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Effects of manual cranial therapy on heart rate variability in children without associated disorders: Translation to clinical practice

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A B S T R A C T

Background: and purpose: Heart rate variability (HRV) represents a marker of autonomic activity, self-regulation and psychiatric illness. Few studies of manual therapy have investigated the neurophysiological effects of manual cranial therapy (MC-t). This study assessed the neurophysiological short/medium-term effects of two manual therapy interventions: massage therapy (Mss-t) and MC-t.

Materials and methods: A double-blind clinical trial was conducted with 50 healthy children, randomized into two groups who received a Mss-t intervention or MC-t. The variables analysed included vital signs (temperature, respiratory rate, heart rate, blood pressure) and HRV components, including the root mean square of successive differences (RMSSD), high frequency (HF), low frequency (LF) and LF/HF ratio.

Results: Both interventions produced short-term parasympathetic effects, although the effects of MC-t were more persistent.

Conclusion: The persistence of the MC-t intervention suggested a prominent vagal control and better self-regulation. Autonomic imbalances in mental pathologies may benefit from the neurophysiological effects of MC-t.

1. Introduction

The autonomic nervous system (ANS) controls unconscious body functions by innervating cardiac and smooth muscles, as well as exocrine and endocrine glands, through three main efferent pathways: sympathetic, parasympathetic, and enteric pathways. The equilibrium between the sympathetic and parasympathetic nervous systems is responsible for maintaining homeostasis and regulating adaptive responses to internal/external environmental changes. Sympathetic activation promotes a 'defence' response, while parasympathetic activation is related to protective and relaxation functions [1]. Mental and internal bodily process interactions are known to maintain body systems within narrow homeostatic bounds while allowing a range of different stable physiological states that are necessary to effectively address changing cognitive and environmental demands [2].

Homeostasis is a dynamic self-regulatory physiological process that aims to achieve a balance between two opposing sets of factors. The human body is a self-adapting system, and as a result of this ability to adapt, new physiological 'steady states' can be achieved and maintained, even in the presence of diseases [3]. Self-regulation refers to automatic regulatory processes, and research on this topic has focused on identifying underlying correlates of self-regulation and reflecting self-regulation capacity at a physiological level; heart rate variability (HRV) has been suggested to serve as a marker of self-regulation [4].

HRV refers to beat-to-beat variations in the heart rate (HR) and reflects the interplay between sympathetic and parasympathetic influences on the HR; HRV seems to be an indicator of ANS flexibility, which is necessary to modulate cardiac activity according to changing situational demands [5]. A reduced HRV corresponds to an ANS imbalance and may be associated with a worse prognosis, whereas a high HRV is associated with a good prognosis in healthy subjects and patients with various diseases [6,7].

Nonetheless, a healthy heart beat ratio is not absolutely regular and varies as a result of many factors, including exercise, physical and mental stress, respiration, blood pressure regulation, and other currently unknown factors [6,7] (see Table 1). As multiple factors may influence autonomic function, standardized test conditions are necessary for comparison [8].

ANS dysfunction involves clinical manifestations that are often undervalued because of their subjectivity, transient nature (in healthy subjects), and difficult evaluation, but autonomic symptoms are important due to their diagnostic implications, effects on quality of life

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Table 1
Factors associated with variations in heart rate variability.

Factors	Type of relationship with HRV
Age [86]	Inverse: advanced age. Correlation with a lower HRV
Sex [87]	Inverse: men. Correlation with a lower HRV Direct: young and pre-menopausal women. Correlation with a higher HRV
Thermoregulatory response [1,3]	Inverse: increase body temperature, increase sweating (to lose heat), and predominance of sympathetic activity. Correlation with a lower HRV Direct: decrease body temperature, decrease sweating (to conserve heat), and predominance of parasympathetic activity. Correlation with a higher HRV
Genetic factors [88]	Inverse: correlation with a lower HRV
Cardiovascular risk factors (metabolic syndrome, smoking, caffeine, alcoholism) [6,16,54]	Inverse: correlation with a lower HRV
Environmental factors [89]	Inverse: hypoxia, extreme temperatures, altitude. Correlation with a lower HRV Direct: acclimatization. Correlation with a higher HRV
Circadian rhythms (sleep-wake cycles) [12,90]	Inverse: insomnia and REM phase. Correlation with a lower HRV Direct: restful sleep and non-REM sleep phase. Correlation with a higher HRV
Physical exercise and lifestyle [14,73,90,91]	Inverse: sedentary lifestyle, overtraining, physical stress. Correlation with a lower HRV Direct: active life style, regular moderate-intensity physical activity, aerobic fitness, submaximal exercise. Correlation with a higher HRV
Postural changes and gravitational stress [92]	Inverse: standing position. Correlation with a lower HRV Direct: supine position, rest. Correlation with a higher HRV
Drugs [93]	Inverse and Direct (not clear evidence): anticholinergics, alpha and beta-adrenergic blockers, adrenergic agonists, drugs associated with ACE. Correlation to a lower and higher HRV
Cognitive functions and emotional intelligence [2,5,7,14,21,50–58]	Inverse: mental stress; burnout; fatigue; pessimism; physical, cognitive and social stressors; psychiatric conditions. Correlation with a lower HRV Direct: development of executive functions and operational memory, cognitive performance, emotional intelligence skills, optimism, mental health. Correlation with higher HRV
Cardiovascular diseases [6,16,54], neurological [94], psychiatric [7,50–58] and rheumatological diseases [69, 70, 95, 96]	Inverse: correlation with a lower HRV
Physiological manifestations: breathing [18,76,78]	Inverse: higher respiratory rate. Correlation with a lower HRV Direct: greater tidal volume. Correlation with a higher HRV
Physiological manifestations: blood pressure [82,83]	Inverse: higher blood pressure. Correlation with a lower HRV
Physiological manifestations: heart rate [76,79,80]	Inverse: higher heart rate. Correlation with a lower HRV
Neurophysiological manifestations of the autonomic nervous system [4,6–11,15,15,21]	Inverse: predominance of sympathetic activity, increase of LF power, increase of LF/HF ratio. Correlation with a lower HRV Direct: predominance of parasympathetic activity, increase of SDNN, RMSSD, PNN50 and HF power. Correlation with higher HRV
Specific interventions of manual therapy [25–35]	Inverse and Direct (not clear evidence): Manual therapy treatments (physiotherapy techniques, manipulations, relaxation techniques, neurodynamics) most confirm direct relationship. Correlation with higher HRV

Type of relationship between the different factors that potentially influence heart rate variability.
Acronyms and abbreviations: Heart rate variability (HRV); rapid eye movement (REM); angiotensin-converting enzyme (ACE); low frequency power (LF); ratio of low frequency (LF) power/high frequency (HF) power (LF/HF ratio); standard deviation from normal to normal (NN) intervals in heartbeats (SDNN); root mean square of successive differences (RMSSD); percentage of the number of NN interval pairs that differ by more than 50 ms (pNN50); high frequency power (HF).

and prognostic significance [1].

Although a clear consensus regarding measures for analysing HRV has not been reached [9,10], several linear (time and frequency domains) and non-linear techniques have been developed to assess ANS function [8,11]. HRV measures in the time and frequency domains (Table 2) have been successfully used as an index of cardiovascular autonomic nervous system activity [8,11], providing information about adaptations to physical and psychological stress, as well as regulation of vegetative activity and relaxation [12–14].

Twenty years ago, the Task Force of Cardiology offered the first solid foundation for HRV research, providing information on which HRV parameters should be considered, defining the standard methods of measurement and describing the most appropriate clinical applications [15]. Other researchers [16] subsequently studied the association between psychophysiology and HRV, developing the following emergent theoretical models: the neurovisceral integration model [7], polyvagal theory [17], biological behavioural model [18], resonance frequency model [19], and psychophysiological coherence model [20]. All these HRV bio-behavioural theories have a common background: their focus on vagal tone [21].

For many years, investigations on physical therapy (PT) and manual therapy (MT) techniques focused on understanding their physio-psychological mechanisms and their clinical effects [22,23]; currently, an increasing number of researchers are investigating different MT types and their effects on the ANS. The proposed mechanism of action of MT focuses on anatomical, physiological and biomechanical changes generated by different MT modalities that cause autonomic activation [22]. HRV has been used to evaluate autonomic HR control under several conditions, but only recently in the MT and PT context, although a consensus regarding the stimulatory or inhibitory effects has not yet been reached [24–35].

Giles et al. [27] focused their research on the relationship between the vagus nerve (VN) and musculoskeletal occiput structures with vagal functions using HRV. The VN is a mixed nerve (10th cranial nerve) joining the glossopharyngeal and accessory nerves to constitute a system with a primarily parasympathetic function. It regulates the motor functions of the larynx, diaphragm, stomach and heart and sensory functions of the ears, tongue and visceral organs, including the liver. As all VN branches with visceral efferent fibres contain afferent sensory fibres, the VN is a highly sensitive nerve [36]. Milnes and Moran [28] investigated the physiological effects of a single cranial technique by analysing the HRV, respiration rate, galvanic skin resistance and skin temperature. Henley et al. [40] studied the association between the cervical myofascial release technique and the ANS by measuring HRV. The relationship between the manual manipulation site (the cervical, thoracic, or lumbosacral region) and its influence on the autonomic HRV response has also been investigated [26,29–33]. Vanoli et al. analysed factors associated with HRV fluctuations after pharmacological and non-pharmacological interventions and their relationships with health status, prognosis, and treatment strategies in

Table 2
Time and frequency domain parameters of heart rate variability.

Variable	Description	Reference values in healthy adults	Reference values in healthy children	Interpretation
HRV, time domain				
RR or NN Interval	Interval between two beats (R peaks on the el EKG)	> 750 ms		High correlation with high frequencies and indicates vagal tone (parasympathetic activity)
SDNN	Standard deviation from normal to normal (NN) intervals in heartbeats	141 ± 39 ms	5–15 years: 48.603–92.96 ms 15–35 years: 38.87531–81.309 ms	Cyclic components responsible for HRV. Global measurement of HRV
RMSSD	Square root of the mean of the sum of the squares of the differences between consecutive NN intervals	27 ± 12 ms	5–15 years: 65.6591–88.482 ms 15–35 years: 62.338–80.189 ms	High correlation with high frequencies and indicates vagal tone (parasympathetic activity)
NN50	Number of NN interval pairs that differ by more than 50 ms	ms		High correlation with high frequencies and indicates vagal tone (parasympathetic activity)
pNN50	Percentage of the number of NN interval pairs that differ by more than 50 ms	%		High correlation with high frequencies and indicates vagal tone (parasympathetic activity)
HRV, frequency domain				
HF power (0.15–0,40 Hz)	High frequency band	975 ± 203 ms^2/Hz		Relationship with vagal tone or parasympathetic activity and respiratory activity; correlation with RMSSD and pNN50
LF power (0.04–0,15 Hz)	Low frequency band	1170 ± 416 ms^2/Hz		Mix of sympathetic and vagal activity, baroreflex activity
VLF (0.0033–0.04 Hz)	Very low frequency band	ms^2		Long-term regulation mechanisms, thermoregulation and the renin-angiotensin system
LF/HF ratio	Ratio LF (ms^2)/HF (ms^2)	1.5–2.0	5–15 years: 1.0591–1.425 15–35 years: 0.88745–1.268	Estimation of sympathetic-vagal balance: vagal influence (related to relaxation and HF) or sympathetic (related to stress and LF).

Description of variables in the time and frequency domains of heart rate variability [8,11,15,16,21].
Acronyms and abbreviations: Heart rate variability (HRV); interval between two consecutive beats (RR or NN); standard deviation from normal to normal (NN) intervals in heartbeats (SDNN); root mean square of successive differences (RMSSD); number of NN interval pairs that differ by more than 50 ms (NN50); percentage of the number of NN interval pairs that differ by more than 50 ms (pNN50); high frequency power (HF); low frequency power (LF); very low frequency power (VLF); ratio of low frequency (LF) power/high frequency (HF) power (LF/HF ratio); milliseconds (ms); electrocardiogram (EKG); Hertz (Hz).

patients with cardiovascular and non-cardiovascular pathologies [41].

Different PT and MT techniques have shown efficacy for certain mental disorders, offering non-pharmacological therapeutic options in a multidisciplinary approach [42–49].

HRV has also been extensively investigated in patients with psychiatric disorders and has been defined as a neurobiological marker of psychiatric illness [50] as HRV variations coexist with altered psychological phenomena, such as social cognition and executive function [21,51–53]. Different psychiatric disorders [54–58] are associated with a reduction in baseline HRV [21,51–53,55]. Because HRV and the clinical features of many psychiatric diseases are related, new and effective treatments designed to concomitantly increase HRV and improve symptoms are being explored.

Few studies have investigated the neurophysiological effects of MT on the cranial field. The relations between the anatomical VN and the musculoskeletal, membranous and myofascial structures in the suboccipital region, from the VN's exit through the jugular foramen and along its route, lend credence to the hypothesis that MT treatments in this location may exert effects on vagal or parasympathetic activity [27,37–39].

The autonomic dysfunctions involved in some mental pathologies represent an opportunity to study the neurophysiological effects of PT and MT [51,52].

The aim of this study was to analyse the effects of two short-term MT programmes (before and after a single intervention) on neurophysiological variables in healthy children and to compare the effects with those of a neutral programme. Additionally, the medium-term persistence of the effects was analysed weekly for up to four weeks post-intervention. The safety of the interventions was studied to translate the outcomes to psychiatric patients with autonomic imbalance.

2. Materials and methods

2.1. Ethics statement

The Parc Tauli Corporation Clinical Research Ethics Board approved this study (#2017311).

2.2. Design and setting

A double-blind, randomized, controlled clinical trial was conducted with healthy children (evaluation: the Swanson, Nolan and Pelham—SNAP-IV—questionnaire). The participants did not know which MT intervention they had received. A clinical psychologist (who collected the variables) also did not know which interventions were assigned to the participants.

2.3. Selection criteria

2.3.1. Inclusion criteria

Healthy children aged between 7 and 11 years with regular physical activity, a normal education, no known diseases or mental health (MH) disorders, and who had not received any pharmacotherapeutic, psychotherapeutic, physiotherapeutic, osteopathic or physical medicines (because these drugs may influence HRV) were included [21].

2.3.2. Exclusion criteria

Children with a chronic organic pathology were excluded.

2.3.3. Withdrawal criteria

The withdrawal criteria were non-compliance, premature abandonment, and adverse events. Recommendations for intervention trials were followed (Table 3) [59].

2.3.4. Potential confounding variables

Potential confounding variables were tracked by an *ad hoc* questionnaire to allow exclusion of participants who did not meet the criteria, to change data collection dates and to analyse outlier conditions. The following potential confounding variables that may influence HRV were considered: the sleep routine on the day before the intervention was assessed (the hours and quality of sleep were recorded), intense physical exercise was avoided on the same day as the assessment, and meals and caffeinated drinks were avoided at least 2 h before the interventions. The participants were asked to empty their bladder before the experiment, and medications and other simultaneous therapeutic treatments were also recorded [21].

2.4. Recruitment method

Potential candidates and parents/guardians were contacted verbally and by phone at a sports facility. Candidates and parents/guardians attended an informative meeting and then confirmed their intention to participate. In a second meeting, information sheets were delivered and informed consent was obtained according to the Good Clinical Practice guidelines (Declaration of Helsinki of the World Medical Association, 2013) and the applicable legal regulations.

2.5. Sample

The sample size was calculated with the Municipal Institute for Medical Research of the Hospital del Mar GRANMO calculator: paired repeated means in two groups with a 0.05 alpha risk, bilateral contrast, 0.20 beta risk, 0.351 main variable standard deviation (from a previous pilot test), 0.20 minimum difference to detect, and 10% anticipated proportion of loss of participants, resulting in n = 27 participants per group (total sample = 54).

Of the sixty possible candidates, five were excluded due to non-compliance, and another five decided not to participate. The final sample included fifty participants (see Fig. 1).

2.6. Randomization

Once the participants were confirmed and informed consent was obtained, randomization was performed using a simple randomization sequence (www.random.org) to produce two possible intervention groups: intervention group 1 (IG1) and intervention group 2 (IG2). Each participant was assigned a number, and the programme randomized all participants under the criterion 'even number: IG1' and 'odd number: IG2'. The homogeneous group distribution reduced possible confounding factors and sources of bias. Finally, the participants were distributed as follows: IG1: n = 25, 18 boys and 7 girls; and IG2: n = 25, 15 boys and 10 girls.

2.7. Procedure design

According to 'vagal tank theory procedures' [21,60], the study was designed with three time points: baseline (the vagal level before a specific MT intervention), event (the specific MT intervention) and post event (persistence of the effects of weekly MT interventions for up to four weeks). Vagal cardiac control reflects the VN contribution (the main parasympathetic nerve) to cardiac regulation, with parasympathetic activity expressed as vagal tone or vagal activity. The investigation was based on an assessment of the roles of tonic and phasic vagal tone [21,60] (see Fig. 4). Tonic cardiac vagal control is defined by the value of HRV measured at a specific time point and applies to baseline, event and post event HRV. Phasic cardiac vagal control is defined by the difference between measurements at two time points: baseline-event changes, which represent reactivity, and baseline-post event changes, which reflect the recovery process [21,60].

Table 3
SPIRIT 2013 statement chronogram.

	Study period						
	Enrolment	Allocation	Post-allocation				Close-out
			Intervention	Follow-up			
Time point	-t1	0	1 wk	2 wks	3 wks	4 wks	5 wks	3 months
Enrolment:								
Eligibility screen	X							
Informed consent	X							
Randomization process	X							
Allocation		X						
Interventions:								
Intervention group 1: a session of a massage therapy program in cervical, dorsal and lumbar musculature (n = 25)			X					
Intervention group 2: a session of a manual cranial therapy program (n = 25)			X					
Groups 1 and 2 (after washout period): Neutral effect program (n = 50)								X
Assessments and variables:								
Age			X					
Sex			X					
BMI			X					
Body fat percentage			X					
WHR			X					
Joint hypermobility			X					
Somatotype			X					
AT			X	X	X	X	X	X
SaO2			X	X	X	X	X	X
Respiratory rate			X	X	X	X	X	X
HR			X	X	X	X	X	X
BP			X	X	X	X	X	X
HRV (frequency domain: LF/HF ratio)			X	X	X	X	X	X
HRV (frequency domain: LF power)			X	X	X	X	X	X
HRV (frequency domain: HF power)			X	X	X	X	X	X
HRV (time domain: RMSSD)			X	X	X	X	X	X

The SPIRIT 2013 statement chronogram provides recommendations that should be addressed in a clinical trial protocol. It also details the scope and systematic development methods [59].
Acronyms and abbreviations: Body mass index (BMI); waist/hip ratio (WHR); axillary temperature (AT); oxygen saturation (SaO2); heart rate (HR); blood pressure (BP); heart rate variability (HRV); low frequency power (LF); high frequency power (HF); square root of the mean of the sum of the squares of the differences between consecutive NN intervals (RMSSD); week (wk); weeks (wks).

2.8. Manual therapy interventions

All interventions were performed under controlled conditions (humidity, temperature, and atmospheric pressure, with no visual or auditory stimuli). The procedures were performed on the same day of the week at the same time and in the same place to reduce bias.

Once randomization and assignments were completed, the neurophysiological effects of two 25-min interventions, including a massage therapy programme (Mss-t) and a manual cranial therapy programme (MC-t), were studied to assess the hypothesis that the MC-t programme may improve vagal heart control as examined by measuring the HRV parameters linked to parasympathetic activity. A physiotherapist administered all interventions, and a 'blinded' psychologist collected all variables. Before and 10 min after receiving the intervention (Mss-t or MC-t), the psychologist collected each participant's variables in the supine position under resting conditions (the baseline value of each variable). Subsequently, the psychologist collected the same variables weekly for up to four weeks after the intervention (post event) to study the persistence of the effects and to determine when the variables returned to the baseline values. After a washout period of 3 months, all participants were included in a neutral effect group (NG) and remained in a relaxed, supine position for 25 min without receiving any intervention; thus, each participant was under his or her own control. The psychologist collected the variables before and after the participant remained in a relaxed state in the supine position under the same conditions.

2.8.1. IG1: (Mss-t)

A single moderate pressure intervention was applied to the cervical, dorsal and lumbar muscles for 25 min with the participant in the prone position. The hypothesis is that massage provides benefits by changing the ANS from a sympathetic state to a parasympathetic state, although a clear consensus has not been reached [22–25,47].

2.8.2. IG2: (MC-t)

A single intervention including a standard sequence of ten cranial techniques was administered for 25 min with the participant in the supine position to promote joint mobility and relaxation of myofascial and membranous structures and thus induce effects on the ANS [61,62].

Based on VN anatomo-physiological relationships, a sequence of techniques was proposed because each technique influences different VN sections after passing through the jugular foramen. The following sequence of techniques was employed: the CV-4 technique, cranial base release (the suboccipital technique), lumbo-sacral decompression (traction of the dural tube), release of the transverse diaphragms, frontal and parietal lift techniques, temporal techniques (temporal wobble, ear pull), myofascial release of the temporo-mandibular joint, the deep cervical fasciae technique and the CV-4 technique (again).

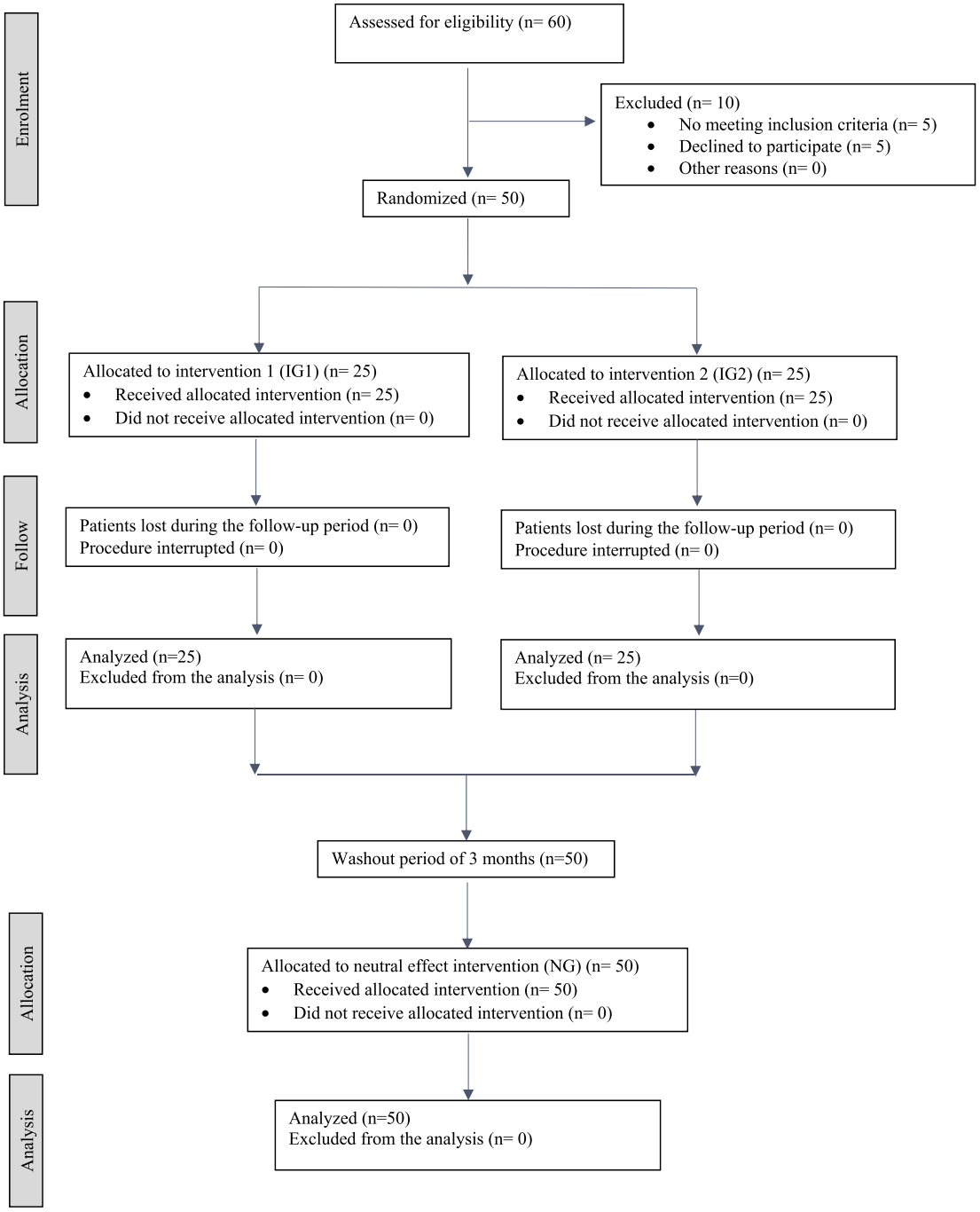


Fig. 1. CONSORT flow diagram.
Acronyms and abbreviations: Intervention group 1 (IG1); intervention group 2 (IG2); neutral group (NG).

These techniques have been reported to exert relaxing effects, lower sympathetic tone, change diaphragm activity and autonomic respiration control, and increase the temperature of the suboccipital region [27,28,34,61–64]. The total time required to apply the ten techniques was 25 min, with each technique requiring approximately 3 min to complete.

An explanation of each technique applied is provided below.

2.8.2.1. The CV-4 technique. The participants were lying in the supine position, and the physiotherapist was located at the head of the bed with his/her elbows on it. Both hands were placed at the lateral protuberances of the squama occipitalis (medially to the occipitomastoid suture) with the thenar eminence. The technique consisted of a compression and a cessation phase and has been described by some authors to follow the rhythmic cranial impulse (osteopathic term) and by others to follow respiratory costal activity when performing occipital compression [28,34,62,65]. During costal expiration, the physiotherapist performed smooth rhythmic occipital compression; the physiotherapist released the compression during the inspiratory phase until slowing of the respiratory rhythm and a decrease in the suboccipital muscle tone were observed [28,61,62]. This CV-4 technique was the first and last (tenth) conducted in the sequence.

2.8.2.2. Cranial base release or the suboccipital technique. The aim was to release myofascial structures in the suboccipital region. The

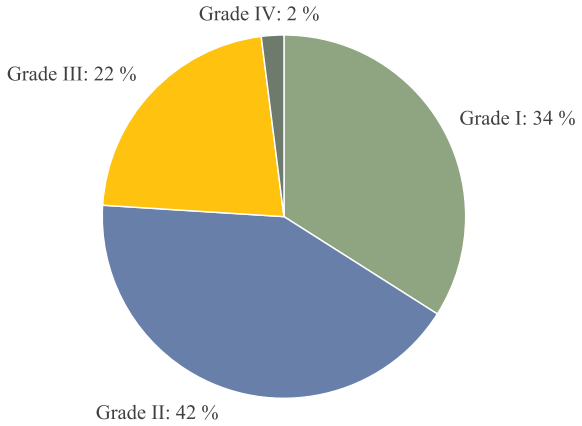


Fig. 2. Percentages of participants displaying joint hypermobility according to Rotés-Querol criteria.

participants were lying in the supine position, and the physiotherapist was located in the same position as described above, with his/her hands placed below the patient's head such that the spinous processes of the cervical vertebrae were able to be palpated with the fingers. The physiotherapist's fingers moved gradually upward to the occipital condyles and then downward to find the hollow space between the condyles and the spinous process of the axis. The physiotherapist raised the skull by bending the metacarpophalangeal joints, while the interphalangeal regions remained in extension, using the index, annular and middle fingers to apply soft pressure that was maintained until the fascia released [37,38].

2.8.2.3. Lumbo-sacral decompression. The participants were lying in the supine position with semiflexion of the knees and hips. With one hand placed on L5 and the other on the sacrum, the physiotherapist applied rhythmic and slow traction with both hands in opposite directions, promoting relaxation of the myofascial structures [61,62].

2.8.2.4. Release of the transverse diaphragms. The phrenic nerve anastomoses with the VN along its pathway, and both innervate the diaphragm. Moreover, the VN is in contact with the spinal trigeminal nucleus through afferent connections. Thus, diaphragmatic dysfunction produces symptoms in the cervical base, mouth floor, dura mater and eyes. The reciprocal tension membranes are innervated by the trigeminal system, VN and hypoglossal nerve. Stimulation generated through transverse diaphragm release reduces tension in the diaphragmatic musculature and improves its activity and the autonomic control of respiration [38,66]. The techniques applied to the transverse diaphragms are described below (pelvic, thoracic and clavicular diaphragms).

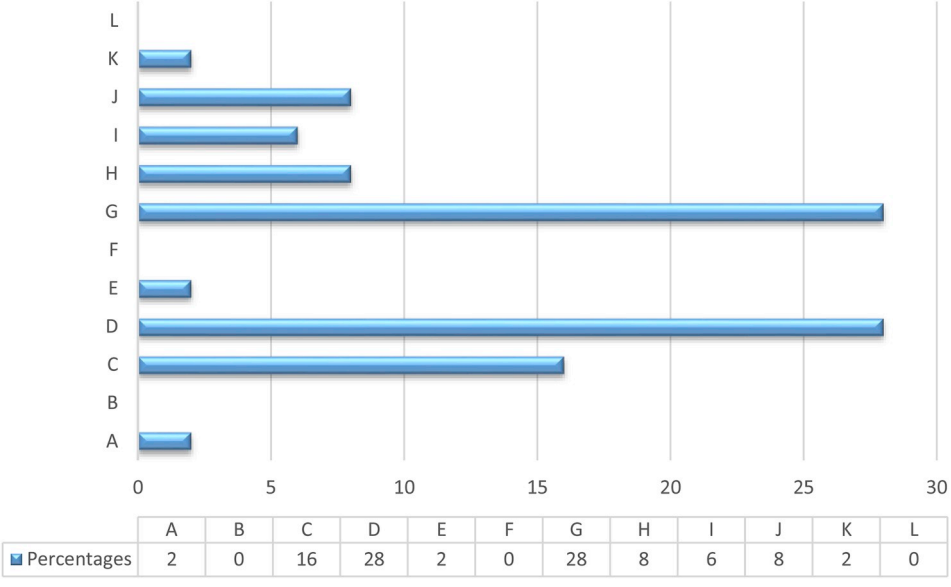
2.8.2.5. Pelvic diaphragm. The participants were lying in the supine position. The physiotherapist placed one hand under the sacral bone and the other on the pubis. When the participant inhaled, the physiotherapist carefully helped the sacral bone rise while simultaneously helping the pubic bone to descend. During exhalation, the technique was performed in the reverse order [38].

2.8.2.6. Thoracic diaphragm. The participants were lying in the supine position. The physiotherapist's thumbs and the whole thenar sides of the hands were placed under the diaphragm in the antero-lateral position and accompanied the patient's respiratory cycle. During inspiration, both hands were separated, and the physiotherapist's hands were brought together on exhalation [38].

2.8.2.7. Clavicular diaphragm. The participants were lying in the supine position, and the physiotherapist placed one hand between both clavicles and the other under the back in a parallel position to accompany the patient's respiratory cycle. During inspiration, the physiotherapist separated his/her hands, and the hands were brought together during exhalation [38].

2.8.2.8. The frontal lift technique. The participants were lying in the supine position, and the physiotherapist was located at the head of the bed with his/her elbows on it, placing his/her fingers just above the eye sockets and applying gentle vertical traction. The falx cerebri inserts in the crest of the frontal bone, in the superior sagittal sinus and in the internal occipital protuberance; thus, this technique may act on the falx cerebri and frontal bone [37,62].

2.8.2.9. The parietal lift technique. The participants were lying in the supine position, with the physiotherapist located in the same position as described above, placing his/her hands on both parietal bones with the fingers extending along the temporoparietal suture, and the thumbs crossed and placed on the opposite sides of the parietal bones. This



	A	B	C	D	E	F	G	H	I	J	K	L
Percentages	2	0	16	28	2	0	28	8	6	8	2	0

Fig. 3. Bar graph showing the percentages of participants with each Heath-Carter somatotype.

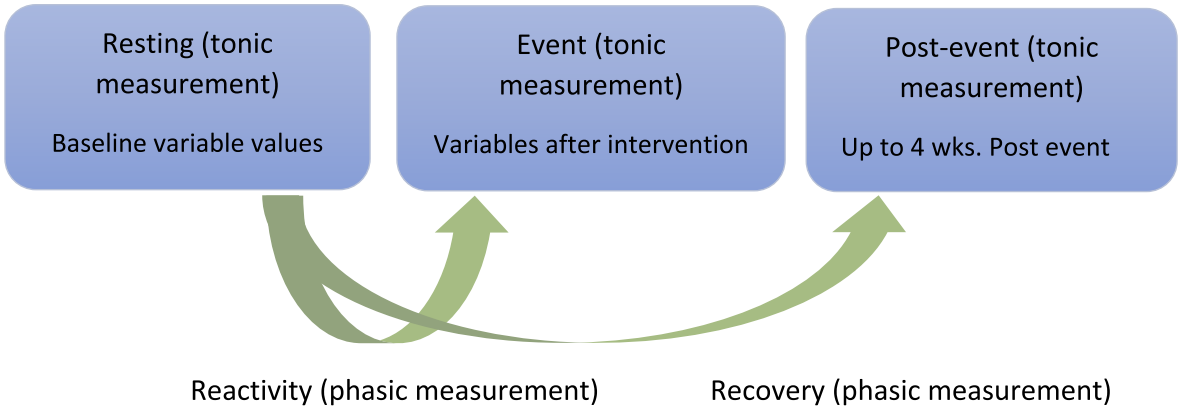


Fig. 4. The three Rs of cardiac vagal control: resting, reactivity and recovery [60].
Acronyms and abbreviations: Weeks (wks).

technique included two phases: first, mild bilateral parietal compression, and then traction of the parietal bones in the cranial direction. As the falx cerebri inserts in the cranial part of the parietal bones and the tentorium cerebelli in the parietal bones' inferoposterior angle and in the lower part of the falx cerebri, this technique may act on the falx cerebri, tentorium cerebelli and parietal bones [37,62].

2.8.2.10. Temporal techniques. As the tentorium cerebelli inserts in the petrous portion and in the mastoid processes of the temporal bones, these techniques may influence the tentorium cerebelli and temporal bones. The temporal techniques applied were wobble and ear pull.

2.8.2.11. Temporal wobble. The participants were lying in the supine position, and the physiotherapist was located at the head of the bed with his/her hands under the skull and thumbs on the mastoid apices, applying gentle lateral pressure towards the midline on one of the mastoid apices, while the other thumb controlled the position of the opposite apsis. The same movement was then performed in the opposite direction [38,62].

2.8.2.12. Ear pull. The participants were lying in the supine position, and a physiotherapist located at the head of the bed performed simultaneous traction of the ear lobes with the index and thumb fingers at a 45° angle by applying symmetrical force until he/she obtained elastic and symmetric resistance on both sides [38,62].

2.8.2.13. Temporo-mandibular joint myofascial release. The participants were lying in the supine position. A physiotherapist at the head of the bed placed both hands on the patient's jaw, with the middle fingers extending along the jaw's vertical ramus and ending under the bilateral angles of the jaw, and applied and maintained gentle caudal traction until temporo-mandibular musculature relaxation occurred [38,39].

2.8.2.14. The deep cervical fasciae technique. Considering the human body as a functional unit where every area is in communication with another through the fascial continuum, the dural fascia system of the skull continues with the deep cervical fascia, then with the endothoracic fascia (anterior) and the thoracolumbar fascia (posterior). The endothoracic fascia continues with the transversalis fascia until the pubis, while the thoracolumbar fascia involves the whole of the posterior area of the body. The aim of the deep cervical fasciae technique was to release the myofascial structures that connect the skull with the trunk structures through the deep cervical fasciae [38]. The participants were lying in the supine position. A physiotherapist at the head of the bed began by releasing the floor of the mouth by placing the fingertips in a position medial to the jawline and applying uniform pressure on both sides to balance the muscle tone. Then, the physiotherapist held the patient's head with one hand under the occiput and raised it with slight extension of the upper cervical vertebrae; the other hand was placed on the sternum. The hand under the occiput applied gentle traction, while the other hand applied caudal pressure on the sternum [37].

Table 4
Variables and measurement instruments.

Variable types	Variables	Scales and measuring instruments
Anthropometric (Sociodemographic)	*Age*	Anamnesis and questioning
	Sex	Anamnesis and questioning
	BMI	Approved scale and telescopic measuring rod
	Body fat percentage	Equations of Slaughter
	WHR	Measuring tape
	Joint hypermobility	Rotés-Querol criteria
	Somatotype	Heath-Carter; ISAK
	AT	Digital thermometer
Physiological variables	*SaO_2*	Pulse oximetry
	Respiratory rate	Breaths/minute
	HR	Approved heart rate monitor
	BP	Approved blood pressure monitor
Neurophysiological variable	*HRV (time and frequency domain parameters related to cardiac vagal tone)*	Wireless monitoring and Polar® thoracic chest strap H7 (following recommendations of psychophysiological research related to HRV and cardiac vagal tone [19,21])

Description of the typology of the variables and their instruments or scales of measurement.
Acronyms and abbreviations: Body mass index (BMI); waist/hip ratio (WHR); axillary temperature (AT); oxygen saturation (SaO2); heart rate (HR); blood pressure (BP); heart rate variability (HRV); ISAK (international standards for anthropometric assessment).

Table 5
Baseline descriptive, physiological and neurophysiological data and normality test.

Variable types	Continuous variables	Mean	Standard Deviation	Min-Max	Kolmogorov-Smirnov test (normality test)	p-value
Descriptive	*Age*	8.860	1.125	7–11	0.1414	1.000
	BMI	18.836	3.699	12.35–26.2	0.5656	0.906
	Body fat percentage	24.599	8.370	9.82–49.51	0.707	0.699
	WHR	0.843	0.059	0.7–1.0	0.989	0.280
Physiological	*AT*	36.132	0.182	35.8–36.6	0.707	0.699
	SaO2	98.48	0.614	97–99	0.1414	1.000
	Respiratory rate	20.22	3.430	14–26	0.848	0.467
	HR	78.88	9.047	60–107	0.989	0.280
	SBP	94.86	7.798	80–113	1.272	0.078
	DBP	60.94	5.839	51–77	0.707	0.699
Neurophysiological	*RMSSD*	70.66	19.710	15–116	0.707	0.699
	LF	637.38	539.76	34–2644	1.131	0.154
	HF	747.54	922.635	70–5745	0.848	0.467
	**LF/HF*	1.2326	0.943	0.2–4.4	1.838	**0.0023***

Initial values of the continuous variables and normality analysis with Kolmogorov-Smirnov test. A p-value > 0.05 indicates a normal distribution. All variables were normally distributed, except the LF/HF ratio*. Asterisks (*) emphasize p-values with statistical significance.
Acronyms and abbreviations: Body mass index (BMI); waist/hip ratio (WHR); axillary temperature (AT); oxygen saturation (SaO2); heart rate (HR); systolic blood pressure (SBP); diastolic blood pressure (DBP); square root of the mean of the sum of the squares of the differences between consecutive NN intervals (RMSSD); low frequency power (LF); high frequency power (HF); ratio of low frequency (LF) power/high frequency (HF) power (LF/HF ratio).

2.9. Variable collection

Each participant attended 6 appointments. In the first session, a physiotherapist performed anamnesis and a physical examination and administered an *ad hoc* questionnaire (collection of anthropometric variables, see Table 4). Then, the participants remained in the supine position and relaxed for 10 min. Next, the psychologist collected physiological and neurophysiological variables before and after the intervention (short-term effects). During the second, third, fourth and fifth sessions (at one, two, three, and four weeks post-intervention, respectively), the same physiological and neurophysiological variables were collected by the psychologist after a 10-min rest in the supine position in a relaxed state (without receiving any intervention) to analyse the persistence of the effects and to determine the ideal treatment cadence. During the sixth session, after a washout period of 3 months, all participants remained at rest for 25 min in the supine position without receiving an intervention (NG); the same variables were collected by the psychologist before and after the rest period to compare the effects between IG1, IG2 and the NG.

2.10. Variables/instruments and scales

2.10.1. Anthropometric (sociodemographic) variables

(see Tables 4 and 5) An abbreviated SNAP-IV questionnaire was administered to exclude any health disorder. The physiotherapist collected data on filiation, age, sex, inclusion and exclusion criteria, potential confounding variables, body mass index (BMI, kg/m^2), percent fat mass (equation for children, Slaughter et al., 1988) [67], the waist/hip ratio (WHR) [68], and joint hypermobility using goniometry according to the 10-item Rotés-Querol criteria for children < 14 years old [69,70] and considering four grades: grade I (2 positive items), grade II (3–5 positive items), grade III (6–8 positive items) and grade IV (8–10 positive items). The Heath-Carter anthropometric somatotype was measured according to the International Society for the Advancement of Kinanthropometry criteria (ISAK) [71,72] and considering ectomorph (somatotype 'C, J, K'), endomorph (somatotype 'B, G, L'), mesomorph ('A, H, I') and combined somatotypes ('E = meso-ectomorph, D = meso-endomorph, F = endo-ectomorph').

2.10.2. Physiological variables

The axillary temperature (AT), oxygen saturation (SaO2), respiratory rate, HR, and systolic and diastolic blood pressure (SBP and DBP, respectively) were collected by the psychologist using approved and reliable devices.

2.10.3. Neurophysiological variables

Time and frequency domain parameters were used to analyse HRV (see Table 2), with a particular focus on the following variables depicting vagal tone: the root mean square of successive differences (RMSSD) as a time domain parameter and the high frequency (HF) component as a frequency domain measure [21]. The low frequency (LF) component and LF/HF ratio were also considered because although a clear consensus has not been achieved [9,10], both variables reflect a mixture of sympathetic and vagal influences, and the LF/HF ratio is classically considered to represent sympathetic-vagal equilibrium. Any change in the LF/HF ratio is interpreted according to increases or decreases in the HF or LF components. Time and frequency domain parameters were collected by the psychologist using wireless monitoring and a Polar® thoracic chest strap H7 (Polar Electro Inc., Bethpage, NY, USA) [73] according to the recommendations for psychophysiological research related to HRV and cardiac vagal tone [21,52].

2.11. Assessment of the safety of the interventions

The psychologist administered an *ad hoc* questionnaire before the patient received the corresponding intervention and recorded attendance, reasons why he or she did not attend any appointments (if necessary), and whether the intervention caused any adverse effects.

2.12. Statistical analysis

Datasets were initially tested for normality using the Kolmogorov-Smirnov test, and then homoscedasticity was measured using Bartlett's and Levene's tests for normally distributed data and for variables that failed the normality test, respectively. A p-value > 0.05 indicated a normal distribution, and Bartlett's and Levene's tests with a p-value > 0.05 indicated equality of variances. All variables were normally distributed with equality of variances, except for the LF/HF ratio, which showed a p-value = 0.0023 in the Kolmogorov-Smirnov test. Levene's test for the LF/HF ratio showed heterogeneity of variances with a p-value = 0.000805 (see Table 5).

Descriptive, univariate and bivariate analyses were performed to evaluate the objectives. The study groups were compared according to the normal distributions of the variables (the *t*-test as a parametric test for normally distributed variables and the Wilcoxon signed-rank test and Mann-Whitney *U* test as non-parametric tests for non-normally distributed variables). Initially, the baseline data from each group (IG1 and IG2) were compared with the same data collected after the specific intervention within each group to assess short-term effects. These data were compared between groups (IG1 and IG2) to determine the effects of the interventions. All variables were collected at one, two, three and four weeks post-intervention under the same conditions and were compared within each group (IG1 and IG2) to study the persistence of the effects. After a washout period of 3 months, the short-term effects observed in IG1 and IG2 were compared with those in the NG to compare the effects of both MT interventions with the effects of remaining in a relaxed state during an equivalent time without receiving any intervention.

For all analyses, significance was set at an alpha level of 0.05, and significance was defined as a p-value < 0.05. Data are reported as means, standard deviations (SDs), minima and maxima.

The effect size was measured using Cohen's d for t-tests (independent samples), where $d \leq 0.20$ represented a small effect size, $0.20 < d \leq 0.50$ represented a medium effect size, $0.50 < d \leq 0.80$ represented a large effect size, and $0.80 < d > 1.30$ represented a very large effect size.

Pearson's correlation coefficient (r, parametric measure) or Spearman's rank correlation coefficient (r_s, non-parametric measure) were used to assess relationships between variables of the frequency domain (+1 indicates a total positive linear correlation, 0 indicates no linear correlation, and −1 represents a total negative linear correlation).

The values used to analyse the strength of the relationship were classified as follows: −0.1 to +0.1 indicating no or a very weak correlation, −0.3 to −0.1 or +0.1 to +0.3 indicating a weak correlation, −0.5 to −0.3 or +0.3 to +0.5 indicating a moderate correlation, and −1.0 to −0.5 or +1.0 to +0.5 indicating a strong correlation.

The coefficient of determination (R^2) was also calculated; once the regression line was adjusted to the observation cloud, R^2 measured the goodness of fit and was defined as the proportion of the variance in the dependent variable that was predictable from the independent variable (s). The Cohen criterion was followed to assess whether this explanatory capacity was low = 0.1, medium = 0.3 or high = 0.5.

3. Results

3.1. Characteristics of the participants

Fifty children were included (66% boys and 34% girls), with an average age of 8.86 ± 1.125 years (minimum 7 years and maximum 11 years). The average BMI was 18.83 ± 3.699 kg/m^2 (12.35–26.2), the average percent fat mass was 24.59 ± 8.37% (9.82–49.51), and the average WHR was 0.843 ± 0.059 (0.7–1.0) (Table 5). An assessment of joint hypermobility showed that most participants were classified as grade II (42%, n = 21), 34% were classified as grade I (n = 17), 22% were classified as grade III (n = 11), and 2% were classified as grade IV (n = 1) (see Fig. 2). Regarding the somatotype, most participants had an endomorphic component (type G = 28%, n = 14 and type D = 28%, n = 14), followed by participants with an ectomorph composition (type C = 16%, n = 8) (Fig. 3).

Table 5 shows all pre-intervention descriptive, physiological and neurophysiological baseline data.

3.2. Short-term effects after the intervention

3.2.1. Short-term univariate analysis within groups (IG1, IG2 and the NG)

See Table 6 Univariate analysis of IG1 showed significant decreases in the AT (p = 0.00001), respiratory rate (p = 0.00001), HR (p = 0.00024), and SBP (p = 0.00001) and significant increases in two HRV parameters: the RMSSD (p = 0.00140) and HF component (p = 0.00082). Univariate analysis of IG2 showed significant decreases in the AT (p = 0.00333), respiratory rate (p = 0.00001), HR (p = 0.00001), SBP (p = 0.00001), DBP (p = 0.00049) and LF/HF ratio (p = 0.0012). Significant increases in the RMSSD (p = 0.00001) and HF component (p = 0.00055) were also observed. The NG showed decreases in the AT (p = 0.00001), respiratory rate (p = 0.00001), HR (p = 0.00001), and SBP (p = 0.0002), with no significant changes in any HRV parameters. All results are shown in Table 6.

3.2.2. Short-term bivariate analysis between groups (IG1, IG2 and the NG) and effect sizes

(see Table 7) Bivariate analysis between IG1 and the NG revealed significant differences in the AT (p = 0.00001, Cohen's d effect size $d = 1.7476$), respiratory rate (p = 0.014366, $d = 0.8673$), and RMSSD (p = 0.0106, $d = 0.8456$). Comparison of IG2 and the NG showed significant differences in the AT (p = 0.00001, $d = 0.4502$), respiratory rate (p = 0.00001, $d = 0.4848$), HR (p = 0.00623, $d = 0$), SBP (p = 0.00018, $d = 1.1217$), DBP (p = 0.00049, $d = 1.1204$), RMSSD (p = 0.00001, $d = 1.614$), HF component (p = 0.0005, $d = 1.0583$), and LF/HF ratio (p = 0.002951, $d = 0.8033$). Comparison of IG1 and IG2 revealed significant differences in the AT (p = 0.000119, $d = 1.1636$), respiratory rate (p = 0.001886, $d = 0.9304$), HR (p = 0.01923, $d = 0.6892$), SBP (p = 0.03056, $d = 0.6389$) and RMSSD (p = 0.006779, $d = 0.7977$). All results are presented in Table 7.

3.3. Medium-term univariate analysis within IG1 and IG2 at one, two, three and four weeks 'post event'

(see Tables 8 and 9) At one week post-intervention, the univariate analysis of IG1 showed a significant decrease in the RMSSD (+8.3 ± 18.222 ms, p = 0.0323), whereas no significant differences were observed in the remaining variables (see Table 8). Univariate analysis of IG2 showed significant increases in the RMSSD (+6.6 ± 12.328 ms, p = 0.01271) and HF component (+805.8 ± 815.257 ms^2/Hz, p = 0.00001) and significant decreases in the AT (−0.2 ± 0.2619 °C, p = 0.00147), respiratory rate (−3.5 ± 4.073 breaths/minute, p = 0.000023), HR (−6.4 ± 5.091 beats/minute, p = 0.00001), SBP (−4.8 ± 5.771 mmHg, p = 0.000323), DBP (−2.8 ± 3.901 mmHg, p = 0.0013), and LF/HF ratio (−0.9 ± 0.979, p = 0.0005) (see Table 9). Two weeks after the intervention, only the significant increase in the RMSSD persisted in IG1 (+9 ± 15.725 ms, p = 0.0088) (see Table 8), while IG2 showed significant increases in the RMSSD (+10.7 ± 17.619 ms, p = 0.00561) and HF component (+604 ± 777.875 ms^2/Hz, p = 0.00078) and significant decreases in the AT (−0.2 ± 0.25 °C, p = 0.00384), respiratory rate (−3.2 ± 3.488 breaths/minute, p = 0.000119), HR (−2.9 ± 5.929 beats/minute, p = 0.0213), SBP (−3.6 ± 5.81 mmHg, p = 0.00533), DBP (−2.1 ± 4.304 mmHg, p = 0.02135), and LF/HF ratio (−0.9 ± 1.058, p = 0.0005) (see Table 9).

At three weeks post event, no significant differences were observed in IG1 (see Table 8), while in IG2, significant increases persisted in the RMSSD p = 0.00351 (+10 ± 15.509 ms, p = 0.00351) and HF component (+359.2 ± 863.113 ms^2/Hz, p = 0.004831), and a significant decrease persisted in the LF/HF ratio (−0.6 ± 1.085, p = 0.0151) (see Table 9). After 4 weeks, all parameters returned to the initial baseline values in both groups (see Tables 8 and 9).

3.4. Medium-term bivariate analysis between groups (IG1 and IG2) at one, two, three and four weeks 'post event' and effect sizes

(see Table 10) At one week, bivariate analysis between IG1 and IG2

Table 6
Modifications of physiological and neurophysiological parameters in the short term (before-after) in intervention group 1, intervention group 2 and the neutral group.

	IG1: massage therapy			IG2: manual cranial therapy			NG: no intervention		
Variable	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value
AT	−0.6	0.396	0.00001*	−0.2	0.282	0.00333*	−0.11	0.02	0.00001*
SaO2	0.1	0.927	0.523	0	0.763	1	0.02	0.05	0.709
Respiratory rate	−2.9	2.538	0.00001*	−5.5	3.029	0.00001*	−4.46	0.17	0.00001*
HR	−4.1	4.733	0.00024*	−7.6	5.401	0.00001*	−7.6	0.67	0.00001*
SBP	−3.9	3.817	0.00001*	−6.9	5.433	0.00001*	−2.56	0.65	0.0002*
DBP	−2.3	5.64	0.0507	−4.3	5.319	0.00049*	−0.08	0.28	0.774
RMSSD	9	12.414	0.00140*	20.8	16.837	0.00001*	1.56	0.84	0.069
LF	168.3	538.633	0.131	123.7	574.249	0.292	2.38	11.11	0.831
HF	253.8	936.688	0.00082*	442.3	556.082	0.00055*	25.92	19.04	0.179
LF/HF	0.3	0.948	0.4254	−0.5	0.72	0.0012*	−0.09	0.05	0.067

Statistical analysis of short-term changes in continuous variables within each group, intervention group 1, intervention group 2 and neutral group, using a *t*-test for mean values of the dependent variables (parametric test for normally distributed variables) and Wilcoxon signed-rank test for the LF/HF ratio (non-parametric test for non-normally distributed variables). A p-value < 0.05 indicates statistically significant results. Asterisks (*) emphasize p-values with statistical significance.
Acronyms and abbreviations: Axillary temperature (AT); oxygen saturation (Sa O2); heart rate (HR); systolic blood pressure (SBP); diastolic blood pressure (DBP); square root of the mean of the sum of the squares of the differences between consecutive NN intervals (RMSSD); low frequency power (LF); high frequency power (HF); ratio of low frequency (LF) power/high frequency (HF) power (LF/HF ratio); intervention group 1 (IG1); intervention group 2 (IG2); neutral group (NG); standard deviation (SD).

Table 7
Short-term comparisons and effect sizes between intervention group 1 and the neutral group, intervention group 2 and the neutral group, and intervention group 1 and intervention group 2.

Continuous Variables	IG1-NG		IG2-NG		IG1-IG2	
	p-value	Cohen's *d*	p-value	Cohen's *d*	p-value	Cohen's *d*
AT	0.00001*	1.7476	0.00001*	0.4502	0.000119*	1.1636
SaO2	0.688	–	1	–	0.619	–
Respiratory rate	0.014366*	0.8673	0.00001*	0.4848	0.001886*	0.9304
HR	0.477	–	0.00623*	0	0.01923*	0.6892
SBP	0.739	–	0.00018*	1.1217	0.03056*	0.6389
DBP	0.072	–	0.00049*	1.1204	0.212	–
RMSSD	0.0106*	0.8456	0.00001*	1.6140	0.006779*	0.7977
LF	0.142	–	0.2871	–	0.778	–
HF	0.244	–	0.0005*	1.0583	0.293	–
LF/HF	0.052	–	0.002951*	0.8033	0.6818	–

Statistical analysis of short-term changes in continuous variables between intervention group 1 and the neutral group; intervention group 2 and the neutral group; and intervention group 1 and intervention group 2 using a *t*-test for the means of independent variables (parametric test for normally distributed variables) and Mann-Whitney *U* test for the LF/HF ratio (non-parametric test for non-normally distributed variables). A p-value < 0.05 indicates statistically significant results. The effect size was measured using Cohen's *d* for t-tests (independent samples), where $d \leq 0.20$ represented a small effect size, $0.20 < d \leq 0.50$ a medium effect size, $0.50 < d \leq 0.80$ a large effect size and $0.80 < d > 1.30$ a very large effect. Asterisks (*) emphasize p-values with statistical significance.
Acronyms and abbreviations: Axillary temperature (AT); oxygen saturation (SaO2); heart rate (HR); systolic blood pressure (SBP); diastolic blood pressure (DBP); square root of the mean of the sum of the squares of the differences between consecutive NN intervals (RMSSD); low frequency power (LF); high frequency power (HF); ratio of low frequency (LF) power/high frequency (HF) power (LF/HF ratio); intervention group 1 (IG1); intervention group 2 (IG2); neutral group (NG).

showed significant differences in the AT (p = 0.04732, Cohen's *d* effect size $d = 0.6854$), respiratory rate (p = 0.000107, $d = 1.1887$), HR (p = 0.00001, $d = 1.5522$), SBP (p = 0.00078, $d = 1.0057$), HF component (p = 0.00341, $d = 0.8712$), and LF/HF ratio (p = 0.00001, $d = 1.3443$). In the second week, bivariate analysis still revealed significant differences in the respiratory rate (p = 0.000349, $d = 1.0888$), HR (p = 0.00997, $d = 0.748$), SBP (p = 0.00149, $d = 0.1331$), HF component (p = 0.049, $d = 0.5713$), and LF/HF ratio (p = 0.0002, $d = 1.211$). In the third week, significant differences only persisted in the LF/HF ratio (p = 0.018, $d = 0.8565$), and in the fourth week, no differences were observed between IG1 and IG2.

3.5. Evolution of the LF/HF ratio from resting (baseline) to reactivity (event) and recovery processes

(see Fig. 5) Although no statistically significant variations were observed, the baseline LF/HF ratio was 0.8 in IG1, which increased to 1.0 (p = 0.4254) after the intervention, remained stable for one week after the intervention (p = 0.258), decreased to the baseline value of 0.8 in the second week (p = 0.5619), increased to 0.9 (p = 0.1976) in the third week, and returned to 0.8 in the fourth week after the intervention (p = 0.659). In IG2, the variations in the basal LF/HF ratio were statistically significant. Initially, the value was 1.7, but it decreased significantly to 1.2 (p = 0.0012) after the intervention, continued to decrease to 1.0 (p = 0.0005) one week later and to 0.8 (p = 0.0005) in the second week, increased to 1.1 (p = 0.0151) in the third week, and the mean value was 1.4 (p = 0.1902) at 4 weeks after the intervention.

3.6. Correlations (R) and coefficients of determination (R^2)

Test–retest differences in the HF component (main variable), LF component and LF/HF ratio were observed in the short term between IG1 and IG2. Table 11 displays the results from IG1, showing a weak positive correlation between the HF and LF components (R = 0.117, p = 0.595, low coefficient of determination $R^2 = 0.0125$). The HF–LF/HF correlation in IG1 was weak and negative (R = −0.107, p = 0.6102, $R^2 = 0.0114$). The results from IG2 revealed a strong

Table 8
Modifications of physiological and neurophysiological parameters in the medium-term in intervention group 1 (received massage therapy).

IG1	1 wk post			2 wks post			3 wks post			4 wks post		
Variable	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value
AT	0	0.318	0.145	0	0.309	0.749	−0.1	0.3318	0.4409	0	0.264	0.457
SaO2	0.1	0.493	0.425	−0.1	0.812	0.626	0.1	0.6	0.3272	0.1	0.665	0.376
Respiratory rate	0.8	3.095	0.208	0.2	2.708	0.327	0.3	2.894	0.6329	0.2	2.677	0.712
HR	1.4	4.958	0.159	1.2	4.993	0.226	2.4	5.721	0.0501	0.7	5.615	0.527
SBP	0	3.5	1	2.1	60.27	0.097	1.6	5.694	0.1834	2	5.909	0.103
DBP	−0.9	5.243	0.389	−0.4	5.887	0.737	1.3	6.606	0.327	0.1	4.6808	0.9326
RMSSD	8.3	18.222	0.0323*	9	15.725	0.0088*	4.3	14.574	0.1505	5	13.296	0.07
LF	99.8	302.933	0.112	120.3	360.183	0.1078	74	228.237	0.8098	11.5	117.59	0.083
HF	181.1	603.07	0.1463	220.1	545.655	0.0559	43.9	506.616	0.668	−73.4	479.227	0.451
LF/HF	0.2	0.617	0.258	0.1	0.494	0.5619	0.1	0.398	0.1976	0	0.244	0.659

Statistical analysis of medium-term changes in continuous variables within intervention group 1 (IG1) using a *t*-test for the means of dependent variables (parametric test for normally distributed variables) and Wilcoxon signed-rank test for the LF/HF ratio (non-parametric test for non-normally distributed variables). A p-value < 0.05 indicates statistically significant results. Asterisks (*) emphasize p-values with statistical significance.
Acronyms and abbreviations: Axillary temperature (AT); oxygen saturation (SaO2); heart rate (HR); systolic blood pressure (SBP); diastolic blood pressure (DBP); square root of the mean of the sum of the squares of the differences between consecutive NN intervals (RMSSD); low frequency power (LF); high frequency power (HF); ratio of low frequency (LF) power/high frequency (HF) power (LF/HF ratio); week (wk); weeks (wks); post-intervention (post); intervention group 1 (IG1); standard deviation (SD).

positive and significant correlation between the HF and LF components (R = 0.5002, p = 0.01108, medium coefficient of determination R^2 = 0.2502). A moderate positive correlation between the HF component and LF/HF ratio was observed in IG2 (R = −0.332, p = 0.104, low coefficient of determination R^2 = 0.104).

3.7. Safety of interventions

The *ad hoc* questionnaire revealed that no adverse effects occurred during the interventions or after the follow-up.

4. Discussion

This study aimed to analyse and compare the immediate effects of two MT modalities (a single session of an Mss-t or MC-t programme) on the physiological and neurophysiological statuses of healthy children and the medium-to long-term persistence of the effects for up to four weeks post-intervention (medium-term effects). Similarly, the short-term effects of both MT modalities were compared with those of a neutral effect programme after a washout period. The power of the effects of the interventions and their safety were also determined.

4.1. Pre-intervention data (at resting state, Table 5)

Reference values for the HRV indices must be established to assess an imbalance in the ANS. Only a limited number of studies have reported such normative/reference HRV values in paediatric populations [74,75]. Acharya et al. [75] analysed HRV in 150 healthy subjects (aged 5–70 years) aiming to establish HRV reference values categorized by age ranges. Our results (see Table 5) place initial baseline HRV values within normal limits.

The baseline average respiratory rate was within the normal respiratory frequency range [76]. Although the literature recommends controlling a subject's respiratory rate, we decided that the participants should be allowed to breath spontaneously because the respiratory rate may be affected by having the participants concentrate on breathing during the treatment. The average baseline HR was also within the normal range [76].

Table 5 displays all descriptive, physiologic and neurophysiologic baseline data and the results of the normality test (Kolmogorov-Smirnov test), showing that all variables were normally distributed, except for the LF/HF ratio.

Table 9
Modifications of physiological and neurophysiological parameters in the medium-term in intervention group 2 (received manual cranial therapy).

IG2	1 wk post			2 wks post			3 wks post			4 wks post		
Variable	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value
AT	−0.2	0.2619	0.00147*	−0.2	0.25	0.00384*	0.1	0.1908	0.087	0.1	0.267	0.129
SaO2	0	0.734	0.7878	0	0.734	0.789	0.2	0.707	0.1701	0	0.645	0.1701
Respiratory rate	−3.5	4.073	0.00023*	−3.2	3.488	0.000119*	0	2.908	0.945	−0.4	2.45	0.378
HR	−6.4	5.091	0.00001*	−2.9	5.929	0.0213*	−1.1	9.962	0.5927	−1.2	4.758	0.2347
SBP	−4.8	5.771	0.000323*	−3.6	5.81	0.00533*	0.4	7.193	0.8045	1.1	7.09	0.4373
DBP	−2.8	3.901	0.0013*	−2.1	4.304	0.02135*	−0.8	4.099	0.3158	0.2	2.91	0.7858
RMSSD	6.6	12.328	0.01271*	10.7	17.619	0.00561*	10	15.509	0.00351*	6.2	18.616	0.1105
LF	159.8	711.615	0.2727	12.5	550.804	0.9104	27.8	570.382	0.8098	92.1	630.25	0.472
HF	805.8	815.257	0.00001*	604	777.875	0.00078*	359.2	863.113	0.04831*	121.7	484.162	0.2209
LF/HF	−0.9	0.979	0.0005*	−0.9	1.058	0.0005*	−0.6	1.085	0.0151*	−0.3	0.915	0.1902

Statistical analysis of medium-term changes in continuous variables within intervention group 2 (IG2) using a *t*-test for the means of dependent variables (parametric test for normally distributed variables) and Wilcoxon signed-rank test for the LF/HF ratio (non-parametric test for non-normally distributed variables). A p-value < 0.05 indicates statistically significant results. Asterisks (*) emphasize p-values with statistical significance.
Acronyms and abbreviations: Axillary temperature (AT); oxygen saturation (SaO2); heart rate (HR); systolic blood pressure (SBP); diastolic blood pressure (DBP); square root of the mean of the sum of the squares of the differences between consecutive NN intervals (RMSSD); low frequency power (LF); high frequency power (HF); ratio of low frequency (LF) power/high frequency (HF) power (LF/HF ratio); week (wk); weeks (wks); post-intervention (post); intervention group 2 (IG2); standard deviation (SD).

Table 10
Medium-term comparisons and effect sizes between intervention group 1 and intervention group 2 at one, two, three and weeks post-intervention.

Continuous Variables	IG1-IG2 1 wk post		IG1-IG2 2 wks post		IG1-IG2 3 wks post		IG1-IG2 4 wks post	
	p-value	Cohen's *d*	p-value	Cohen's *d*	p-value	Cohen's *d*	p-value	Cohen's *d*
AT	0.04732*	0.6854	0.0849	–	0.123	–	0.5612	–
SaO2	0.496	–	0.586	–	0.668	–	0.5207	–
Respiratory rate	0.000107*	1.1887	0.000349*	1.0888	0.698	–	0.3823	–
HR	0.00001	1.5522	0.00997*	0.7480	0.1408	–	0.2077	–
SBP	0.00078*	1.0057	0.00149*	0.1331	0.5162	–	0.6357	–
DBP	0.1484	–	0.2441	–	0.171	–	0.9424	–
RMSSD	0.711	–	0.711	–	0.1838	–	0.80766	–
LF	0.7001	–	0.4168	–	0.708	–	0.5326	–
HF	0.00341*	0.8712	0.049*	0.5713	0.1218	–	0.1587	–
LF/HF	0.00001*	1.3443	0.0002*	1.2111	0.018*	0.8565	0.2501	–

Statistical analysis of medium-term changes in continuous variables between intervention group 1 (IG1) and intervention group 2 (IG2) using t-tests for the means of independent variables (parametric test for normally distributed variables) and Mann-Whitney *U* test for the LF/HF ratio (non-parametric test for non-normally distributed variables). A p-value < 0.05 indicates statistically significant results. The effect size was measured using Cohen's *d* for t-tests (independent samples), where $d \leq 0.20$ represented a small effect size, $0.20 < d \leq 0.50$ a medium effect size, $0.50 < d \leq 0.80$ a large effect size and $0.80 < d > 1.30$ a very large effect size. Asterisks (*) emphasize p-values with statistical significance.
Acronyms and abbreviations: Axillary temperature (AT); oxygen saturation (SaO2); heart rate (HR); systolic blood pressure (SBP); diastolic blood pressure (DBP); square root of the mean of the sum of the squares of the differences between consecutive NN intervals (RMSSD); low frequency power (LF); high frequency power (HF); ratio of low frequency (LF) power/high frequency (HF) power (LF/HF ratio); week (wk); weeks (wks); post-intervention (post); intervention group 1 (IG1); intervention group 2 (IG2).

4.2. Short-term results and effect sizes (see Tables 6 and 7)

The short-term results showed immediate effects of a single Mss-t or MC-t intervention on HRV indices, which are linked to cardiac vagal tone. Significant increases in parameters related to parasympathetic activity (the RMSSD and HF component) were observed after the administration of both MT programmes, and both groups exhibited significantly decreased values for some short-term physiological parameters immediately after the intervention, indicating that an immediate increase in parasympathetic activity occurred after both MT programmes. Notably, in the short term, the MC-t programme modified a greater number of variables and caused changes of greater magnitudes (see Table 6).

After a washout period of three months, the short-term results were compared among the programmes (Mss-t, MC-t and the NG) to determine whether the interventions had a real neurobiological impact beyond the effects of simple relaxation while lying in the supine position. After the neutral intervention (resting in the supine position for 25 min), significant decreases in some vital signs were observed, but no variations were observed in any of the HRV indicators under the conditions used in the study (see Table 6).

According to a limited number of studies, MTs affect vagal control; many manipulative medicine techniques have been postulated to affect the ANS, including stimulatory and inhibitory effects on the respective branches of the ANS [27]. Our findings are comparable to those of studies in the area of manual medicine and its relationship with the

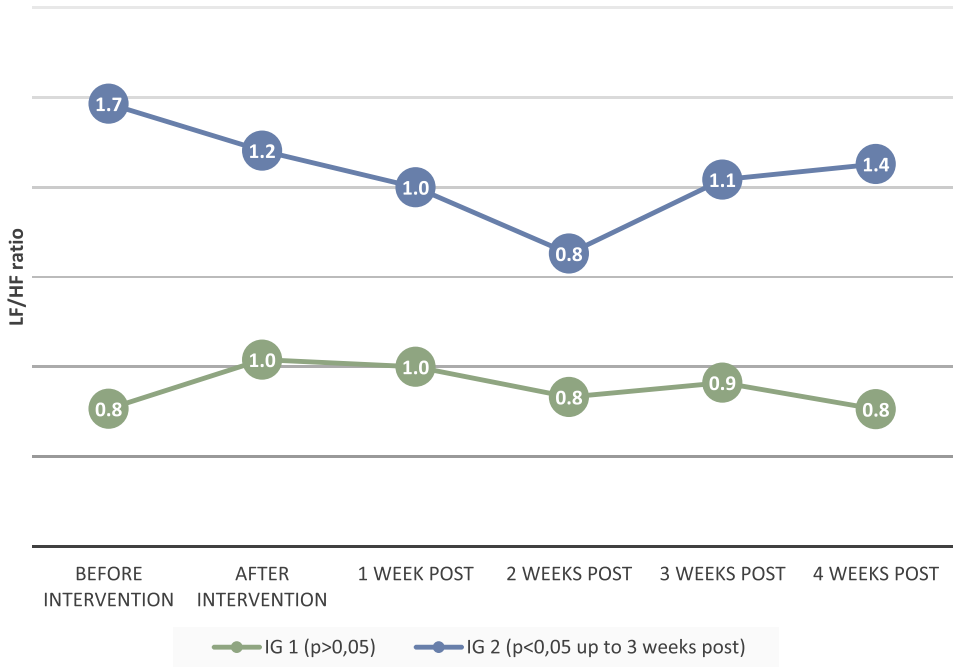


Fig. 5. Evolution of the LF/HF ratio post-intervention.
Acronyms and abbreviations: High frequency power (HF); low frequency power (LF); ratio of low frequency (LF) power/high frequency (HF) power (LF/HF ratio); post-intervention (post); intervention group 1 (IG1); intervention group 2 (IG2); p-value (p).

Table 11
Correlations and coefficient of determination of test–retest differences in HF with LF and LF/HF ratio in the short-term.

Intervention group 1 Intervention group 2												
HRV frequency domain parameters	LF			LF/HF			LF			LF/HF		
	R	p-value	R^2	r_s	p-value	R^2	R	p-value	R^2	r_s	p-value	R^2
HF	0.1117	0.595	0.0125	−0.107	0.6102	0.0114	0.5002	**0.0108***	0.2502	0.332	0.10459	0.1104

Pearson's correlation coefficient (R) as a parametric measure or Spearman's rank correlation coefficient (*rs*) as a non-parametric measure were used to assess the relationships between variables of the frequency domain, considering HF as the dependent variable and LF and the LF/HF ratio as independent variables. +1: indicates a total positive linear correlation; 0 indicates no linear correlation and −1 represents a total negative linear correlation. A p-value < 0.05 indicates statistically significant results. The coefficient of determination (R^2) was calculated to measure the goodness of fit. The Cohen criterion for R^2 was low = 0.1, medium = 0.3 or high = 0.5. Asterisks (*) emphasize p-values with statistical significance.
Acronyms and abbreviations: Heart rate variability (HRV); high frequency power (HF); low frequency power (LF); ratio of low frequency (LF) power/high frequency (HF) power (LF/HF ratio).

ANS. As shown in the study by Giles et al., upper cervical spine manipulation and suboccipital decompression acutely affected HRV measures in healthy individuals, with increases in the SDNN and HF component and a decrease in the LF/HF ratio [27]. Milnes and Moran investigated the physiological effects of the cranial manipulation technique on healthy individuals but found minimal physiological changes in the autonomic measures [28]. Budgell and Polus studied the effects of thoracic manipulation on HRV and observed short-term changes in HRV by identifying significant increases in the LF component and LF/HF ratio and decreases in the HF component, indicating an increase in sympathetic output to the heart [29]. Zhang et al. [30] asserted that the manipulation site may influence the autonomic response. When manipulation is performed in the cervical region, the parasympathetic nervous system is activated due to the response of the VN [29,30], and parasympathetic activation is mediated by parasympathetic nerves in this region when lumbosacral manipulation is performed [30,31,33]. Regarding thoracic manipulation, an increase in the sympathetic response occurs since sympathetic roots are located at this level [29,30,32]. Relaxation promoted by myofascial techniques may also facilitate the parasympathetic response [34,35,40,77]. These findings are consistent with our results showing changes in HRV indices related to an increase in vagal tone (parasympathetic activity) reflected in the increase in the RMSSD and HF component (in both groups) and a decrease in the LF/HF ratio (in MC-t group) throughout the process, which appears to be related to an increase in and maintenance of the relaxation state [34,35,40,77].

Breathing is an important factor that significantly influences HR and HRV [78]. In a population with a known fast breathing rate, such as children, the respiratory depth and frequency are associated with HR fluctuations and may influence HRV data [76], and HRV-related studies recommend that respiration should considered [21,52]. In our study, the respiratory rate was monitored before and after the intervention to determine whether any changes occurred after the intervention. As we mentioned, the breathing rate was not regulated as recommended in the literature because participants may be less likely to relax when receiving treatment, and the respiratory rate may be influenced. All groups displayed a significantly decreased respiratory rate after a single session of Mss-t, MC-t or the neutral intervention, indicating a state of relaxation after any procedure.

HRV is significantly associated with the average HR; therefore, HRV provides information on the HR and its variability [76]. Different authors have shown an inverse correlation between HRV and HR [79,80]. The influence of HR on HRV in children has also been studied. Jarrin et al. concluded that HR is the strongest factor determining HRV and presented their normative values adjusted for HR [81]. In our study, HR was monitored before and after the intervention to detect any changes after the specific interventions. All groups presented a significantly decreased HR after receiving a single session of Mss-t or MC-t or after remaining in a relaxed position without receiving any intervention.

Blood pressure is another factor that is significantly associated with HRV, and a large number of studies have reported that HRV is associated with cardiovascular diseases and mortality. Hypertension is associated with increased sympathetic activity and decreased parasympathetic activity. A reduced HRV has been reported in adults with hypertension; however, studies investigating the association between HRV and paediatric hypertension are lacking. Gui-Ling Xie et al. [82] studied HRV levels in children with hypertension and hypothesized that a reduced HRV represents a potential pathophysiological biomarker of adulthood cardiovascular diseases. Aourell et al. [83] studied the effects of massage on blood pressure and observed decreases in SBP without changes in DBP. Our short-term results are partially consistent with these findings, revealing a significant short-term decrease in SBP in the Mss-t group (without changes in DBP), but significant decreases in both SBP and DBP were observed in the MC-t group (see Table 6). Weerapong et al. asserted that although the mechanisms of different massage techniques have not been widely investigated, massage has been shown to increase parasympathetic activity as measured by HR, BP and HRV values, reflecting a state of relaxation [84]. Our study specifically incorporated time and frequency domain HRV indicators that are exclusively linked to cardiac vagal tone and provided evidence for the parasympathetic predominance.

Some relaxation techniques associate HRV parameters with temperature changes. Díaz-Rodríguez et al. [85] suggested an inverse relationship between temperature and the LF component after the application of relaxation techniques. In our study, a significant decrease in AT was observed immediately after any intervention, which is characteristic of the relaxation state.

When comparing Mss-t with the neutral programme, the short-term results revealed that Mss-t affected the HRV indices, with a predominance of parasympathetic activity, since a significant increase in the RMSSD was observed immediately after the intervention. However, in the NG treated under the conditions of the present study, simple relaxation did not produce changes in HRV parameters (only a significant reduction in some vital signs). Changes evoked in the RMSSD by the Mss-t programme are illustrated in Table 7, showing a very large effect size of the intervention (greater than 0.80).

When comparing MC-t with the neutral programme, the short-term results showed that MC-t produces significant changes in a greater number of variables related to vagal activity, all of which displayed a very large effect size (greater than 0.80), reflecting an increase in HR parasympathetic control by predominant vagal activity (see Table 7).

For comparison of both MT interventions (Mss-t with MC-t), significant short-term differences were observed in some vital signs and in the RMSSD (the HRV parameter linked to parasympathetic activity), with a very large effect size (greater than 0.80, see Table 7).

4.3. Medium-term results obtained for up to four weeks and effect sizes (see Tables 8–10)

In the Mss-t group, only the RMSSD effects persisted for up to two weeks after the intervention (see Table 8), revealing maintenance of the parasympathetic activity that was reflected in only one variable. In contrast, the MC-t group displayed significant decreases in some vital signs that persisted for up to two weeks after the intervention (see Table 9). In addition, differences in the indicators of HRV cardiac vagal control remained significant for up to three weeks, showing a parasympathetic predominance reflected in the increases in the RMSSD and HF component, as well as the significant decrease in the LF/HF ratio. Thus, the effects on vagal cardiac activity seemed to persist for up to three weeks only in the MC-t group (see Table 9).

Comparing both groups, significant differences in the respiratory rate, HR, SBP, HF component and the LF/HF ratio were observed for up to two weeks. At 3 weeks post event, significant differences only persisted in the LF/HF ratio, showing the predominance and maintenance of parasympathetic activity in the MC-t group. At 4 weeks, no differences were observed between groups as the values had returned to baseline. In all cases, the effect size of the intervention was considered large or very large (see Table 10).

4.4. Evolution of the LF/HF ratio from resting (baseline) to reactivity (event) and recovery processes (see Figs. 4 and 5)

Based on 'vagal tank theory' research, higher resting cardiac vagal control is associated with better self-regulation [60]. Regarding reactivity (a change between baseline and the effects produced after the specific MT intervention), higher resting cardiac vagal control predicts better self-regulation during reactivity [60]. Recovery is defined as the process of restoration to the initial condition (a change between weekly values and baseline values). This theory interprets the values obtained during the recovery process as follows: if cardiac vagal control increases during the event, better self-regulation occurs as the time during which the cardiac vagal tone remains above baseline levels increases [60].

Classically, the LF/HF ratio is considered to represent the sympathetic-vagal balance, but a consensus is lacking [9,10]. A decrease in the LF/HF ratio indicates a predominance of parasympathetic activity that is potentially due to an increase in the HF component, a decrease in the LF component, or both; it may also occur as a result of a simultaneous increase in the LF component and a decrease in the HF component if the decrease in the HF component is greater than the increase in the LF component [21,60]. In the present study, the baseline mean LF/HF ratios were slightly higher in IG2 than those in IG1, which initially predicted worse vagal heart control in IG2. Nevertheless, after the intervention (reactivity process), the ratio in IG2 significantly decreased, while it had a tendency to increase in IG1. Thus, IG2 (MC-t) displayed a significant increase in parasympathetic activity, which may indicate better self-regulation. In addition, the recovery process was also measured weekly in both groups to assess the persistence of the effects and to precisely determine when the values returned to the baseline levels. In IG2, the LF/HF ratio continued to display a significant decrease up to the third week, showing a predominance of parasympathetic activity during this process, whereas in IG1, the values had returned to the baseline one week after the intervention. Based on these findings, better self-regulation was induced in IG2 because after four weeks, the LF/HF ratio did not return to its baseline values, reflecting a predominance of vagal cardiac control. MC-t induced a predominance of parasympathetic activity after the intervention, and the neurophysiological effects persisted for up to three weeks.

4.5. Short-term correlations (R) and coefficient of determinations (R^2) for test–retest differences in the HF and LF components and the LF/HF ratio between IG1 and IG2 (see Table 11 and Fig. 5)

The HF component was considered the dependent variable because it reflects cardiac vagal tone, and the LF component and the LF/HF ratio were considered independent variables because the literature considers these parameters to be a mixture of sympathetic and parasympathetic influences. In IG1 (Mss-t), no significant correlations were observed between the variables. In contrast, IG2 (MC-t) showed a strong positive and significant correlation between the HF and LF components, with a medium coefficient of determination ($R^2 = 0.2502$); therefore, the HF and LF parameters were strongly correlated. Notably, 25.02% of the variance in the HF variable is explained by the LF variable, indicating that an increase in the HF component also increases the LF component. However, a higher increase was observed in the HF component because the LF/HF ratio was significantly decreased in IG2 in the short and medium term (see Fig. 5), indicating a predominance of parasympathetic activity. The correlation between the HF component and LF/HF ratio in IG2 had a tendency to be moderate and positive, with a low coefficient of determination. The fact that two variables tended to increase or decrease simultaneously does not indicate that one has a direct or indirect effect on the other. Both are likely influenced by other variables (confounding factors), generating a mathematical relationship between them.

4.6. Strengths and limitations

Based on our data, the MC-t programme generated neurophysiological effects on HRV by increasing parasympathetic activity, and the effects persisted up to three weeks. These results were observed in healthy children without concomitant comorbidities; thus, the positive effect was not due to a treatment effect associated with an underlying musculoskeletal condition. Therefore, the short- and medium-term effects of MC-t appeared to be related to specific treatment techniques.

A homogeneous sample was obtained in terms of age through the use of specific selection criteria, and each participant was treated on the same day of the week and at the same time to attenuate sources of measurement bias. We attempted to control any confounding variables that may affect HRV parameters to the greatest extent possible by tracking them with an *ad hoc* questionnaire (sleep routine, physical activity, meals and caffeinated drinks, medications and co-interventions). The participants attended a weekly follow-up visit for up to four weeks, and both MT interventions were compared with a neutral intervention after a washout period. Furthermore, the psychologist who assessed the results of the tests and the participants were blinded, providing more methodological rigor to the study.

Despite these strengths, several limitations must be noted. First, the desired sample size was not achieved. Second, when controlling for the confounding variables, some children's appointments were changed to another day in the same week because they did not meet the criteria mentioned above.

4.7. Implications for clinical practice

Certain specific populations may require HRV modulation in some situations, such as to control the adverse effects of some medications and the clinical manifestations of one or several disorders or to monitor cardiovascular activity and some MH disorders that show neurovegetative dysregulation with a basal sympathetic predominance.

Our findings are potentially useful for translational research in the use of this MC-t programme in paediatric populations with autonomic

imbalances as such populations may benefit from this intervention through the positively influence on neurovegetative parameters and presumably other associated symptoms.

5. Conclusion

Although both programmes (MC-t and Mss-t) appeared to induce a state of relaxation after the intervention, which was plausible based on the modifications in some variables linked to parasympathetic activity and decreases in some vital signs, MC-t exerted significantly greater effects on a larger number of neurophysiological variables, and these effects persisted for up to three weeks according to the time and frequency domain parameters analysed. According to these results, providing such interventions every three weeks may be ideal.

The decrease in the LF/HF ratio observed in the MC-t group throughout the process may explain the predominance of parasympathetic activity, the state of relaxation after the intervention and the better self-regulation in this group.

These findings support the theory that the proposed MC-t programme may exert effects on vagal functions since the applied techniques had a direct or indirect influence on the VN pathways. We recommend that future research studies examining neurovegetative responses to MT techniques include a larger sample size and assess longer treatment and follow-up periods. While maintaining the strengths of the study design, we recommend translation of the cranial manual therapy programme, including other neurophysiological and neuropsychological variables, to specific populations with autonomic imbalances caused by mental health disorders to study the neurophysiological effects of MT interventions and their possible relationships with psychological variables and behaviour.

Conflict of interest

None declared.

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