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Hydrotherapy with hydrogen-rich water compared with RICE protocol following acute ankle sprain in professional athletes: a randomized non-inferiority pilot trial

Dejan Javorac[a], Valdemar Stajer[a], Laszlo Ratgeber[b], Andras Olah[b], Jozsef Betlehem[b], Pongras Acs[b], Boris Vukomanovic[c] and Sergej M. Ostojic[a,b]

[a]Faculty of Sport and Physical Education, University of Novi Sad, Novi Sad, Serbia; [b]Faculty of Health Sciences, University of Pecs, Pecs, Hungary; [c]Department of Orthopedic Surgery, University of Belgrade, School of Medicine, Belgrade, Serbia

ABSTRACT

We analysed the effects of an experimental novel protocol of intensive hydrotherapy with hydrogen-rich water (HRW) on injury recovery in athletic men who suffered an acute ankle sprain (AAS) and compared it with a RICE protocol (rest, ice, compression, elevation). Professional athletes (age 23.7 ± 4.0 years; weight 78.6 ± 5.7 kg, height 182.5 ± 4.3 cm; professional experience 5.9 ± 3.9 years) who incurred AAS during a sport-related activity were randomly assigned immediately after the injury to either hydrogen group (n = 9) or a conventional RICE treatment group (n = 9). Hydrogen group received six 30-min ankle baths with HRW throughout the first 24 h post-injury, with hydrotherapy administered every 4 hours during the intervention period. RICE group stood off the injured leg, with ice packs administered for 20 min every 3 hours, with the injured ankle compressed with an elastic bandage for 24 hours and elevated at all possible times above the level of the heart. HRW was equivalent to RICE protocol to reduce ankle swelling (2.1 ± 0.9% vs. 1.6 ± 0.8%; P = 0.26), range of motion (2.4 ± 1.3 cm vs. 2.7 ± 0.8 cm; P = 0.60), and single-leg balance with eyes opened (18.4 ± 8.2 sec vs. 10.7 ± 8.0 sec; P = 0.06) and closed (5.6 ± 8.4 sec vs. 3.9 ± 4.2 sec; P = 0.59). This non-inferiority pilot trial supports the use of HRW as an effective choice in AAS management. However, more studies are needed to corroborate these findings in other soft tissue injuries.

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Ankle sprain; molecular hydrogen; swelling; interleukin-1 beta; rice; non-inferiority trial

Introduction

Acute ankle sprain (AAS) is an extremely common injury among athletes and other physically active individuals that requires a prompt, convenient, safe and effective treatment. The method known as RICE has been traditionally recognized as "the gold standard" therapeutic option following AAS, with this first-aid intervention focused to reduce joint swelling and pain, and speed up healing (Wolfe et al., 2001). This method was introduced to the sports medicine community by Mirkin and Hoffman (1978) over

CONTACT Sergej M. Ostojic sergej@panet.rs Applied Bioenergetics Lab, Faculty of Sport and Physical Education, University of Novi Sad, Novi Sad 21000, Serbia

40 years ago. The acronym RICE is a mnemonic to remember four elements of immediate care for sprains, strains and other soft tissue injuries, including Rest, Ice, Compression, and Elevation (for a detailed review see ET Chen et al., 2019). The National Athletic Trainers' Association position statement on conservative management and prevention of ankle sprains in athletes enlisted RICE among best practices employed by athletic trainers and other health-care professionals immediately after AAS (Kaminski et al., 2013). However, many recent studies proposed several therapeutic alternatives to RICE for AAS management (Wells et al., 2019; Zhao et al., 2018). In particular, different treatment approaches with gaseous hydrogen have been recently put forward in musculoskeletal and sports medicine (Ostojic, 2016; Ostojic et al., 2014). A recent case report by our group indicated that multi-session hydrotherapy with hydrogen-rich water (HRW) might be a helpful intervention in terms of pain, swelling reduction and regaining range of motion after AAS in a professional athlete (Javorac et al., 2020). HRW positively affected AAS-induced signs and symptoms at 24-h follow up perhaps due to its antioxidant, anti-inflammatory and anti-apoptotic effects. Due to its small size and high diffusing capacity, hydrogen originating from HRW could be easily transported to hard-to-reach tissues (including areas of injury) where it might prevent or neutralize the generation of toxic compounds that occur after initial injury due to subsequent cell damage and tissue hypoxia, often referred as a secondary injury (Ostojic, 2015). Nevertheless, no studies so far validated the effectiveness and safety of a novel experimental treatment with hydrogen against the standard clinical procedure of RICE for AAS management in a randomized clinical trial. This non-inferiority pilot study thus aimed to monitor the impact of intensive ankle baths with HRW administered throughout the first 24 h post-injury on tissue recovery and inflammatory biomarkers in athletic men who suffered AAS and compare it with the RICE regimen in a randomized controlled parallel-group trial.

Methods

Participants

A total of eighteen healthy male professional athletes (age 23.7 ± 4.0 years; weight 78.6 ± 5.7 kg, height 182.5 ± 4.3 cm; professional experience 5.9 ± 3.9 years) who incurred AAS during a sport-related activity signed an informed consent to voluntarily participate in this randomized controlled non-inferiority parallel-group trial. All participants were aged 18 to 30 years, with normal weight (body mass index 18.5–24.9 kg/m^2), free of major chronic diseases or acute disorders, and with no history of a previous AAS during the past 6 months. The study protocol was approved by the local IRB at the University of Novi Sad (ABL11-HRW/2019), with the study protocol systematized in accordance with the Declaration of Helsinki. The trial was registered at *ClinicalTrials.gov* (NCT04167202).

Experimental design

All participants were evaluated immediately after AAS by a health-care professional, with the type and degree of injury (AAS grade I or II) confirmed by a physical examination. At this initial examination, the participants also completed a visual analog score (VAS) score for pain at rest and during movement (Hawker et al., 2011). The figure-of-eight method of measuring ankle joint swelling and weight-bearing lunge test (WBLT) for ankle range of motion were conducted as previously described (Petersen et al., 1999), with the single leg balance test (SLBT) executed with eyes open and closed (Trojian & McKeag, 2006).

Venous blood was collected and serum immediately analysed for biomarkers of inflammation, including C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1ß) using commercial ELISA kits with an automated analyser (Hitachi, Tokyo, Japan). After this baseline analyzes, all participants were randomly assigned to either the hydrogen group (n = 9) or the conventional RICE treatment group (n = 9). The first session of either treatment was given after ~60 min after the injury. Hydrogen group received six 30-min ankle baths with HRW throughout the first 24 h post-injury, with hydrotherapy administered every 4 hours during the intervention period. HRW was produced by putting a magnesium-producing formulation into a 3-L stationary whirlpool with tap water of neutral temperature (20°C). Hydrogen was produced by the following reaction: $Mg + H_2O \rightarrow H_2 + Mg(OH)_2$, with the concentration of hydrogen in a whirlpool ~8 ppm. The intervention was provided by HRW Natural Health Products Inc. (New Westminster, BC, Canada). The conventional RICE treatment group stood off the injured leg, with ice packs administered for 20 min every 3 hours (total of 8 sessions), with the injured ankle compressed with an elastic bandage for 24 hours and elevated at all possible times above the level of the heart. This non-inferiority pilot trial attempted to show that the new treatment (hydrotherapy with HRW) is not an unacceptably worse alternative to the standard RICE protocol so no other interventions were provided during the period of evaluation to either group. Ankle swelling, pain at rest and during movement, range of movement, balance and serum inflammatory markers were re-assessed at 24-h follow-up. In addition, the participants were instructed to report any adverse effects of the intervention (e.g., tingling, skin discolouration, burning, itching, rash) through an open-ended questionnaire.

Statistical analyses

The number of participants was calculated using power analysis (G*Power 3.1, Heinrich Heine University, Düsseldorf, Germany), with effects size set at 0.70, alpha error probability 0.05, power 0.80 for two groups and two measurements of study outcomes. The primary outcome was the change in ankle joint swelling assessed at baseline and 24-h post-injury. A comparison between outcomes in each interventional group at baseline and 24-h follow up was conducted using the paired-samples T-test. Two-way ANOVA for repeated measures (treatment vs. time) was used to establish if any significant differences existed between two interventions administered during the experiment. Where significant differences were found, the Tukey post-hoc test was employed to identify the differences. The significance level was set at $P \leq 0.05$. Data were analysed using the SPSS program (version 21.0) (SPSS Inc., Chicago, IL, USA).

Results

All participants completed the trial, with no patient reported any relevant side effects of either intervention. The changes in ankle swelling, pain at rest and during movement, range of movement, balance with eyes opened and closed, and serum inflammatory markers during the trial are depicted in Table 1. Hydrotherapy with HRW significantly reduced ankle

Table 1. Changes in injury outcomes and serum inflammatory markers during the trial. Values are mean ± SD.

	Baseline	24 h follow-up	*P* *
Ankle swelling (cm)			
HRW (*n* = 9)	55.4 ± 3.0	54.2 ± 2.7 †	0.26
RICE (*n* = 9)	55.3 ± 3.3	54.5 ± 3.4 †	
Pain at rest (score)			
HRW	48.9 ± 10.7	29.9 ± 8.1 †	0.12
RICE	47.6 ± 11.4	37.1 ± 9.1 †	
Pain at movement (score)			
HRW	69.3 ± 16.8	44.7 ± 14.9 †	0.13
RICE	70.2 ± 13.1	54.4 ± 13.3 †	
WBLT (cm)			
HRW	4.1 ± 1.7	6.5 ± 1.9 †	0.60
RICE	4.1 ± 2.0	6.7 ± 2.0 †	
SLBT with eyes open (s)			
HRW	24.6 ± 10.3	43.0 ± 14.4 †	0.06
RICE	31.4 ± 13.0	42.1 ± 11.9 †	
SLBT with eyes closed (s)			
HRW	8.8 ± 6.2	14.3 ± 7.5	0.59
RICE	8.7 ± 5.6	12.6 ± 6.8 †	
CRP (mg/dL)			
HRW	7.1 ± 3.9	6.8 ± 2.5	0.55
RICE	6.8 ± 3.6	6.0 ± 2.6	
TNF-α (pg/mL)			
HRW	27.1 ± 14.6	23.3 ± 13.7	0.45
RICE	25.8 ± 7.2	23.8 ± 9.7	
IL-1ß (pg/mL)			
HRW	0.6 ± 0.4	0.5 ± 0.3	0.17
RICE	0.4 ± 0.2	0.4 ± 0.2	

Abbreviations: HRW, hydrogen-rich water; RICE, rest, ice, compression, elevation; WBLT, weight-bearing lunge test; SLBT, single-leg balance test; CRP, C-reactive protein; TNF-α, tumour necrosis factor-alpha; IL-1ß, interleukin-1 beta. † Indicates significant difference baseline *vs*. follow-up at $P \leq 0.05$ for each intervention. * *P* values from independent samples two-way ANOVA time x intervention for repeated measures (HRW *vs*. RICE).

swelling for 2.1% at 24 h follow-up (95% confidence interval [CI] from 1.4 to 2.9; $P = 0.0002$). Individual changes in the primary outcome (ankle swelling) between hydrotherapy with HRW and RICE are presented in Figure 1. Pain at rest and during movement significantly dropped after HRW intervention ($P < 0.001$), while the range of motion evaluated with WBLT improved for 2.4 cm (95% CI from 1.4 to 3.4; $P = 0.0005$) at follow-up. In addition, single-leg balance with eyes open and closed significantly improved for 18.4 sec (95% CI from 12.1 to 24.7; $P = 0.0001$) or tended to improve (5.6 sec on average; 95% CI from – 0.9 to 12.1; $P = 0.08$) after HRW intervention, respectively. A significant reduction in ankle swelling and pain at rest and during movement were also found in the RICE group ($P < 0.05$), accompanied by augmented WBLT and SLBT scores at 24 h follow-up ($P < 0.05$). Neither intervention affected serum CRP, TNF-α and IL-1ß ($P > 0.05$), although HRW treatment tended to reduce circulating IL-1ß levels at post-administration (for 10.8% on average; 95% CI from – 6.0 to 27.6; $P = 0.07$). A two-way ANOVA with repeated measures revealed no significant differences between hydrotherapy with HRW and RICE treatment for reducing ankle swelling and pain at rest and during movement, and improving range of motion and balance after AAS ($P > 0.05$).

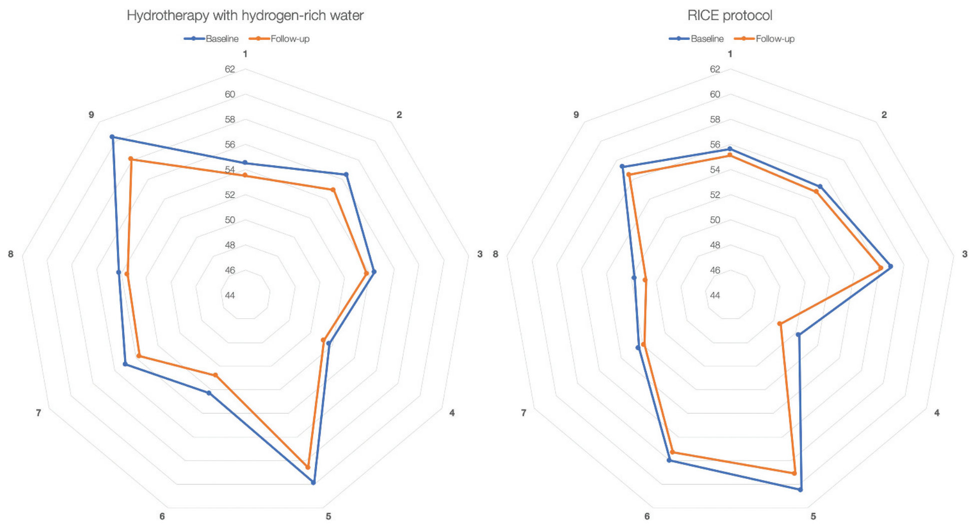

Figure 1. Individual changes in the primary outcome (ankle swelling) between hydrotherapy with hydrogen-rich water (left panel, $n = 9$) and RICE protocol (right panel, $n = 9$). Values are shown in cm of ankle circumference measured by the figure-of-eight method at baseline and 24-h follow-up.

Discussion

This randomized controlled pilot trial demonstrated the non-inferiority of HRW hydrotherapy to RICE protocol for post-injury recovery in young professional athletes who suffered an acute ankle sprain. It appeared that ankle bathing with HRW is equally effective as the gold-standard therapeutic option to reduce joint swelling and pain while regaining range of motion and balance when administered intensively during the first 24 hours after the damage. This perhaps advances hydrotherapy with HRW as a novel approach to tackle AAS in sports medicine.

While found non-inferior than standard RICE protocol in terms of efficacy and safety, hydrotherapy with HRW appeared comparatively easy to administer. It requires only one straightforward procedure (ankle bathing) while RICE being more demanding with regard to various techniques employed (ET Chen et al., 2019). As an easy-to-follow method, HRW hydrotherapy might therefore be highly relevant to professional athletes (and health-care professionals) looking for a fast and helpful strategy in AAS management and recovery, fitting their busy schedule while requiring minimal resources. On the other hand, due to the fact that hydrogen in water tends to rapidly evaporate over time (CS Huang et al., 2010), hydrotherapy with HRW should be prepared at the site and monitored for hydrogen concentration to keep it above a therapeutically effective level of ≥1 ppm. The rate of hydrogen exsolution and dissipation from the water is directly affected by time, temperature, agitation, and surface area, with an open container of dissolved HRW has a half-life of about 2 hours (Molecular Hydrogen Institute, 2019).

Being prompted by the evident effects of H_2 on disuse muscle atrophy, cartilage trauma, and osteopenia in animal studies, a number of trials in the past decade or so evaluated the effectiveness of hydrogen therapeutics in patients suffering from different muscle, ligament and bone conditions: from sprains and strains to chronic joint disorders to acute muscular injuries (for a detailed review see Ostojic, 2016). Case in point, hydrogen reduces disease activity (tenderness and swelling) in 28 joints in patients with rheumatoid arthritis (Ishibashi et al., 2012). In the case of acute injuries, topical hydrogen appears to augment biomarkers of inflammation and provide a faster return to normal joint range of motion in professional athletes who suffered sports-related soft tissue damage (Ostojic et al., 2014). The hydrogen-rich formulation was found beneficial in attenuating acute muscle damage in a rat model of skeletal muscle injury induced by 3-h tourniquet occlusion and 4-h reperfusion (T Huang et al., 2015). Favourable effects of H_2 were reported in another experimental model of muscular damage (Watanabe et al., 2017), with mice that had been treated with H_2 recovered faster from acute muscle injury and achieved smoother walking at post-administration. Our study corroborates previous findings, with ankle bathing with super-saturated HRW improved several outcomes of functional recovery after AAS. Ankle swelling dropped rapidly for ~2% after hydrogen treatment, with one patient (soccer player, age 24) experienced even 3.8% reduction in joint swelling at 24-h follow up (equivalent to 2.3 cm decrease in ankle circumference). Since joint swelling compromises adequate recovery after musculoskeletal injury (Van den Bekerom et al., 2012), intensive hydrotherapy with HRW might be put forward as an advanced intervention that reverses acute swelling and thus promotes rapid recovery after an ankle sprain in an athletic environment. A possible anti-oedematous action of hydrogen could be due to anti-inflammation effects mediated by various mechanisms that involve H_2 (for a review see Qiu et al., 2019), with a previous study proven that different parameters of inflammation (including foot volume) were attenuated by hydrogen treatment in carrageenan-induced paw oedema (Xu et al., 2012). We also found that ankle bathing with HRW diminishes both pain at rest and during movement. The pain-relieving effects of hydrogen were substantiated in many conditions, including painful bladder syndrome (Matsumoto et al., 2013), neuropathic pain (Kawaguchi et al., 2014), and post-herpetic neuralgia (Ma et al., 2017), perhaps by counteracting pain-provoking oxidative stress (Y Chen et al., 2015) and cytokine release (Y Chen et al., 2015). Besides, we demonstrated that intense HRW hydrotherapy improved weight-bearing lunge test performance (2.4 cm on average, corresponding to ~9 degrees of ankle dorsiflexion), suggesting a better range of motion at the ankle joint at 24-h follow-up. This was accompanied by a regained balance performance at post-administration (particularly for a single-leg balance test performance with eyes opened). Both advancements are probably due to a hydrogen-driven swelling reduction that enables a better amount of movement of ankle joint and faster recovery of functional ankle stability after AAS. Finally, we found no significant changes in serum inflammatory markers during the trial although HRW hydrotherapy tended to reduce circulating IL-1ß levels at 24-h follow-up. There is a vast literature reporting hydrogen-induced changes in various inflammation-related biomarkers in blood serum and plasma (for a review see Qiu et al., 2019; Li et al., 2018), with hydrogen exerts a therapeutic anti-inflammatory effect in many conditions. A possible explanation for this discrepancy could be the duration of the monitoring period used in our study and the route of hydrogen administration. A rather short-term trial employed here (e.g., 24 hours) may not be long enough to enable appropriate monitoring of circulating dynamics for CRP, TNF-α, and IL-1ß in response to acute injury-induced inflammation and succeeding HRW intervention. In addition, topical hydrogen applied to a localized area of the body (e.g., foot and ankle) might not be the most suitable technique to produce systemic action.

Several limitations should be considered when our study findings are interpreted. First, we exclusively recruited male patients for this pilot trial therefore no information has been provided for a possible gender-specific response to hydrotherapy with HRW after AAS. Second, our study evaluated intensive HRW hydrotherapy in patients with mild-to-moderate sports-related AAS while no data have been provided for athletes who experienced a severe sprain (grade III) with complete ligament rupture, large swelling, high-tenderness loss of function, and marked instability, or non-athletic populations with AAS. Third, our patients were followed for a comparatively short period of time (corresponding to the acute phase of injury) while the possible impact of hydrotherapy with HRW for subacute and/or chronic phase of AAS remained unaddressed at this moment. Fourth, the changes in H_2 concentration in HRW whirlpool throughout the 30-min ankle bath remained unknown. Finally, only a few clinician- and patient-reported outcomes were analysed in this trial, and no biomarkers addressing a possible mechanism of hydrogen action in AAS were assessed.

Conclusion

Hydrotherapy with hydrogen-rich water is non-inferior to RICE protocol in terms of reducing joint swelling and pain while regaining range of motion and balance when administered intensively during the first 24 hours after AAS in professional athletes. This pilot RCT brings forward intensive ankle bathing with HRW as an effective, safe and convenient approach to tackle AAS in sports medicine, and being superior in terms of practical implications for a sporting/clinical context. However, more studies are required to corroborate these findings in other soft tissue injuries and non-athletic populations.

Disclosure statement

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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References

Chen, E. T., Borg-Stein, J., & McInnis, K. C. (2019). Ankle sprains: Evaluation, rehabilitation, and prevention. *Current Sports Medicine Reports*, *18*(6), 217–223. https://doi.org/10.1249/JSR.0000000000000603

Chen, Y., Chen, H., Xie, K., Liu, L., Li, Y., Yu, Y., & Wang, G. (2015). H2 treatment attenuated pain behavior and cytokine release through the HO-1/CO pathway in a rat model of neuropathic pain. *Inflammation*, *38*(5), 1835–1846. https://doi.org/10.1007/s10753-015-0161-x

Hawker, G. A., Mian, S., Kendzerska, T., & French, M. (2011). Measures of adult pain: Visual analog scale for pain (VAS Pain), Numeric rating scale for pain (NRS Pain), McGill pain questionnaire (MPQ), Short-form McGill pain questionnaire (SF-MPQ), Chronic pain grade scale (CPGS), Short form-36 bodily pain scale (SF-36 BPS), and Measure of intermittent and constant osteoarthritis pain (ICOAP). *Arthritis Care & Research*, *63*(S11), S240–252. https://doi.org/10.1002/acr.20543

Huang, C. S., Kawamura, T., Toyoda, Y., & Nakao, A. (2010). Recent advances in hydrogen research as a therapeutic medical gas. *Free Radical Research*, *44*(9), 971–982. https://doi.org/10.3109/10715762.2010.500328

Huang, T., Wang, W., Tu, C., Yang, Z., Bramwell, D., & Sun, X. (2015). Hydrogen-rich saline attenuates ischemia-reperfusion injury in skeletal muscle. *Journal of Surgical Research*, *194*(2), 471–480. https://doi.org/10.1016/j.jss.2014.12.016

Ishibashi, T., Sato, B., Rikitake, M., Seo, T., Kurokawa, R., Hara, Y., Naritomi, Y., Hara, H., & Nagao, T. (2012). Consumption of water containing a high concentration of molecular hydrogen reduces oxidative stress and disease activity in patients with rheumatoid arthritis: An open-label pilot study. *Medical Gas Research*, *2*(1), 27. https://doi.org/10.1186/2045-9912-2-27

Javorac, D., Stajer, V., & Ostojic, S. M. (2020). Case report: Acute hydrotherapy with super-saturated hydrogen-rich water for ankle sprain in a professional athlete. *F1000 Research*, *9*, 245. https://doi.org/10.12688/f1000research.22850.1

Kaminski, T. W., Hertel, J., Amendola, N., Docherty, C. L., Dolan, M. G., Hopkins, J. T., Nussbaum, E., Poppy, W., & Richie, D., & National Athletic Trainers' Association. (2013). National Athletic Trainers' Association position statement: Conservative management and prevention of ankle sprains in athletes. *Journal of Athletic Training*, *48*(4), 528–545.

Kawaguchi, M., Satoh, Y., Otsubo, Y., & Kazama, T. (2014). Molecular hydrogen attenuates neuropathic pain in mice. *PLoS One*, *9*(6), e100352. https://doi.org/10.1371/journal.pone.0100352

Li, H. M., Shen, L., Ge, J. W., & Zhang, R. F. (2018). The transfer of hydrogen from inert gas to therapeutic gas. *Medical Gas research*, *7*(4), 265–272. https://doi.org/10.4103/2045-9912.222451

Ma, H., Chen, H., Dong, A., Wang, Y., Bian, Y., & Xie, K. (2017). Hydrogen-rich saline attenuates hyperalgesia and reduces cytokines in rats with post-herpetic neuralgia via activating autophagy. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi = Chinese Journal of Cellular and Molecular Immunology*, *33*(2), 155–158.

Matsumoto, S., Ueda, T., & Kakizaki, H. (2013). Effect of supplementation with hydrogen-rich water in patients with interstitial cystitis/painful bladder syndrome. *Urology*, *81*(2), 226–230. https://doi.org/10.1016/j.urology.2012.10.026

Mirkin, G., & Hoffman, M. (1978). *The sports medicine book*. Little Brown & Co.

Molecular Hydrogen Institute. *Concentration and solubility of H_2. Half life of H_2 in a solution*. Retrieved Jan 24, 2019, from http://www.molecularhydrogeninstitute.com/concentration-and-solubility-of-h2

Ostojic, S. M. (2015). Molecular hydrogen in sports medicine: New therapeutic perspectives. *International Journal of Sports medicine*, *36*(4), 273–279. https://doi.org/10.1055/s-0034-1395509

Ostojic, S. M. (2016). Should hydrogen therapy be included in a musculoskeletal medicine routine? *F1000Res*, *5*, 2659. https://doi.org/10.12688/f1000research.9758.1

Ostojic, S. M., Vukomanovic, B., Calleja-Gonzalez, J., & Hoffman, J. R. (2014). Effectiveness of oral and topical hydrogen for sports-related soft tissue injuries. *Postgraduate Medicine*, *126*(5), 187–195. https://doi.org/10.3810/pgm.2014.09.2813

Petersen, E. J., Irish, S. M., Lyons, C. L., Miklaski, S. F., Bryan, J. M., Henderson, N. E., & Masullo, L. N. (1999). Reliability of water volumetry and the figure of eight method on subjects with ankle joint swelling. *Journal of Orthopaedic & Sports Physical Therapy*, *29*(10), 609–615. https://doi.org/10.2519/jospt.1999.29.10.609

Qiu, P., Liu, Y., & Zhang, J. (2019). Recent advances in studies of molecular hydrogen against sepsis. *International Journal of Biological Sciences*, *15*(6), 1261–1275. https://doi.org/10.7150/ijbs.30741

Trojian, T. H., & McKeag, D. B. (2006). Single leg balance test to identify risk of ankle sprains. *British Journal of Sports Medicine*, *40*(7), 610–613. https://doi.org/10.1136/bjsm.2005.024356

Van den Bekerom, M. P., Struijs, P. A., Blankevoort, L., Welling, L., Van Dijk, C. N., & Kerkhoffs, G. M. (2012). What is the evidence for rest, ice, compression, and elevation therapy in the treatment of ankle sprains in adults? *Journal of Athletic Training*, *47*(4), 435–443. https://doi.org/10.4085/1062-6050-47.4.14

Watanabe, M., Kamimura, N., Iuchi, K., Nishimaki, K., Yokota, T., Ogawa, R., & Ohta, S. (2017). Protective effect of hydrogen gas inhalation on muscular damage using a mouse hindlimb ischemia-reperfusion injury model. *Plastic and Reconstructive Surgery*, *140*(6), 1195–1206. https://doi.org/10.1097/PRS.0000000000003878

Wells, B., Allen, C., Deyle, G., & Croy, T. (2019). Management of acute grade II lateral ankle sprains with an emphasis on ligament protection: A descriptive case series. *International Journal of Sports Physical Therapy*, *14*(3), 445–458. https://doi.org/10.26603/ijspt20190445

Wolfe, M. W., Uhl, T. L., Mattacola, C. G., & McCluskey, L. C. (2001). Management of ankle sprains. *American Family Physician*, *63*(1), 93–104.

Xu, Z., Zhou, J., Cai, J., Zhu, Z., Sun, X., & Jiang, C. (2012). Anti-inflammation effects of hydrogen saline in LPS activated macrophages and carrageenan induced paw oedema. *Journal of Inflammation*, *9*, 2. https://doi.org/10.1186/1476-9255-9-2

Zhao, M., Gao, W., Zhang, L., Huang, W., Zheng, S., Wang, G., Hong, B. Y., & Tang, B. (2018). Acupressure therapy for acute ankle sprains: A randomized clinical trial. *Pm R*, *10*(1), 36–44. https://doi.org/10.1016/j.pmrj.2017.06.009