patients with Special Needs, Cruzeiro do Sul University, Rua Galvão Bueno 868, Liberdade, São Paulo-SP 01506-000, Brazil

<sup>3</sup> Associação de Assistência à Criança Deficiente (AACD), Av. Professor Ascendino Reis, 724 - Ibirapuera, São Paulo-SP P 04027-000, Brazil

<sup>4</sup> Sergipe Federal University, Hospital Universitario (HU), Campus Prof. João Cardoso Nascimento Rua Cláudio Batista, s/n, Cidade Nova, Aracaju SE CEP 49060-108, Brazil

<sup>5</sup> School of Dentistry, São Paulo University (USP), Av. Prof. Lineu Prestes 2227 Cidade Universitária, São Paulo SP CEP 05508-900, Brazil

compromised function because of a diminished inhibition of muscle contraction, range of motion, voluntary strength, and movement [4].

Compromised oral health is commonly associated with CP because of the spasticity effect on the masseter and temporalis muscles, such as limitation of mouth opening amplitude; difficulty in efficiently maintaining oral hygiene, chewing, speaking, and swallowing; oral trauma caused by the presence of pathological oral reflexes, particularly the biting reflex during jaw closing; and motor weakness and lower maximal bite force [5–9].

Various spasticity treatments are available for reducing muscle spasms [10]. The pharmacological options to treat spasticity include anti-spastic drugs, of which the most widely used are baclofen, diazepam, and dantrolene. Baclofen, a gamma-aminobutyric acid, is an oral medication that exerts its effects by activating GABA-B receptors, thus, inhibiting presynaptic calcium influx, which blocks the release of excitatory neurotransmitters in the spinal cord. Nevertheless, its use is limited by the difficulty of determining the dose for pediatric populations and reducing cerebral penetration and its half-life [11]. The adverse effects include somnolence, confusion, and headaches [11].

Neuromuscular blocking with alcohol, phenol, and local anesthetics has been performed for the last 20 years. Recently, botulinum toxin has, in selected cases, demonstrated usefulness in preventing deformities secondary to spasticity, improving the quality of life of children with CP. Botulinum toxin is an injectable medication for local spasticity that acts on the neuromuscular junction to inhibit the release of acetylcholine, and the major side effect is excessive weakness of the treated muscle [11].

Photobiomodulation therapy (PBMT) is the effective and critical therapeutic application of light that affects biologic systems [12]. PMBT can modulate both phases of acute and chronic inflammation [13], interfering with the progression of the disease, improving inflammatory conditions [14], and stimulating the angiogenesis [15]. Regarding the effects of PBMT, this therapeutic modality increases the activity of the enzyme cytochrome c oxidase (COXI) on skeletal muscle, [16] improves skeletal muscle performance, recovery and gain of muscular strength when applied before exercises [17–19], and improvement of the rehabilitation after muscle injury [18].

In a previous study conducted by our group, we evaluated the effect of low-level laser therapy (LLLT) on the spasticity of the masseter muscle in children with CP. The results showed an increase in the amplitude of mouth opening and a decrease in the masseter muscle tonus in children with spastic CP over 3 weeks of LLLT [20]. However, the study did not determine the effects of LLLT on the thickness of the masseter muscle, which may explain the increase in mouth opening and the decrease of the masseter tonus.

A systematic review of the literature revealed no studies after searching for published articles containing the terms masseter muscle thickness, low-level laser therapy/photobiomodulation therapy, and cerebral palsy. In this context, the aims of the present study were as follows: (i) to evaluate the effect of PBMT on bilateral masseter thickness and the amplitude of mouth opening in children with spastic CP; and (ii) to compare answers to the Parental-Caregiver Perceptions Questionnaire (P-CPQ) before and after PBMT was applied to the bilateral masseter of the children with spastic CP.

It was hypothesized that greater masseter muscle spasticity generates a less thick masseter muscle and that a relaxed masseter muscle tonus would be observed after six applications of PBMT.

## Material and methods

This study was approved by the Human Research Ethics Committee of the Association Assistance to Disabled Children (AACD), São Paulo, Brazil (# 1.103.429). Written informed consent for participation was obtained from the adult caregiver responsible for each patient.

## Study design

This was a three-arm clinical trial, registered with the World Health Organization (Universal Trial Number U1111-1171-2795) and Brazilian Clinical Trials Registry (RBR-5N3MTJ). This longitudinal study was conducted on children with spastic CP who were referred to a specialized center in São Paulo, Brazil, and children without neurological damage from the Pediatric Dentistry Clinic at Cruzeiro do Sul University, São Paulo, Brazil.

## Sample size

Statistical power analysis revealed that with a sample size of 26 subjects to each group and 80% power of detection, a clinically relevant difference would be present at an alpha level of 0.05. This was a superiority study designed to demonstrate that LLLT is more effective (70% chance of success) than no treatment (30% chance of success).

## Subjects

Fifty-two patients were recruited from the Department of Dentistry of the AACD, Ibirapuera Unity, São Paulo, Brazil. The inclusion criteria were (1) age between 5 and 17 years, (2) medical diagnosis of spastic CP (hemiplegic, quadriplegic, or diplegic), and (3) with partially preserved cognitive function. The exclusion criteria included (1) genetic or acquired clinical

conditions (acute or chronic), (2) with muscular impairment, and (3) patients making use of analgesic medication.

These children were divided into two groups: an experimental group (EG), comprising children with spastic CP whose caregivers reported difficulty in maintaining oral hygiene because of diminished mouth opening amplitude, recurrent trauma in the lips, tongue, and cheek, ( $n = 26$ ); and a positive control group (PCG), comprising children with spastic CP whose caregivers did not report difficulties in maintaining oral hygiene, trauma, or feeding practices ( $n = 26$ ).

Twenty-six children without neurological damage, paired by sex and age, composed a negative control group (NCG) and were recruited from the Pediatric Dentistry Clinic at Cruzeiro do Sul University, São Paulo, Brazil.

A flow chart of the progress of this clinical study is included (Fig. 1).

## Ultrasonography

Masseter muscle thickness was measured using ultrasonography examination for all participants. The imaging was conducted by the same radiologist at the Diagnostic Center of the AACD, using a real-time 8-MHz ACUSON X300™ Ultrasound System, Premium Edition (Siemens AG, Healthcare Sector, Erlangen, Germany).

The imaging and measurements were performed with the children in a sedation position, with plane of the head aligned with the rest of the body and the masseter muscle in a relaxed habitual position. A water-based gel (Mercur®, São Paulo, São Paulo, Brazil) was applied to a transducer, and the masseter muscles on both sides were scanned perpendicular to the anterior border of the muscle and the surface of the mandibular ramus, 2–5 cm above the inferior border of the mandible with minimum pressure. The masseter muscle thickness

was measured directly from the image at the time of scanning, in millimeters. For the EG, the ultrasound evaluations of the masseter muscles were conducted before and after the six applications of laser treatment. For the PCG and NCG, ultrasound evaluations were performed only once.

## Photobiomodulation therapy

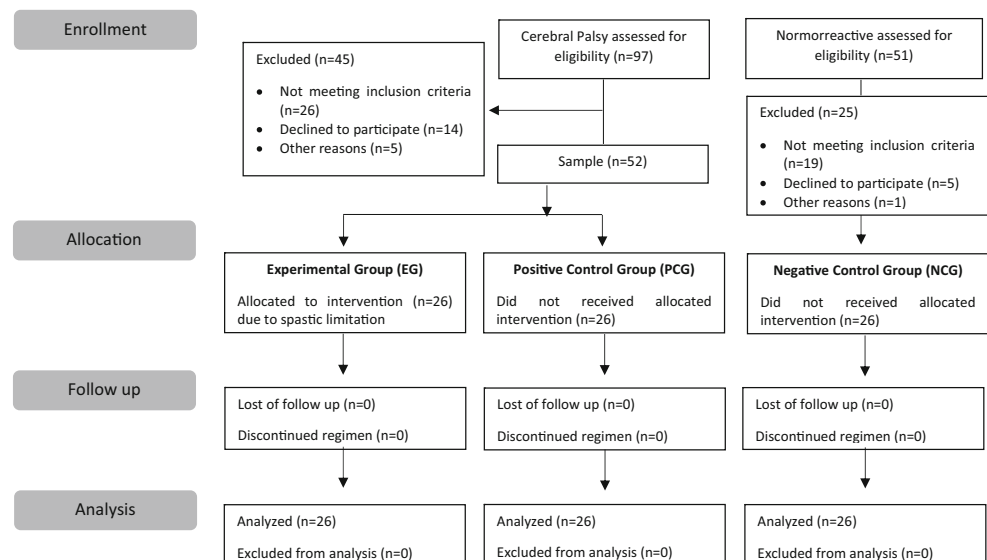
The same calibrated examiner (MTBRS) performed all of the clinical examinations, using the same methodology as described in a previous study by our group [20], who assessed the muscle by palpation through a 2 s application of pressure [21]. The location of PBMT irradiation was the point of greatest contraction determined by palpation.

In the EG, the masseter muscles on both sides of the face were irradiated in the middle of the muscle once a week for six consecutive weeks. Laser irradiation was performed with a continuous wave (CW) infrared low-level Ga-Al-As laser ( $\lambda = 808 \pm 3$  nm, 120 mW, Twin Flex Evolution Laser MMOptics, São Paulo, São Paulo, Brazil), using a  $3 \text{ J/cm}^2$  energy dose per site, with a 20 s exposure time per site (spot area:  $4 \text{ mm}^2$ ; irradiance:  $3 \text{ W/cm}^2$ ; energy delivery per point: 2.4 J). The parameters of PBMT were determined based on a previous study [13].

## Measurement of the amplitude of mouth opening

The amplitude of mouth opening was measured by a caliper [5] (Digimess®, São Paulo, São Paulo, Brazil, capacity 200 mm/8, reproducibility 0.01 mm and accuracy  $\pm 0.03$  mm) whose stems touched the upper and lower incisors, as previously described [20]. The subjects were requested to open their mouths to the maximum amplitude possible.

**Fig. 1** Flow diagram



**Table 1** Descriptive characteristics of children with and without spastic cerebral palsy

Groups					
Variables	EG ( <i>n</i> = 26)	PCG ( <i>n</i> = 26)	NCG ( <i>n</i> = 26)	Total ( <i>n</i> = 78)	<i>P</i> value
Sex, ( <i>n</i> , %)					0.949 <sup>a</sup>
Female	12 (46.2)	13 (50.0)	13 (50.0)	38 (48.7)	
Male	14 (53.8)	13 (50.0)	13 (50.0)	40 (51.3)	
Age in years (mean ± SD)	11.2 ± 4.3	11.1 ± 2.2	10.6 ± 3.4	9.5 ± 2.2	0.826 <sup>b</sup>
Clinical pattern, ( <i>n</i> , %)					<0.001 <sup>a*</sup>
Quadriplegic	24 (92.3)	10 (38.5)	0 (0.0)	34 (65.4)	
Diplegic	2 (7.7)	10 (38.5)	0 (0.0)	12 (23.1)	
Hemiplegic	0 (0.0)	6 (23.0)	0 (0.0)	6 (11.5)	

EG experimental group, PCG positive control group, NCG negative control group

The data were compared with the following: <sup>a</sup> chi-square test, <sup>b</sup> ANOVA, \**P* < 0.05

For the EG, measurement of the amplitude of mouth opening was performed before and after the six applications of PBMT. For the PCG and NCG, the amplitude of mouth opening was evaluated only once.

### Parental-caregiver perception questionnaire

The instrument used in this study was a validated Brazilian version of the **P-CPQ, a 35-item questionnaire [22]. This instrument evaluated the perception of the parents regarding the impact of oral diseases on the quality of life of the participants.** The questions addressed the frequency of oral symptoms/complains in the last 3 months. The items were scored using a five-point Likert scale (response options: *never* = 0, *once or*

*twice* = 1, *sometimes* = 2, *often* = 3, *every day or almost every day* = 4).

Subscale scores were obtained by summing the responses to the following conceptually based, discrete subsets of items: oral symptoms (6 items), functional limitations (8 items), emotional well-being (8 items), and social well-being (11 items).

Parents and caregivers were also asked to provide overall or global assessments of the participants' oral health ("How would you rate the health of your child's teeth, lips, jaws, and mouth?") and the extent to which the oral or orofacial condition in question affected the child's overall well-being ("How much is your child's overall well-being affected by the condition of his/her teeth, lips, jaws, and mouth?"). These global

**Table 2** Measures of right and left masseter thickness and mouth opening amplitude before and after six applications of PBMT for the EG, PCG, and NCG

Groups				
Variables	EG ( <i>n</i> = 26)	PCG ( <i>n</i> = 26)	NCG ( <i>n</i> = 26)	<i>P</i> value
Right masseter				
Before	8.16 ± 0.87a	–	–	
After	8.93 ± 1.18A, b	9.18 ± 1.14A	10.64 ± 2.41B	<i>P</i> < 0.01*
<i>P</i> value	<i>P</i> < 0.001*			
Left masseter				
Before	8.15 ± 0.86a	–	–	
After	8.92 ± 1.25A, b	9.15 ± 1.18A	10.65 ± 2.42B	<i>P</i> < 0.01*
<i>P</i> value	<i>p</i> < 0.001*			
Mouth opening amplitude				
Before	22.4 ± 3.6a	–	–	
After	29.8 ± 4.4A, b	30.5 ± 2.7A	41.04 ± 3.43B	<i>P</i> < 0.01*
<i>P</i> value	<i>P</i> < 0.001*			

Thickness and mouth opening amplitude were measured in millimeters (mm). Different capital letters in the same row indicate a statistically significant difference (ANOVA, Tukey). Different lower case letters in the same column indicate a statistically significant difference (paired *t* test)

EG experimental group, PCG positive control group, NCG negative control group

\**P* < 0.05

ratings were part of the P-CPQ. These questions were answered using a five-point response format, from “excellent” to “poor” for the child’s oral health and from “not at all” to “very much” for overall well-being [23].

Total questionnaire scores and subscales scores are the sum of the numerical response codes. Higher scores indicate worse oral health-related quality of life (OHRQOL) [23].

### Statistical analyses

The primary outcomes of this study were the masseter thickness and amplitude of mouth opening, which were considered as continuous variables. The independent variables were sex (male or female), age (continuous variable in years), and clinical patterns of CP (diplegic or hemiplegic patterns versus quadriplegic pattern). The overall P-CPQ based on the five-point Likert scale responses was considered a discrete variable. A chi-square test, an ANOVA (post hoc Tukey), a paired *t* test (for intragroup), and an unpaired *t* test (intergroup) were performed.

In analyzing the measurements of masseter thickness, it was considered the left and right side masseter muscles; therefore, two measurements were obtained for each subject, and this cluster nature of this data was further considered in the analysis.

In the EG, analyses were performed before and after the six applications of PBMT. Consequently, multilevel analyses were performed. Therefore, for the masseter analysis, three levels were considered: the side of the muscle, the measurements before and after the six PBMT applications, and the group distribution. The measurements underwent a Kolmogorov-Smirnov normality test, and they adhered to a normality curve determined by multilevel linear regression. This approach was adopted to calculate the linear regression coefficients, standard error values, and *P* values using the maximum likelihood estimation. The values were obtained primarily through simple linear regression and group comparisons, and these values were then adjusted in a multiple model for other variables of interest.

A multilevel analysis was also conducted to assess the mouth opening measurements obtained before and after the six PBMT applications. After normal distribution was confirmed using the Kolmogorov-Smirnov test, linear regression multilevel analysis was also performed in the same way as described above.

For the analysis of the different P-CPQ domains, as well as the total score, Poisson multilevel regression analysis was used to calculate between-group *P* values. Estimation of the power analysis for sample size was performed for ANOVA and *t* test. For all analyses, the significance level was set at 5%, and the statistical software Stata 13.0 (Stata Corp LP, College Station, TX, USA) was used.

### Results

The EG, PCG, and NCG did not differ regarding sex ( $P = 0.949$ ) or age ( $P = 0.826$ ). However, the EG presented a higher percentage of quadriplegic children ( $P < 0.001$ ) (Table 1).

The EG presented significantly higher values for right and left masseter thickness ( $P < 0.001$ ) and for amplitude of mouth opening ( $p < 0.001$ ) after the six PBMT applications (intragroup). The EG (after the six PBMT applications) and PCG differed regarding masseter thickness ( $P < 0.001$ ) and amplitude of mouth opening ( $p < 0.001$ ) compared to the NCG, which showed significantly higher values for both variables (Table 2).

The P-CPQ scores of the EG before and after the six PBMT applications decreased significantly ( $P < 0.001$ ) for the subscales of global perception, oral symptoms, functional limitations, and emotional well-being, as well as the total P-CPQ scores. The comparison of P-CPQ scores between the post-treatment EG and the PCG did not reveal differences in any subscale (Table 3).

Unadjusted multilevel analyses for masseter thickness and for amplitude of mouth opening were used to compare the study groups. The multilevel analyses adjusted for age and

**Table 3** Parental-Caregiver Perceptions Questionnaire scores before and after the six applications of PBMT for spastic cerebral palsy groups

P-CPQ domains	EG before	EG after	PCG	<i>P</i> value
Global perception	4.96 ± 1.89a	2.69 ± 0.68b	2.50 ± 1.07b	<0.001*
Oral symptoms	9.46 ± 4.24a	4.85 ± 1.57b	4.62 ± 1.72b	<0.001*
Functional limitations	8.77 ± 3.95a	4.54 ± 2.30b	4.38 ± 3.03b	<0.001*
Emotional well-being	6.85 ± 2.13a	2.31 ± 1.87b	2.38 ± 2.43b	<0.001*
Social well-being	0.81 ± 2.30a	0.77 ± 0.65a	0.73 ± 0.96a	0.985
CPQ total	30.8 ± 7.0a	15.2 ± 4.1b	14.6 ± 6.2b	<0.001*

Different lower case letters in the same row indicate a statistically significant difference. Paired *t* test (for intragroup) and unpaired *t* test (intergroup)

EG experimental group, PCG positive control group

\* $P < 0.05$

**Table 4** Multilevel linear regression for masseter thickness before and after the six applications of PBMT for the EG, PCG, and NCG

Groups	Masseter thickness mean (standard deviation)			Unadjusted $\beta$ (SE)	Adjusted $\beta$ (SE) <sup>b</sup>
	Right	Left	Total		
EG					
Before	8.16 (0.87)	8.15 (0.86)	8.15 (0.85)	-0.76 (0.08) <sup>a</sup>	-0.77 (0.08) <sup>a</sup>
After	8.93 (1.18)	8.92 (1.25)	8.92 (1.20)	Ref.	Ref.
PCG	9.18 (1.14)	9.15 (1.18)	9.17 (1.15)	0.24 (0.44)	0.28 (0.37)
NCG	10.64 (2.41)	10.65 (2.42)	10.65 (2.39)	1.73 (0.44) <sup>a</sup>	1.87 (0.37) <sup>a</sup>

EG experimental group, PCG positive control group, NCG negative control group,  $\beta$  coefficient of multilevel linear regression, SE standard error

<sup>a</sup> Statistically significant at 5%

<sup>b</sup> Adjusted for age and masseter muscle side

the side of the masseter muscle demonstrated that the EG presented an average increase of 0.77 mm in masseter thickness after the six applications of PBMT, whereas the NCG presented an increase of 1.87 mm in masseter thickness (Table 4). The multilevel analyses adjusted for age demonstrated that the EG presented an average increase of 7.39 mm for amplitude of mouth opening after the six applications of PBMT, whereas the NCG presented an increase of 11.68 mm in the amplitude of mouth opening (Table 5).

Figures 2, 3, and 4 show the ultrasonography evaluations of thickness of the masseter muscles in both sides of the face in the EG (before and after treatment), PCG, and NCG, respectively. The EG presented a lower masseter thickness before treatment (right = 7.3 mm and left = 7.6 mm) when compared to the PCG (right = 8.2 mm and left = 8.2) and NCG (right = 13.0 mm and left = 13.0 mm). After PBMT, the EG presented higher masseter thickness (right = 8.2 mm and left = 8.3 mm).

## Discussion

This study shows for the first time the therapeutic efficacy of PBMT on spastic masseter muscle thickness, evaluated using

ultrasonography, as well as improvement of OHRQOL in individuals with CP with masticatory muscle hypertonia.

Ultrasonography has been described as an effective, non-invasive, and accurate method for evaluating masseter muscle thickness in subjects with myositis [24]. This methodology was adopted in the current study, and all of the participants permitted the evaluations, considering the presence of neurological damage. In addition to the measurement of masseter thickness, this examination enables the evaluation of internal muscle structure. In this study, substitution of muscular fiber by conjunctive tissue was observed in three subjects, necessitating a discussion with the rehabilitation team regarding the importance of spastic masticatory muscles, because such changes are irreversible.

Muscle spasticity causes stiffness, imprecise movement, compromised oral motor and speech functions [1], and pain [4]. Therefore, new modalities for spasticity treatment are necessary. Conventional treatment using anti-spastic drugs [10], neuromuscular blocking [11], the most up-to-date intrathecal baclofen [25], repetitive transcranial magnetic stimulation [26], and a combination of prolonged passive muscle stretching and whole body vibration [27] remain the focus of attention in this field of knowledge. However, all of these treatment options present limitations related to side effects, high cost of treatment, or the necessity for constant dose adjustment.

**Table 5** Multilevel linear regression for mouth opening before and after the six applications of PBMT for the EG, PCG, and NCG

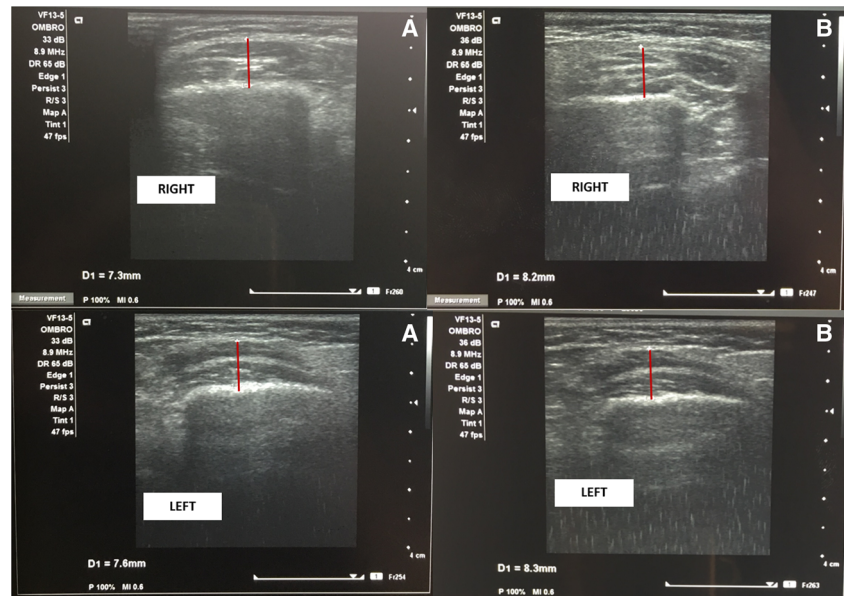
Groups	Mouth opening mean (SD)	Unadjusted $\beta$ (SE)	Adjusted $\beta$ (SE) <sup>b</sup>
EG			
Before	22.4 (3.6)	-7.39 (0.61) <sup>a</sup>	-7.39 (0.58) <sup>a</sup>
After	29.8 (4.4)	Ref.	Ref.
PCG	30.5 (2.7)	0.73 (0.95)	0.80 (0.84)
NCG	41.2 (3.4)	11.40 (0.95) <sup>a</sup>	11.68 (0.37) <sup>a</sup>

EG experimental group, PCG positive control group, NCG negative control group,  $\beta$  coefficient of multilevel linear regression, SE standard error

<sup>a</sup> Statistically significant at 5%

<sup>b</sup> Adjusted for age

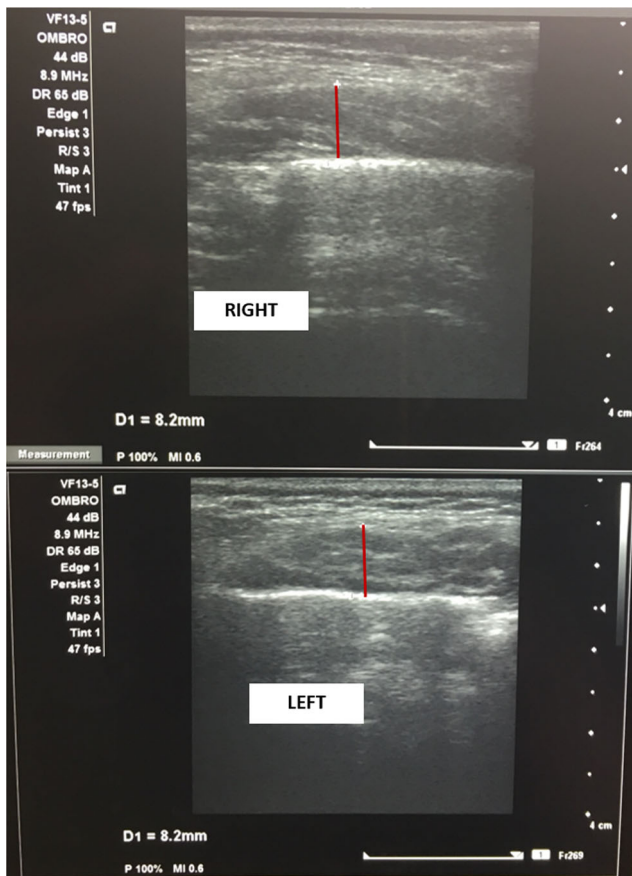
**Fig. 2** Masseter thickness in millimeters before (a) and after (b) PBMT in an 8-year-old spastic CP children (EG)



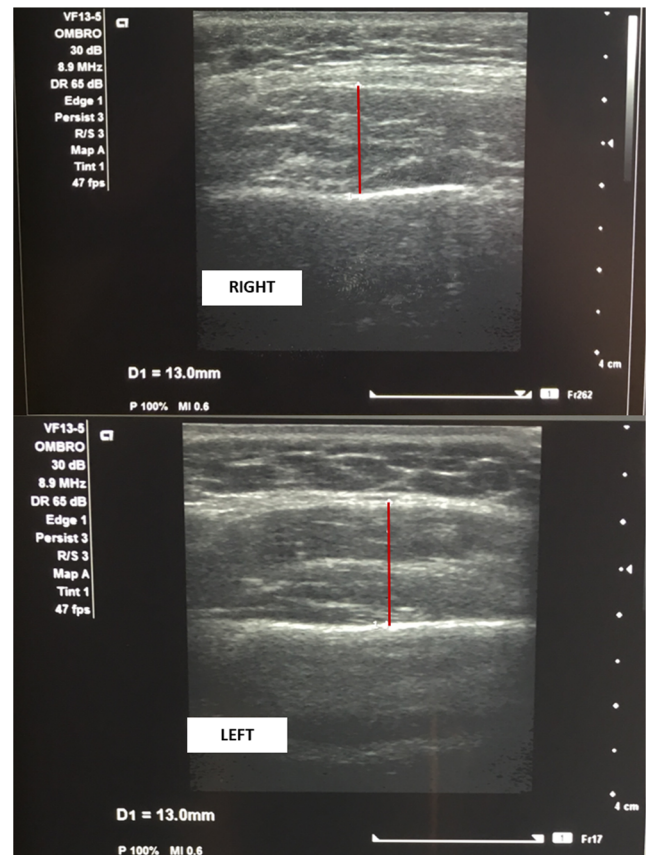
Non-ambulatory children with spastic CP and a quadriplegic clinical pattern have been described as more prone to episodes of musculoskeletal pain, with greater intensity, frequency, and duration [28]. The EG comprised a majority of

subjects with a quadriplegic clinical pattern and whose caregivers reported higher levels of oral symptoms and functional limitations before PBMT.

PBMT seems to be a promising non-invasive therapy, delaying the development of fatigue and preservation against



**Fig. 3** Masseter thickness in millimeters in 11-year-old spastic CP children (PCG)



**Fig. 4** Masseter thickness in millimeters in 13-year-old children without CP (NCG)

muscle injuries, improving muscular performance [17–19, 29, 30]. The PBMT proved to be an effective treatment option for the spastic masseter muscles of the subjects with CP. For the EG, the values of masseter muscle thickness before treatment were lower than after the six applications of PBMT. A possible explanation for this observation is that the spasticity in the masseter muscles increased the tonus and was responsible for its contraction, and the six applications of PBMT promoted inflammation reduction, increasing the masseter thickness and amplitude of mouth opening. The results obtained in this study are in accordance with the properties of PBMT as a treatment for injury-induced inflammation and disorders of the central nervous system [31].

It is essential to improve the knowledge for the use of PBMT in this population. Thus, the masseter muscles of the patients in this study were irradiated using an 808 nm infrared low-level laser that enhanced neuropeptide substance P (SP) secretion compared to blue LED, red LED, and red laser irradiation [32]. SP is released from the terminals of specific sensory nerves and is associated with inflammatory processes and pain. Its action can suppress inflammation and mobilize stem cells to exert anti-inflammatory effects by inducing regulatory T cells and M2 macrophages, increasing interleukin-10 production, and reducing tumor necrosis factor alpha concentration *in vivo* and *in vitro* [22]. It is also pertinent to discuss the neurosuppressive effect of infrared laser energy on the hippocampus with an increase of GABA [33]. This PBMT activity may explain the significant reduction in the majority of the subscale scores evaluated using the P-CPQ compared to the pre-treatment values.

The caregivers' perception of the OHRQOL was investigated in this study because of the severity of the impairment of this ability in CP children. Statistically higher values were observed for the subscales of global perception, oral symptoms, functional limitations, and emotional well-being in the pre-treatment EG, indicating the impact of the muscle spasticity and oral pain in this group [34]. The subscale of social well-being did not change after treatment, because unlike other people with special needs, individuals with CP have social conviviality only during their rehabilitation treatment, such as music therapy, arts, toys for use in play therapy, and clown and superhero visits. The subscales of oral symptoms and functional domains were the most affected by CP before treatment, as shown by Abanto et al. [35] and Baens-Ferrer et al. [36] with a variety of daily problems.

In this study, PBMT was applied with the same laser parameters as used previously [20], only changing the interval between applications: from six applications over 3 weeks to six applications over 6 weeks (once a week). The reasoning for modifying the intervals between applications was based on our previous results, when spastic CP subjects were followed for more than 3 weeks without laser applications. In that study, after the fourth week, the positive effects of PBMT decreased

until the values were similar to the initial pre-treatment values for amplitude of mouth opening and bite force. Thus, we chose to irradiate for 6 weeks using PBMT, making it possible to achieve an increase in the metabolic pattern of muscle fibers [37].

The limitations of this study are related to the fact that the CP participants could not be randomized to receive the PBMT for ethical reasons; this is because they are part of a special group according to the Brazilian Institutional Review Board and must be treated when presenting symptoms. Another limitation was the impossibility of conducting long-term follow-up of all subjects. Future randomized double-blind clinical trial is critical to establish an ideal interval between PBMT applications to maintain the long-term effects of this therapeutic technical procedure. However, it should be stated that eight subjects are being followed for 5 months after the end of the study protocol, and they are receiving PBMT at 3-week intervals. All of the subjects exhibit the same beneficial effects of PBMT regarding quality of life reported by the caregivers, even though no ultrasonography or amplitude of mouth opening evaluations are being performed. The great advantage of these findings is the importance to dental surgeons who treat individuals with neurological sequelae, because PBMT showed benefits, being non-invasive, inexpensive, and without side effects.

Future investigations are recommended in order to evaluate different optimal doses of PMBT with specific wavelength and combination of different light sources used synergistically to improve the effects on muscle fatigue and performance, as previous suggested by Santos et al. [30] and Antonialli et al. [38].

## Conclusion

The six applications of PBMT with an 808 nm CW diode laser increased masseter thickness, and the amplitude of mouth opening, and reduced the impact of spastic CP on OHRQOL.

**Acknowledgments** This study was supported by the São Paulo Research Foundation (Fundação de Amparo à Pesquisa do Estado de São Paulo), FAPESP #2014-15662-1.

## Compliance with ethical standards

**Ethics statement** All experiments were conducted and approved by the Human Research Ethics Committee of the Association Assistance to Disabled Children (AACD), São Paulo, Brazil (# 1.103.429).

All participants provided their written consent to participate in the study. The written consent form had been approved by the ethics committee.

The study has been registered with the World Health Organization (Universal Trial Number U1111-1171-2795) and Brazilian Clinical Trials Registry assigned under number RBR-5N3MTJ.



**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Rosenbaum P, Paneth N, Leviton A, Goldstein M, Bax M, Damiano D, Dan B, Jacobsson B (2007) A report: the definition and classification of cerebral palsy April 2006. *Dev Med Child Neurol Suppl* 109:8–14S
- Ronan S, Gold JT (2007) Nonoperative management of spasticity in children. *Childs Nerv Syst* 23:943–956
- Koman LA, Smith BP, Shilt JS (2004) Cerebral palsy. *Lancet* 15: 1619–1631
- Gracies JM (2005) Pathophysiology of spastic paresis. I: paresis and soft tissue changes. *Muscle Nerve* 31:535–551
- Manzano FS, Granero LM, Masiero D, dos Maria TB (2004) Treatment of muscle spasticity in patients with cerebral palsy using BTX-A: a pilot study. *Spec Care Dentist* 24:235–239
- dos Santos MT, de Oliveira LM (2004) Use of cryotherapy to enhance mouth opening in patients with cerebral palsy. *Spec Care Dentist* 24:232–234
- Santos MT, Manzano FS, Genovese WJ (2008) Different approaches to dental management of self-inflicted oral trauma: oral shield, botulinum toxin type A neuromuscular block, and oral surgery. *Quintessence Int* 39:e63–e69
- Santos MT, Manzano FS, Chamlian TR, Masiero D, Jardim JR (2010) Effect of spastic cerebral palsy on jaw-closing muscles during clenching. *Spec Care Dentist* 30:163–167
- Botti Rodrigues Santos MT, Duarte Ferreira MC, de Oliveira GR, Guimarães AS, Lira Ortega A (2015) Teeth grinding, oral motor performance and maximal bite force in cerebral palsy children. *Spec Care Dentist* 35:170–174
- Chung CY, Chen CL, Wong AM (2011) Pharmacotherapy of spasticity in children with cerebral palsy. *J Formos Med Assoc* 110: 215–222
- Rabchevsky AG, Kitzman PH (2011) Latest approaches for the treatment of spasticity and autonomic dysreflexia in chronic spinal cord injury. *Neurotherapeutics* 8:274–282
- Anders JJ, Lanzafame RJ, Arany PR (2015) Low-level light/laser therapy versus photobiomodulation therapy. *Photomed Laser Surg* 33:183–184
- Casalechi HL, Leal-Junior EC, Xavier M, Silva JA Jr, de Carvalho PT, Aimbire F, Albertini R (2013) Low-level laser therapy in experimental model of collagenase-induced tendinitis in rats: effects in acute and chronic inflammatory phases. *Lasers Med Sci* 28:989–995
- Tomazoni SS, Leal-Junior EC, Pallotta RC, Teixeira S, de Almeida P, Lopes-Martins RA (2017) Effects of photobiomodulation therapy, pharmacological therapy, and physical exercise as single and/or combined treatment on the inflammatory response induced by experimental osteoarthritis. *Lasers Med Sci* 32:101–108
- da Rosa AS, dos Santos AF, da Silva MM, Facco GG, Perreira DM, Alves AC, Leal Junior EC, de Carvalho PT (2012) Effects of low-level laser therapy at wavelengths of 660 and 808 nm in experimental model of osteoarthritis. *Photochem Photobiol* 88:161–166
- Albuquerque-Pontes GM, Vieira RP, Tomazoni SS, Caires CO, Nemeth V, Vanin AA, Santos LA, Pinto HD, Marcos RL, Bjordal JM, de Carvalho PT, Leal-Junior EC (2015) Effect of pre-irradiation with different doses, wavelengths, and application intervals of low-level laser therapy on cytochrome c oxidase activity in intact skeletal muscle of rats. *Lasers Med Sci* 30:59–66
- de Paiva PR, Tomazoni SS, Johnson DS, Vanin AA, Albuquerque-Pontes GM, Machado CD, Casalechi HL, de Carvalho PT, Leal-Junior EC (2016) Photobiomodulation therapy (PBMT) and/or cryotherapy in skeletal muscle restitution, what is better? A randomized, double-blinded, placebo-controlled clinical trial. *Lasers Med Sci* 31:1925–1933
- Vanin AA, Miranda EF, Machado CS, de Paiva PR, Albuquerque-Pontes GM, Casalechi HL, de Tarso Camillo de Carvalho P, Leal-Junior EC (2016) What is the best moment to apply phototherapy when associated to a strength training program? A randomized, double-blinded, placebo-controlled trial: phototherapy in association to strength training. *Lasers Med Sci* 31:1555–1564
- Leal-Junior EC, Vanin AA, Miranda EF, de Carvalho PT, Dal Corso S, Bjordal JM (2015) Effect of phototherapy (low-level laser therapy and light-emitting diode therapy) on exercise performance and markers of exercise recovery: a systematic review with meta-analysis. *Lasers Med Sci* 30:925–939
- Santos MT, Diniz MB, Gouw-Soares SC, Lopes-Martins RA, Frigo L, Baeder FM (2016) Evaluation of low-level laser therapy in the treatment of masticatory muscles spasticity in children with cerebral palsy. *J Biomed Opt* 21:28001
- Schiffman E, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, List T, Svensson P et al (2014) Diagnostic criteria for temporomandibular disorders (DC/TMD) for clinical and research applications: recommendations of the international RDC/TMD consortium network\* and orofacial pain special interest group†. *Journal of oral & facial pain and headache* 28:6–27
- Barbosa TS, Steiner-Oliveira C, Gavião MBD (2010) Tradução e adaptação brasileira do Parental-Caregiver Perceptions Questionnaire (P-CPQ). *Saúde e Sociedade* 19:698–708
- Barbosa TS, Tureli MC, Gavião MB (2009) Validity and reliability of the Child Perceptions Questionnaires applied in Brazilian children. *BMC Oral Health* 18:9–13
- Rai S, Ranjan V, Misra D, Panjwani S (2016) Management of myofascial pain by therapeutic ultrasound and transcutaneous electrical nerve stimulation: a comparative study. *Eur J Dent* 10:46–53
- Bonouvrié L, Becher J, Soudant D, Buizer A, van Ouwkerk W, Vles G, Vermeulen RJ (2016) The effect of intrathecal baclofen treatment on activities of daily life in children and young adults with cerebral palsy and progressive neurological disorders. *Eur J Paediatr Neurol* 20:538–544
- Gupta M, Lal Rajak B, Bhatia D, Mukherjee A (2016) Effect of r-TMS over standard therapy in decreasing muscle tone of spastic cerebral palsy patients. *J Med Eng Technol* 40:210–221
- Tupimai T, Peungsuwan P, Prasertnoo J, Yamauchi J (2016) Effect of combining passive muscle stretching and whole body vibration on spasticity and physical performance of children and adolescents with cerebral palsy. *J Phys Ther Sci* 28:7–13
- Barney CC, Krach LE, Rivard PF, Belew JL, Symons FJ (2013) Motor function predicts parent-reported musculoskeletal pain in children with cerebral palsy. *Pain Res Manag* 18:323–327
- Pinto HD, Vanin AA, Miranda EF, Tomazoni SS, Johnson DS, Albuquerque-Pontes GM, Junior AIO, Grandinetti VD, Casalechi HL, de Carvalho PT, Leal-Junior EC (2016) Photobiomodulation therapy improves performance and accelerates recovery of high-level rugby players in field test: a randomized, crossover, double-blind, placebo-controlled clinical study. *Journal of strength and conditioning research* 30:3329–3338
- Santos LA, Marcos RL, Tomazoni SS, Vanin AA, Antoniali FC, Grandinetti Vdos S, Albuquerque-Pontes GM, de Paiva PR, Lopes-Martins RA, de Carvalho PT, Bjordal JM, Leal-Junior EC (2014) Effects of pre-irradiation of low-level laser therapy with different doses and wavelengths in skeletal muscle performance, fatigue, and skeletal muscle damage induced by tetanic contractions in rats. *Lasers Med Sci* 29:1617–1626
- Jiang MH, Chung E, Chi GF, Ahn W, Lim JE, Hong HS, Kim DW, Choi H, Kim J, Son Y (2012) Substance P induces M2-type macrophages after spinal cord injury. *Neuroreport* 23:786–792

32. Hochman B, Pinfildi CE, Nishioka MA, Furtado F, Bonatti S, Monteiro PK, Antunes AS, Quieregatto PR, Liebano RE, Chadi G, Ferreira LM (2014) Low-level laser therapy and light-emitting diode effects in the secretion of neuropeptides SP and CGRP in rat skin. *Lasers Med Sci* 29:1203–1208
33. Ahmed NA, Radwan NM, Ibrahim KM, Khedr ME, El Aziz MA, Khadrawy YA (2008) Effect of three different intensities of infrared laser energy on the levels of amino acid neurotransmitters in the cortex and hippocampus of rat brain. *Photomed Laser Surg* 26:479–488
34. Breau LM, Camfield CS, McGrath PJ, Finley GA (2003) The incidence of pain in children with severe cognitive impairments. *Arch Pediatr Adolesc Med* 157:1219–1226
35. Abanto J, Carvalho TS, Bönecker M, Ortega AO, Ciamponi AL, Raggio DP (2012) Parental reports of the oral health-related quality of life of children with cerebral palsy. *BMC Oral Health* 18:12–15
36. Baens-Ferrer C, Roseman MM, Dumas HM, Haley SM (2005) Parental perceptions of oral health-related quality of life for children with special needs: impact of oral rehabilitation under general anesthesia. *Pediatr Dent* 27:137–142
37. Rizzi EC, Issa JP, Dias FJ, Leão JC, Regalo SC, Siéssere S, Watanabe IS, Iyomasa MM (2010) Low-level laser intensity application in masseter muscle for treatment purposes. *Photomed Laser Surg Suppl* 2:S31–S35
38. Antonialli FC, De Marchi T, Tomazoni SS, Vanin AA, dos Santos GV, de Paiva PR, Pinto HD, Miranda EF, de Tarso Camillo de Carvalho P, Leal-Junior EC (2014) Phototherapy in skeletal muscle performance and recovery after exercise: effect of combination of super-pulsed laser and light-emitting diodes. *Lasers Med Sci* 29: 1967–1976