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Osteopathic Manipulative Treatment and Cardiovascular Autonomic Parameters in Rugby Players: A Randomized, Sham-Controlled Trial

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1 **ABSTRACT**

2 **Objectives:** The purpose of this study was to investigate the effects of osteopathic
3 manipulative treatment (OMT) on cardiovascular autonomic parameters after a rugby match.

4 **Methods:** Resting and reactivity (i.e., response to orthostasis) measures of mean arterial
5 pressure, heart rate, and heart rate variability were assessed in twenty-three male players
6 following a single session of OMT, both 18-20 hours after a rugby match and in a
7 corresponding no-match condition, in a randomized, sham-controlled, crossover design.

8 **Results:** Signs of reduced heart rate variability, and elevated mean arterial pressure and
9 heart rate were found 18-20 hours after a rugby match compared with a no-match condition.

10 A significant increase in heart rate variability and a significant reduction in mean arterial
11 pressure were observed following OMT both in the after match and no-match conditions.
12 Heart rate and heart rate variability responses to orthostasis were not affected by previous
13 match competition, but were significantly larger following OMT compared with sham
14 treatment.

15 **Conclusion:** This study documents the presence of cardiovascular autonomic alterations in
16 rugby players in the aftermath of a competitive match, which may be indicative of prolonged
17 fatigue and incomplete recovery. In these players, favorable changes in cardiovascular
18 autonomic parameters were observed following a single session of OMT, suggesting that
19 OMT may be implemented as a recovery strategy to restore players' cardiovascular
20 autonomic homeostasis after a rugby match.

21

22 Keywords: autonomic; cardiovascular; osteopathy; recovery; rugby

23

24

25

26

27 INTRODUCTION

28 It is well known that substantial cardiac autonomic adjustments must occur during exercise
29 to meet the competing demands of working muscles (metabolic demands) and skin blood
30 flow (thermoregulatory demands), while maintaining blood pressure and adequate perfusion
31 to other organs.¹ Upon exercise termination, there is an immediate cessation of mechanical,
32 chemical and thermal stimuli on the body that leads to partial cardiac autonomic recovery.²
33 However, complete recovery occurs only after chemical, humoral and thermoregulation
34 factors have returned to normal levels.^{3,4}

35 A few studies have found that higher exercise intensities delay the recovery of heart rate
36 (HR) and HR variability (HRV, a surrogate marker of cardiac autonomic function) in the
37 immediate hours that follow exercise cessation in both athletes and sedentary subjects.⁴⁻⁷

38 These cardiac autonomic changes are particularly evident in situations where the physical
39 demands associated with high exercise intensities are associated with high psychological
40 demands, as, for example, during competitive match play.^{8,9} Indeed, elevated HR and
41 reduced HRV have been described in soccer and rugby league players, and soccer referees
42 for up to 10-24 hours following the match.¹⁰⁻¹² Moreover, a previous study documented a
43 reduced ability of the autonomic nervous system (ANS) (i.e., smaller HRV changes) to
44 respond to an orthostatic challenge (i.e., active standing) on the days following a competitive
45 rugby league match.¹²

46 Importantly, similar alterations in resting and reactivity measures of cardiac autonomic
47 function have been associated with overtraining syndrome and illness in elite athletes,¹³⁻¹⁵
48 suggesting that incomplete cardiac autonomic recovery after competitive match play may
49 have potential negative consequences on subsequent training and overall performance. This
50 warrants the investigation of effective interventions aimed at facilitating a faster recovery of
51 athletes' cardiac autonomic homeostasis in the aftermath of a competitive match.

52 Osteopathic manipulative treatment (OMT) is a form of non-invasive manual treatment that
53 uses a set of touch, manipulation and mobilization procedures to diagnose, treat, and
54 prevent illness or injury.¹⁶ For many years, investigations on manual therapy techniques

55 focused on understanding their psychophysiological mechanisms and their clinical effects.¹⁷⁻
56 ¹⁹ Specifically, a theoretical basis for the effects of OMT on the body has been advanced
57 based on its action on the ANS, which causes concomitant vasodilatation, smooth muscle
58 relaxation, and increased blood flow, resulting in improved range of motion, decrease in pain
59 perception, and/or change in tissue.^{20,21} However, it was not until recently that the theoretical
60 association between OMT and ANS activity was supported by empirical evidence showing
61 that OMT leads to an increase in HRV at rest and counteracts stress-induced HRV reduction
62 in healthy individuals.²²⁻²⁵ These results appear consistent with a larger body of literature
63 which documents similar ANS responses (e.g., increased HRV and decreased systolic blood
64 pressure) to other forms of manual therapy, particularly spinal manipulative therapy, both in
65 asymptomatic individuals and patients complaining of neck or back pain.²⁶⁻³¹ The
66 documented ability of manual therapy techniques to induce ANS activation under several
67 conditions represents an opportunity to study the effects of OMT in the context of
68 cardiovascular autonomic recovery after competitive match play.
69 Specifically, the main objective of the current study was to investigate the effects of a single
70 session of OMT on resting and reactivity measures of cardiovascular autonomic function
71 (i.e., HRV, HR and blood pressure) in trained adult male players long after (18-20 hours) a
72 rugby union match. Anticipating signs of reduced HRV, and elevated HR and blood pressure
73 in the aftermath of the match, we hypothesized a normalization of these resting parameters
74 following OMT. Moreover, we tested the hypothesis that OMT would be associated with
75 greater cardiac autonomic reactivity to an orthostatic challenge (i.e., head-up tilt test).

76

77 **METHODS**

78 **Participants**

79 **Recruitment.** Volunteers from three male rugby union teams competing in the second (i.e.,
80 “Serie A”) and third (i.e., “Serie B”) tier of the 2017-2018 Italian Rugby Union championship
81 were recruited from October 01, 2017 to October 31, 2017 through direct contact. Initially, all

82 players of these teams received written information and a verbal explanation about the
83 nature and purpose of the study and were invited to take part in it.

84 ***Inclusion/Exclusion Criteria.*** Inclusion criteria included: (i) being healthy and injury-free, (ii)
85 age \geq 18 years old, (iii) being Caucasian (to reduce variability related to ethnic differences in
86 HRV and blood pressure regulation),^{32,33} (iv) regular participation in competitive matches of
87 rugby union consisting of 2, 40-min halves, and (v) regular training for an average of six
88 hours/week during the last four weeks. Exclusion criteria included: (i) history of
89 cardiovascular disease or traumatic brain injury, (ii) chronic drug treatment, (iii) use of any
90 medications during the last week, and (iv) having received OMT before.

91 ***Study settings.*** The study took place at the Italian College of Osteopathy in Parma, Italy.

92 ***Ethics.*** Written informed consent was obtained from all players prior to participation with
93 ethics approval granted from the independent institutional review board of the Foundation
94 COME Collaboration (authorization n° 032017) in accordance with the 1964 Helsinki
95 declaration and its later amendments. This trial has been registered at <http://clinicaltrials.gov/>
96 (identifier NCT04242485).

97

98 **Study Design**

99 The present study adopted a randomized, double-blind, sham-controlled, crossover design
100 to test each player four times in two different conditions, namely after match and no-match
101 (Figure 1). In the after match condition, players undertook a recording session (see below)
102 18 to 20 hours after their participation in a competitive match of rugby union, which took
103 place between 3 pm and 6 pm of the preceding day. In the no-match condition, players
104 undertook a recording session (see below) the day after a resting day in which they had not
105 done any intense or sustained physical activity as determined from a pre-screening
106 questionnaire. Every player was tested twice in the after match condition and twice in the no-
107 match condition, at the same time of the day (between 10 am and 1pm).

108 For each condition, after baseline recordings, players were randomly assigned to OMT or
109 sham treatment (Figure 2). Randomization of conditions and treatments was performed

110 according to a computer-generated table by a researcher not involved in the intervention
111 sessions. Thus, each player undertook four recording sessions: (1) after match + OMT, (2)
112 after match + sham, (3) no-match + OMT, and (4) no-match + sham (Figure 1). In addition, a
113 one-week washout period between recording sessions was employed. The after match
114 recording session (see below) could be rescheduled if the participants had not completed a
115 minimum of 60 min of match play. Subjects were considered drop-out in case they did not
116 meet this requirement at the rescheduled session or in case of nonattendance.

117

118 **Sample Size**

119 The primary study outcome measure of HRV was used for calculation of the required sample
120 size using G*Power Version 3.1.³⁴ This was based on pilot study data suggesting an effect
121 size (Cohen's *d*) of 0.65 for HRV measured before and after a single session of OMT in the
122 aftermath of a rugby match. To achieve a power of 80% at an alpha level of 0.05 a total of 21
123 players was required to detect meaningful within-subject changes in HRV. Therefore, to
124 account for up to a 20% withdrawal rate, a total of 26 players were recruited.

125

126 **Blinding**

127 Players were blinded to study design and treatment allocation (OMT or sham). Osteopaths
128 were blinded to players' conditions (i.e., after match or no-match). Data collection and
129 analysis were conducted by a researcher who was blinded to players' conditions (after
130 match or no-match) and treatments (OMT or sham).

131

132 **Procedure**

133 Participants were asked not to perform physical activity the day of the recording session and
134 to refrain from caffeine and alcohol consumption or smoking at least 12 hours prior to the
135 recording session, as these variables may have transient effects on cardiovascular
136 measurements.³⁵ The sequence of procedures adopted in this study is depicted in Figure 2.
137 Upon their arrival to the laboratory, players were instrumented with a BT16Plus device

138 (Francesco Marazza Hardware & Software, Monza, IT), which allows real time acquisition of
139 the ECG signal (sampling frequency: 250 Hz) through 2 electrodes secured to the right and
140 left parasternal regions and a third reference electrode secured on the right side of the groin
141 area. Moreover, an electronic sphygmomanometer (A&D Medical: Model UA-631 V) was
142 positioned on players' non-dominant arm. Players were then asked to lie supine, with a
143 pillow supporting the head, and refrain from moving or talking for the entire duration of the
144 recording session. After this adaptation phase which lasted 15 min, the cuff inflated and
145 systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured three
146 times alongside recording of the ECG signal for 10 min. Subsequently, players received
147 either OMT or sham treatment (see below for details) for 30 min, in a randomised sequence.
148 After the treatment, the cuff inflated and SBP and DBP were measured again for three times.
149 ECG recordings resumed during the following three 5-min phases: post-treatment, with the
150 players lying supine; tilt test, with the players being tilted head-up at 60° on a special
151 motorized table; recovery, with the players back in supine position.

152

153 **Intervention**

154 Players received either OMT or sham manipulative treatment protocols, both of which lasted
155 for 30 min and were performed by two osteopaths with the same educational curricula and
156 experience (each osteopath was randomly assigned to a player and followed him to the end
157 of the study). At the start of each OMT or sham protocol session, players received a 10-min
158 structural evaluation of the pelvis, abdomen, cervical spine, dorsal spine, lumbar spine,
159 sacrum, and upper and lower limbs to diagnose somatic dysfunction as characterized by
160 altered TART (tenderness, asymmetry, range of motion, tissue texture alteration)
161 parameters.³⁶ Subsequently, for the OMT protocol, the osteopaths treated the specific
162 somatic dysfunction found on structural evaluation for 20 min using OMT techniques that
163 were left at their discretion, including the myofascial release and cranial sacrum
164 techniques,³⁷ following a rationale grounded on the notion that "*the therapeutic application of*
165 *manually guided forces...[is crucial] to improve physiological function and homeostasis that*

166 *has been altered by somatic dysfunction*".³⁸ Alternatively, for the sham protocol, the
167 osteopaths placed both hands on a sequence of body regions of the players that included
168 the pelvis, abdomen, cervical spine, dorsal spine, lumbar spine, sacrum, and upper and
169 lower limbs. Each region was gently touched for 2-3 min without the use of any specific
170 treatment,³⁹ and total contact time with the osteopath was the same as the OMT condition
171 (20 min). It is worth noting that before the start of the study, the two osteopaths underwent a
172 4-hour training session to familiarise with and consistently reproduce the sham protocol.

173

174 **Outcomes**

175 Resting measures of HRV, HR and blood pressure in the aftermath of a rugby match and
176 following a single session of OMT were considered the primary outcomes of the study. HRV
177 and HR responses to the tilt test in the aftermath of a rugby match and following a single
178 session of OMT were considered the secondary outcomes of the study.

179 **HR and HRV Analysis.** ECG signals were amplified, converted to digital, and analysed with
180 Chart5 software (ADInstruments, Sydney, Australia) in 5-min epochs. Initially, each raw ECG
181 signal was manually inspected to ensure that all R-waves were correctly detected. Then, we
182 calculated HR plotting the number of R waves per unit time (reported in beats per minute;
183 bpm). Subsequently, we quantified time- and frequency-domain parameters of vagally-
184 mediated HRV, namely the root mean square of successive beat-to-beat interval differences
185 (RMSSD, ms) and the power of the high frequency band (HF; 0.15–0.4 Hz; ms²),
186 respectively.⁴⁰

187 **Blood Pressure Analysis.** SBP and DBP values obtained via sphygmomanometry were
188 averaged and transformed into mean arterial pressure (MAP) using a standard formula: \sum
189 (DBP + 1/3 (SBP - DBP)).

190

191 **Data Processing and Statistical Analysis**

192 Statistical analyses were performed using SPSS 25 software package (SPSS Inc., Chicago,
193 IL, USA). Statistical significance was set at $p < .05$. Assumptions for normality were tested for

194 all continuous variables using the Shapiro-Wilk test. We accounted for non-normal
195 distribution of RMSSD and HF values ($p < .001$) by calculating their natural logarithm
196 (\ln RMSSD and \ln HF). Pearson correlation coefficients were computed between resting (i.e.,
197 pre-treatment) MAP, HR, \ln RMSSD, and \ln HF values corresponding to the two after match
198 and the two no-match conditions, respectively. Given the high correlation coefficients
199 ($0.75 < r < 0.83$) found between cardiovascular parameters obtained in the same resting
200 condition, average MAP, HR, \ln RMSSD, and \ln HF resting values were calculated for each
201 player for the after match and no-match condition.

202 Subsequently, to evaluate alterations in resting cardiovascular parameters following a rugby
203 union match, a series of general linear models (GLMs) were applied, with “condition” being
204 the within-subject factor (two levels: after match or NO-MATCH), controlling for the effects of
205 smoking status.

206 The effects of OMT and sham treatment on resting cardiovascular parameters were
207 calculated as delta values (i.e., the differences between post-treatment MAP, HR, \ln RMSSD,
208 and \ln HF values and the corresponding pre-treatment values) and analysed by means of a
209 series of 2 (after match or no-match) \times 2 (OMT or sham) GLMs, controlling for the effects of
210 smoking status.

211 Finally, HR, \ln RMSSD and \ln HF responses to the tilt test and the following recovery phase
212 were evaluated as delta values (i.e., tilt test value – pre-tilt resting value and recovery value
213 – pre-tilt resting value) and analysed by means of a series of 2 (after match or no-match) \times 2
214 (OMT or sham) \times 2 (tilt test or recovery) GLMs, controlling for the effects of smoking status.

215

216 **RESULTS**

217 **Participants Characteristics**

218 In total 23 participants completed the study and provided data for the analysis (Figure 1).
219 Their average age and body mass index were, respectively, 24.1 (SD=4.8) years and 30.8
220 (SD=4.9) kg/m²; 26% of them were occasional smokers.

221

222 **Resting Cardiovascular Parameters After a Rugby Union Match**

223 The after match condition was characterized by significantly higher resting values of MAP
224 ($F=5.3$, $p<.05$, $\eta_p^2=0.201$) (Figure 3A) and HR ($F=9.3$, $p<.01$, $\eta_p^2=0.307$) (Figure 3B), and
225 significantly lower resting values of lnRMSSD ($F=8.43$, $p<.01$, $\eta_p^2=0.286$) (Figure 3C) and
226 lnHF ($F=11.1$, $p<.01$, $\eta_p^2=0.346$) (Figure 3D) compared with the no-match condition.

227

228 **Resting Cardiovascular Parameters Following OMT**

229 The GLM applied to delta MAP values yielded a significant effect of treatment ($F=12.7$,
230 $p<.01$, $\eta_p^2=0.256$), with a significantly larger reduction in resting MAP values after OMT than
231 sham treatment both in the after match ($p<.01$, $\eta_p^2=0.185$) and no-match ($p<.05$, $\eta_p^2=0.121$)
232 condition (Figure 4A).

233 As for delta HR values, we found a significant effect of condition ($F=4.9$, $p<.05$, $\eta_p^2=0.117$),
234 and only a marginally significant effect of treatment ($F=3.3$, $p=.07$, $\eta_p^2=0.082$). In other
235 words, the reduction in resting HR values was larger in the after match condition compared
236 to the no-match condition independently from OMT or sham treatment (after match: -5.2 ± 0.9
237 bpm vs no-match: -2.9 ± 0.9 bpm, $F=9.8$, $p<.05$, $\eta_p^2=0.121$) (Figure 4B).

238 The GLM applied to delta lnRMSSD values yielded a significant effect of treatment ($F=6.8$,
239 $p<.05$, $\eta_p^2=0.347$), with a significantly larger increase in resting lnRMSSD values after OMT
240 than sham treatment both in the after match ($p<.05$, $\eta_p^2=0.114$) and no-match ($p<.05$,
241 $\eta_p^2=0.138$) condition (Figure 4C).

242 As for delta lnHF values, we found a significant effect of treatment ($F=5.6$, $p<.05$, $\eta_p^2=0.132$),
243 with a significantly larger increase in resting lnHF values after OMT than sham treatment
244 only in the after match condition ($p<.05$, $\eta_p^2=0.124$) (Figure 4D).

245

246 **Cardiac Autonomic Responses to the Tilt Test**

247 The GLM applied to delta HR values yielded a significant effect of time ($F=43.9$, $p<.01$, η_p^2
248 $=0.543$). As expected, there was an increase in HR values during the tilt phase, with no

249 significant effects of condition (after match or no-match) or treatment (sham or OMT) (Figure
250 5A).

251 As for delta lnRMSSD values, we found a significant effect of time ($F=45.0$, $p<.01$,
252 $\eta_p^2=0.554$) and a time x treatment interaction ($F=4.2$, $p<.05$, $\eta_p^2=0.107$). As expected, there
253 was a decrease in lnRMSSD values during the tilt phase, which was significantly larger after
254 OMT than sham treatment independently from condition (i.e., after match or no-match)
255 (OMT= -0.78 ± 0.09 vs sham= -0.48 ± 0.08 , $F=5.9$, $p<.05$, $\eta_p^2=0.138$) (Figure 5B). Similarly, the
256 GLM applied to delta HF values yielded a significant effect of time ($F=46.6$, $p<.01$,
257 $\eta_p^2=0.557$) and a significant time x treatment interaction ($F=5.5$, $p<.05$, $\eta_p^2=0.129$). As
258 expected, there was a decrease in lnHF values during the tilt phase, which was significantly
259 larger after OMT than sham treatment independently from condition (i.e., after match or no-
260 match) (OMT= -1.49 ± 0.25 vs sham= -0.77 ± 0.20 , $F=5.1$, $p<.05$, $\eta_p^2=0.122$) (Figure 5C).

261

262 **Harms**

263 **No adverse events or unintended effects were reported following OMT or sham treatment.**

264

265 **DISCUSSION**

266 The major and novel finding of the current study is the presence of increased resting and
267 reactivity measures of HRV and reduced resting MAP following a single session of OMT in
268 rugby players, both in the aftermath of a competitive match and in a no-match control
269 condition.

270 Rugby union is a high-intensity team sport during which players perform repeated high
271 intensity efforts such as sprints, high-impact collisions, and rapid changes of direction. Not
272 surprisingly, we found signs of reduced vagally-mediated HRV, and elevated MAP and HR,
273 during the assessment of resting cardiovascular parameters 18-20 hours after a rugby union
274 match in trained players. These results confirm, and further extend, previous research
275 demonstrating lower vagal-related HRV indexes for up to 10-24 hours following soccer or

276 rugby league matches.¹⁰⁻¹² Other studies have shown that while the decrease of HR and
277 sympathetic predominance is fast at the end of an exercise, it takes several minutes, even
278 several hours to recover the initial level of resting cardiac autonomic balance.^{41,42} This
279 physiological adaptation is essential to maintain an important blood flow in active muscles to
280 remove metabolic waste produced during exercise, especially during competitive
281 conditions.⁴³ The delay of returning to a basal state of sympathovagal balance seems
282 dependent on the intensity of the exercise, because it was found to be more important after
283 higher than lower exercise intensities, and may involve the arterial baroreflex and its
284 sensibility.⁴ Therefore, signs of cardiac autonomic imbalance in the aftermath of an intense
285 exercise performed during a competitive match may indicate incomplete recovery and
286 prolonged fatigue. Indeed, physical markers of fatigue (e.g., reduced jump peak power) have
287 been described in rugby league players for up to 48 hours after match play.⁴⁴

288 The combative nature of rugby union combined with high intensity efforts is synonymous
289 with repeated blunt force trauma and post-match muscle soreness. Given the negative
290 relationship found between acute and chronic pain and vagally-mediated HRV,^{45,46} it is
291 plausible that signs of reduced vagally-mediated HRV after a rugby match may also be a
292 consequence of post-match aches and pains. Notably, a sustained elevation of cortisol
293 levels has also been described in the 24 hours that follow a rugby league match.⁴⁷ Taken
294 together, these findings indicate an incomplete return to hormonal and cardiovascular
295 homeostasis for at least 24 hours after exposure to physical and psychological stress
296 associated with rugby match play.

297 Contrary to our expectations, we did not find post-match changes in cardiac autonomic
298 reactivity to an orthostatic challenge. This is in disagreement with a previous study
299 documenting a reduced ability of the ANS (i.e., smaller HRV changes) to respond to
300 orthostasis on the days following a competitive rugby league match.¹² A potential
301 explanation for this discrepancy lies in the difference between the orthostatic challenge
302 adopted by the Edmonds' study (i.e., active standing) and the present study (i.e., 60°
303 passive head-up tilting). It must also be acknowledged that the assessment of blood

304 pressure changes, as well as of baroreflex activity, during the orthostatic challenge would
305 have provided a more complete picture of the ability of the cardiovascular system to adapt to
306 postural changes the day after a rugby match.

307 The implementation of recovery strategies in the aftermath of a rugby match may be
308 beneficial to restoring players' physiological systems for greater adaptations to subsequent
309 training and enhanced overall performance. In this context, the main objective of this study
310 was to evaluate the effects of a single session of OMT on resting and reactivity measures of
311 cardiovascular autonomic function after a rugby union match. Notably, we found an increase
312 in resting measures of vagally-mediated HRV following OMT compared to sham treatment,
313 particularly in the after match condition. Moreover, a significant decrease in resting MAP
314 values was found following OMT, but not sham treatment, both in the after match and no-
315 match condition. Changes in resting HR after OMT were somewhat less evident. This was
316 due to the fact that the sham treatment that we adopted in this study (i.e., "soft touch"
317 without applying any pressure) was also associated with a reduction in HR and, partly, an
318 increase in vagally-mediated HRV, which is in line with previous studies.^{23,25} Lastly, we found
319 greater cardiac autonomic responses (i.e., larger HRV changes) to the orthostatic challenge
320 following OMT compared to sham treatment, independently from previous competitive
321 participation in a rugby union match.

322 These results are in line with a growing body of research showing an increase in vagally-
323 mediated HRV at rest or during a stressful challenge after OMT, independently from the part
324 of the body to which it is applied.²²⁻²⁵ Moreover, we extend this previous research by showing
325 that OMT is associated with reduced MAP in healthy normotensive individuals. Moreover,
326 these results are consistent with previous research documenting an increase in vagally-
327 mediated HRV and a decrease in systolic blood following other forms of manual therapy,
328 particularly spinal manipulative therapy, both in asymptomatic individuals and patients
329 complaining of neck or back pain.²⁶⁻³¹ Taken together, these pieces of evidence support the
330 view that manual therapy techniques, including OMT, can induce ANS activation under
331 several conditions.

332 In the current study we did not investigate the mechanisms underlying the changes in
333 cardiovascular autonomic parameters following OMT. A previous study, which explored the
334 association between OMT and hypertension, found that OMT (in addition to routine care)
335 was associated with improved intima media thickness and systolic blood pressure after one-
336 year follow-up.¹⁸ The authors of this study suggested that in the presence of trauma or
337 somatic dysfunction changing the structure of the tissue, OMT, consistently with in-vitro
338 models,⁴⁸ may decrease the production of inflammatory cytokines, generating a cascade
339 effect on mechanisms that generally improve the metabolism of the arterial wall and activate
340 the ANS.^{18,23,49} Consequently, we speculate that the cardiovascular changes observed
341 following OMT in this sample of rugby players may involve indirect mechanisms, including a
342 reduction of post-match soreness and inflammation through correction of somatic
343 dysfunctions, or direct mechanisms, including activation of c-tactile afferent projections to
344 brain stem nuclei involved in the autonomic control of cardiovascular function, or a
345 combination of both.^{50,51}

346

347 **Limitations**

348 The results of this study must be interpreted within the context of their limitations. First, we
349 did not quantify other subjective (e.g., rating of perceived exertion) and/or objective (e.g.,
350 blood lactate) measures to characterize the psychophysiological overload caused by the
351 rugby match. Also, we did not measure pain or soreness, which may have helped elucidate
352 a potential indirect effect of OMT on cardiovascular function. Similarly, measurements of
353 blood lactate or pH may have helped raise hypotheses about another potential indirect effect
354 of OMT on the ANS via improvements of lactate removal and overall metabolic recovery.
355 Second, the sample size is relatively small and a type II error is possible. However, we
356 adopted a within-subjects design to reduce error variance associated with individual
357 differences and increase statistical power. Nevertheless, future research should involve a
358 randomized clinical trial with a larger sample size. Third, we did not examine the time course
359 of post-match changes in cardiovascular parameters, but restricted our observation to a

360 specific time window. Further, due to practical/logistic reasons, OMT and sham treatment
361 were performed by two osteopathic practitioners, which might have affected protocol
362 homogeneity. Lastly, players' perception of treatment allocation was not assessed and may
363 have changed during the study, even if "treatment context" (similar contact time with
364 osteopaths and settings) was similar between OMT and sham treatment. Therefore, it would
365 be important to ascertain whether changes in cardiovascular parameters might be ascribable
366 to changes in attributed self-relevance and efficacy of the received treatment. Future studies
367 are needed to clarify all these aspects.

368

369 **CONCLUSION**

370 The present results add to a growing body of literature documenting the development of
371 alterations in cardiovascular autonomic parameters following a competitive rugby union
372 match in trained players. Moreover, these results suggest the presence of increased resting
373 and reactivity measures of HRV and reduced resting MAP following a single session of OMT
374 in rugby players, both in the aftermath of a competitive match and in a no-match condition.
375 This warrants the investigation of the potential underlying mechanisms, which at present are
376 largely unknown. Notwithstanding the above reported limitations, OMT holds promise as a
377 strategy to promote a faster cardiovascular autonomic recovery from a rugby union match,
378 but its efficacy and applicability to other sports and/or conditions characterized by
379 cardiovascular alterations and autonomic imbalance requires further investigation in larger
380 studies.

381

382 **Figure legends**

383 Figure 1. Flow chart.

384

385 Figure 2. Timeline of the experimental procedures adopted in this study. Abbreviations: OMT
386 = osteopathic manipulative treatment.

387

388 Figure 3. Resting cardiovascular parameters in the no-match and after match conditions.
389 Data are expressed as means \pm SEM. Abbreviations: MAP = mean arterial pressure;
390 lnRMSSD = natural logarithm of the root mean square of successive beat-to-beat interval
391 differences; lnHF = natural logarithm of the high frequency band. # indicates a significant
392 difference between the two conditions ($p < .05$).

393

394 Figure 4. Changes induced by OMT and sham treatment on resting cardiovascular
395 parameters in the no-match and after match conditions. Data are expressed as means \pm
396 SEM. Abbreviations: OMT = osteopathic manipulative treatment; MAP = mean arterial
397 pressure; lnRMSSD = natural logarithm of the root mean square of successive beat-to-beat
398 interval differences; lnHF = natural logarithm of the high frequency band. * indicates a
399 significant difference between OMT and the “same condition” sham treatment ($p < .05$); #
400 indicates a significant difference between the no-match and after match conditions ($p < .05$).

401

402 Figure 5. Cardiac autonomic responses to the 60° head-up tilt test. Data are expressed as
403 means \pm SEM of delta values, which were calculated as the difference between tilt/recovery
404 values and their respective pre-tilt resting values. Abbreviations: OMT = osteopathic
405 manipulative treatment; lnRMSSD = natural logarithm of the root mean square of successive
406 beat-to-beat interval differences; lnHF = natural logarithm of the high frequency band. *
407 indicates a significant difference between OMT and sham treatment ($p < .05$).

408

409

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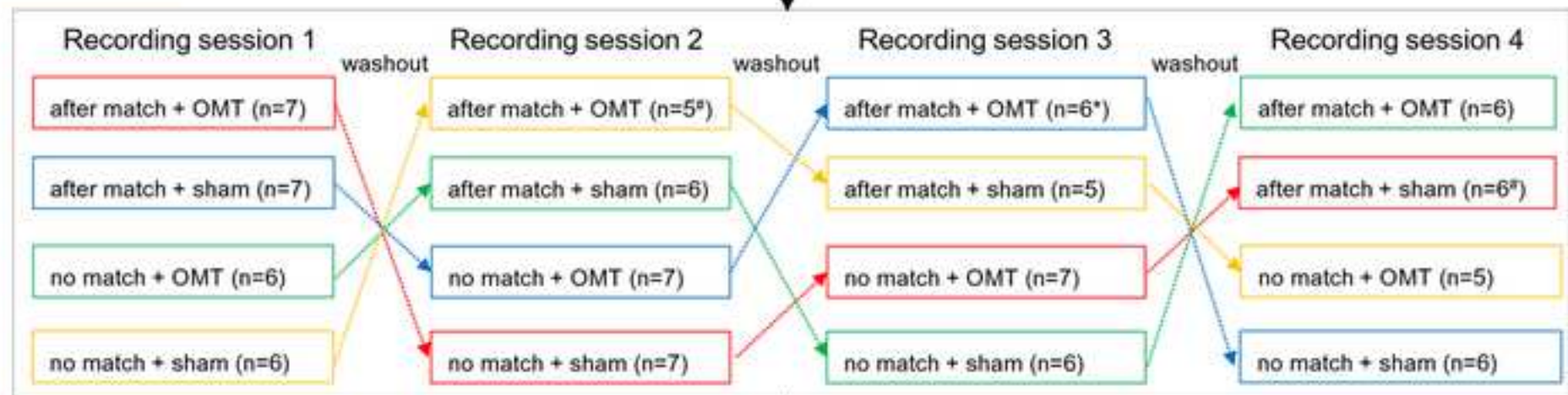
ENROLLMENT

Assessed for eligibility
(n=65)

Excluded (n=39)
 - Not meeting inclusion criteria (n=27)
 - Declined to participate (n=12)
 - Other reasons (n=0)

ALLOCATION

Randomized to condition and
treatment (n=26)



FOLLOW UP

Completed all recording sessions (n=23)
 Not attending all recording sessions due to
 injury development (* n=1) or work
 commitments or tardiness (# n=2)

ANALYSIS

Analysed (n=23)
 - Excluded from analysis (n=0)

