Effectiveness of Oral and Topical Hydrogen for Sports-Related Soft Tissue Injuries

DOI: 10.3810/pgm.2014.09.2813

Sergej M. Ostojic, MD, PhD[1,2]
Boris Vukomanovic, MD[1,3]
Julio Calleja-Gonzalez, PhD[1,4]
Jay R. Hoffman, PhD, FACSM, FNSCA[5]

[1]Center for Health, Exercise, and Sport Sciences, Stari DIF, Belgrade, Serbia; [2]Faculty of Sport and Physical Education, University of Novi Sad, Novi Sad, Serbia; [3]Institute of Orthopaedic-Surgical Diseases "Banjica," Belgrade, Serbia; [4]Faculty of Sport Sciences, University of the Basque Country, Vitoria-Gasteiz, Spain; [5]Institute of Exercise Physiology and Wellness, University of Central Florida, Orlando, FL

Abstract

Background: Because hydrogen therapy has been found beneficial for the treatment of inflammation, ischemia-reperfusion injury, and oxidative stress in humans, it seems useful to evaluate the effects of exogenously administered hydrogen as an element in the immediate management of sports-related soft tissue injuries. The main aim of this pilot study was to examine the effects of 2-week administration of hydrogen on the biochemical markers of inflammation and functional recovery in male professional athletes after acute soft tissue injury. **Method:** During the 2013 season (from March to May), 36 professional athletes were recruited as participants and examined by a certified sports medicine specialist in the first 24 hours after an injury was sustained. Subjects were allocated to 3 randomly assigned trials in a single-blind design. Those in the control group received a traditional treatment protocol for soft tissue injury. Subjects in the first experimental group followed the same procedures as the control group but with additional administration throughout the study of oral hydrogen-rich tablets (2 g per day). Subjects in the second experimental group also followed the procedures of the control group, with additional administration throughout the study of both oral hydrogen-rich tablets (2 g per day) and topical hydrogen-rich packs (6 times per day for 20 minutes). Participants were evaluated at the time of the injury report and at 7 and 14 days after baseline testing. **Results:** Oral and topical hydrogen intervention was found to augment plasma viscosity decrease as compared with the control group ($P = 0.04$). Differences were found for range-of-motion recovery between the 3 groups; oral and topical hydrogen intervention resulted in a faster return to normal joint range of motion for both flexion and extension of the injured limb as compared with the control intervention ($P < 0.05$). **Conclusion:** These preliminary results support the hypothesis that the addition of hydrogen to traditional treatment protocols is potentially effective in the treatment of soft tissue injuries in male professional athletes. **Trial identification:** Clinicaltrials.gov number NCT01759498.

Keywords: plasma viscosity; interleukin-6; range of motion; RICE protocol; hydrogen

Introduction

Increased participation in sports during the last 2 decades has been accompanied by an increase in rates of sports injuries among both professional and recreational athletes, with soft tissue injuries (eg, muscle sprain, ligament strain, tendonitis, contusion) accounting for > 75% of all injuries.[1–3] Timely and effective management of sports-related soft tissue injuries is a key factor contributing to a quicker recovery and return to regular training and competition.[4] Soft tissue repair is often facilitated by conservative procedures such as the RICE (rest, ice, compression, and elevation) protocol and topical or oral administration of nonsteroidal anti-inflammatory drugs to relieve pain, swelling, or bruising and to improve functional movement.[5] An added problem

Correspondence: Sergej M. Ostojic, MD, PhD,
Center for Health, Exercise, and Sport Sciences,
Stari DIF,
Deligradska 27,
Belgrade 11000, Serbia.
Tel: (++381)-11-2643-242
Fax: (++381)-11-2643-242
E-mail: sergej.ostojic@chess.edu.rs

with soft tissue injuries is the further cell damage that can be caused by tissue hypoxia and acute reactive oxygen species (ROS) produced at the site of the injury. This subsequent tissue damage is often referred to as the secondary zone of injury, to distinguish it from the initial damage caused by the actual mechanism of injury.[6] As secondary injury following musculoskeletal trauma causes serious damage to soft tissues,[6] an intervention that focuses on the damaging effects of ROS may have several potential advantages over current therapies for achieving prompt recovery. Hydrogen therapy has been found to be beneficial in the treatment of inflammation, ischemia-reperfusion injury, and oxidative stress in humans,[7] and it therefore seems useful to evaluate the effects of hydrogen as an element in the management of acute sports-related soft tissue injuries.

Hydrogen is known to act as a potent antioxidant that rapidly diffuses to subcellular compartments and directly eliminates hydroxyl radical, a highly cytotoxic species produced in inflamed tissues.[8] Other biochemical effects of hydrogen therapy may also be relevant (eg, hydrogen functioning as a gaseous signaling molecule).[9] The prominent effects of hydrogen in preventive and therapeutic applications have previously been observed in cases of cerebral infarction,[10] chronic inflammation in patients with hemodialysis,[11] inflammatory myopathies,[12] metabolic syndrome,[13] diabetes mellitus,[14] Parkinson's disease,[15] and rheumatoid arthritis.[16] It seems likely that hydrogen treatment might effectively protect cells, tissues, and organs against oxidative injury and help them to recover from dysfunction. In particular, hydrogen delivery to cardiomyocytes has efficiently ameliorated secondary injury of muscle cells due to ischemia and reperfusion,[17] suggesting a possible therapeutic application for hydrogen in common soft tissue injuries. However, no study has so far validated this therapeutic potential for the treatment of soft tissue injuries in the field of sports medicine. The main aim of this preliminary study was to examine the effects of 2-week oral and/or topical administration of hydrogen on inflammation, recovery, functional ability, and pain intensity in competitive male athletes after acute soft tissue injury. We hypothesized that the addition of hydrogen to traditional treatment protocol would enhance recovery and reduce inflammation in male athletes following a sports-related soft tissue injury.

Method

Study Population

Athletes were eligible to participate in the study if they had a recent history of acute soft tissue sports injury and clinical findings consistent with trauma. Acute soft tissue sports injury was defined as a direct or indirect trauma incurred during any sports-related activity that caused absence from training or competition. During the 2013 season (from March to May), 36 professional athletes were recruited and examined by a certified sports medicine specialist in the outpatient clinics of the Center for Health, Exercise, and Sport Sciences (Belgrade, Serbia) within 24 hours of sustaining an injury. Characteristics of the participants are presented in Table 1.

Based on amount of pain, weakness, and loss of motion, clinical findings were categorized as follows: grade I, mild, with some swelling and pain on stretch, but function and strength are mostly unaffected; grade II, moderate, with pain and swelling at the site, and some loss of function and strength; and grade III, severe, with considerable loss of function and strength, and with injuries that often need surgical repair.[18] Patients who were not ambulatory or who had clinical findings classified as more significant than grade II were excluded from the study. For diagnostic consistency of inclusion criteria for soft tissue injury and the grading of clinical findings, the same observer evaluated all study participants; all participants provided informed consent and volunteered to participate in the study. The protocol was approved by the local institutional review board in accordance with the Declaration of Helsinki. At the first assessment session, participants were fully informed, verbally and in writing, about the nature and demands of the study as well as the known health risks. They completed a health history questionnaire and were informed that they could withdraw from the study at any time, even after giving their written consent. All subjects were in good health (eg, no evidence of diabetes, heart disease, or cancer), were nonsmokers, participated in regular training (average of 12 hours per week) for the past 5+ years, and were not currently taking a drug or dietary supplement that contained hydrogen (or any similar preparation).

Table 1. Characteristics of the Study Population (n = 36)

Age (mean ± SD)	23.1 ± 2.3 years
Professional experience (mean ± SD)	5.2 ± 1.1 years
Sport played	Soccer (n = 17)
	Basketball (n = 10)
	Track and field (n = 4)
	Other (n = 5)
Type of injury	Ligament sprain (n = 21)
	Muscle strain (n = 8)
	Contusion (n = 6)
	Other (n = 3)
Location of injury	Lower limb (n = 19)
	Upper limb (n = 10)
	Other (n = 7)

Experimental Procedures

This is an early evaluation study of hydrogen effectiveness for sports injuries, with follow-up at 2 weeks. In a single-blind design, participants were randomly assigned to 1 of 3 trials using a computer-generated list. During the 2-week study period, subjects in the control group received a traditional treatment protocol for soft tissue injuries that included the RICE protocol (rest, ice packs for 20 minutes every 2 hours, compression with elastic bandage, and elevation at all possible times of the injured area above the level of the heart) during the first 48 hours, and a subacute protocol thereafter (passive stretching 3 times per day for 90 seconds, 3 sets of isometric strength exercises with 15 repetitions, and 30 minutes of pain-free weight-bearing exercises).

Subjects in the first experimental group (HYD1) followed the procedures for the control group, with the additional administration throughout the study of oral hydrogen-rich tablets (2 g per day). Subjects in the second experimental group (HYD2) also followed the control group procedures, with the additional administration throughout the study of both oral hydrogen-rich tablets (2 g per day) and topical hydrogen-rich packs (6 times per day for 20 minutes). The oral hydrogen treatment formulation was provided in tablet form by SevenPoint2 (7.2 Recovery with HydroFX, Newport Beach, CA), and participants were instructed to take 4 tablets 3 times a day, before main meals. The topical hydrogen treatment formulation was provided by NORP Inc. (San Diego, CA), and participants were instructed to administer the hydrogen pack directly to the skin above the site of the injury, using elastic wrap to secure the pack. During the administration period, all subjects refrained from training. No other interventions were performed.

Participant Evaluation

Participants were evaluated at the beginning of the study (at the time of injury report) and at 7 and 14 days after the report of injury. For baseline testing prior to administration, fasting blood was collected from a radial vein into a gel Vacutainer for biochemical measures; serum C-reactive protein (CRP) and serum interleukin-6 (IL-6) were determined using a highly sensitive enzyme-linked immunosorbent assay (ELISA) procedure (eBioscience, San Diego, CA); and plasma viscosity at 25°C was measured using a capillary viscometer (Coulter Viscometer II, Electronics Ltd., Luton, UK). Pain intensity was assessed using a visual analogue scale of 1 to 15.[19] Participants completed 2 visual analogue assessments at each visit, 1 representing pain intensity while at rest, and the other representing pain while walking. Passive joint flexibility of the injured limb in the sagittal plane was measured using a modified goniometer with spirit level (Creative Health Inc., Plymouth, CA), recording deficits of flexion and extension. The degree of limb swelling at the site of injury was measured with anthropometric tape (Creative Health Inc.) and compared with the uninjured limb. To assess potential side effects of the treatment regimen, all subjects were instructed to report any adverse effects of administration (eg, skin irritation, rush) during each visit to the medical center.

Statistical Analyses

The primary efficacy outcome was change in serum CRP level at 2 weeks after administration (effect size of 1.0) in the HYD1 group as compared with the control group. Allowing for $>$ 80% power, it was estimated that 10 participants per group would be required in the final analyses; this was adjusted to 12 subjects per group to accommodate a predicted 20% dropout rate. All results were expressed as mean ± standard deviation. For group comparison at a series of time points during intervention, the area under the curve (AUC) was first identified and calculated for all dependent variables for each subject.[20] The Shapiro-Wilk test was then applied to summary measures (mean AUC) for each group to assess normality of distribution, and Bartlett's test was used to assess homogeneity of variances. Where homogeneous variances were verified for normally distributed data, summary measures were compared by analysis of variance (ANOVA). In the event of a significant F ratio (the ratio of the variance between groups to the variance within groups), post-hoc Tukey honest significant difference tests were employed to identify differences between individual sample pairs. Where nonhomogeneous variances were identified, mean AUCs were compared using the 3 independent samples Kruskal-Wallis test, and the Games-Howell post-hoc test was used to identify significant differences between any 2 groups. Effect size (Cohen's d) was calculated for all variables; a Cohen's $d > 0.5$ and ≤ 0.8 is considered moderately strong, and a value > 0.8 is considered strong. For all statistical tests, a criterion alpha level of $P \leq 0.05$ was used to determine statistical significance. All statistical analyses were performed using SPSS (Version 21, SPSS Inc., Chicago, IL).

Results

A total of 36 participants completed the study, with no participants lost on follow-up. Most participants received all interventions regularly, but a few omitted some quantity of tablets and/or packs. Total compliance with the hydrogen regimen

was 83% for the HYD1 group and 75% for the HYD2 group. Two participants from the HYD1 group reported mild diarrhea during the first 2 days of the intervention. No additional side effects were reported, and no serious adverse events occurred during the study.

Changes in plasma inflammatory markers during the study are presented in Table 2. The HYD2 intervention was found to augment plasma viscosity decrease as compared with the control group ($P = 0.04$), whereas the magnitude of alteration for other markers of inflammation (CRP, IL-6) did not differ significantly between the control group and hydrogen regimens ($P > 0.05$). However, Table 3 shows that small-to-medium effect sizes were found for plasma viscosity and IL-6 for both hydrogen protocols ($d > 0.35$).

In all 3 groups, significant decreases in pain scores, at rest and while walking, were observed after the first and second week, respectively (Figure 1). No differences were found for pain score changes between the groups ($P > 0.05$). However, for pain scores at walking, a moderate-to-large effect size was found for the HYD2 intervention as compared with the control group ($d = 0.74$). Injured limb swelling decreased throughout the study (Figure 2), but no differences were found between groups for degree of swelling reduction ($P > 0.05$). Finally, differences were found between the 3 groups for range of motion (ROM) recovery (Figure 3); as compared with the control intervention, the HYD2 intervention resulted in faster return to normal joint ROM for both flexion and extension of the injured limb ($P < 0.05$).

Discussion

In this preliminary study, we had the unenviable task of trying to improve upon an already very effective traditional treatment for mild-to-moderate soft tissue sports injuries with the addition of hydrogen. We have shown that 2-week oral and topical hydrogen intervention augments the plasma viscosity decrease and enhances recovery of joint flexibility in male athletes following a sports-related soft tissue injury, as compared with the control intervention. Hydrogen administration (in either tablet or topical form) did not result in a statistically significant difference in plasma CRP, IL-6, pain scores, or limb swelling as compared with control. The primary findings here provide evidence that oral and topical hydrogen may be effective as an adjunct agent in traditional conservative treatment of soft tissue injuries.

The medical application of hydrogen in humans was first reported nearly 40 years ago,[21] and has subsequently been evaluated in a number of experimental and clinical contexts. Although research on the health benefits of hydrogen remains limited, with scant data on long-term effects, hydrogen has been identified as beneficial in the prevention and treatment of a wide range of diseases.[10–16] The therapeutic effects of hydrogen have been attributed to 4 major molecular mechanisms: specific scavenging activities of hydroxyl radical and of peroxynitrite, alterations of gene expression, and signal-modulating activities.[7] Because hydrogen is known to scavenge toxic ROS and to induce a number of antioxidant proteins during inflammation,[22] its use is likely to have a significant impact, especially

Table 2. Changes in Plasma Inflammatory Markers During the Study[a]

	Baseline	Week 1	Week 2	AUC	*P* value	Post-hoc differences[b]
C-reactive protein (mg/L)						
CON	60.2 ± 38.3	34.3 ± 21.3	18.7 ± 10.4	73.7 ± 44.4	0.97[c]	–
HYD1	75.0 ± 71.1	47.5 ± 44.5	29.4 ± 29.8	99.7 ± 93.4		
HYD2	62.6 ± 36.0	37.0 ± 24.3	21.9 ± 12.1	79.2 ± 47.1		
Interleukin-6 (pg/mL)						
CON	92.5 ± 24.3	72.3 ± 10.5	68.6 ± 6.9	152.9 ± 24.4	0.45[c]	–
HYD1	105.7 ± 35.7	77.2 ± 11.5	68.1 ± 8.6	164.1 ± 31.2		
HYD2	101.0 ± 22.8	74.1 ± 12.5	67.3 ± 7.7	158.3 ± 25.4		
Viscosity (mPa·s)						
CON	1.45 ± 0.12	1.34 ± 0.10	1.26 ± 0.10	2.70 ± 0.20	0.04[d]	[e]
HYD1	1.42 ± 0.15	1.26 ± 0.08	1.19 ± 0.07	2.57 ± 0.17		
HYD2	1.39 ± 0.14	1.25 ± 0.07	1.16 ± 0.06	2.52 ± 0.15		

[a]Values are mean ± SD (n = 36). AUC is defined as the area under the plot of serum concentration of selected outcome (not logarithm of the concentration) against time after intervention administration.
[b]Significant difference at $P < 0.05$.
[c]*P* value from independent samples Kruskal-Wallis test.
[d]*P* value from 3-sample unpaired ANOVA test.
[e]CON vs HYD2.
Abbreviations: AUC, area under the curve; CON, control group; HYD1, group supplemented with oral hydrogen; HYD2, group supplemented with oral hydrogen and topical hydrogen packs.

Table 3. Effect Size Between Groups for Mean Gain Scores During the Study[a]

	HYD1 vs CON	HYD2 vs CON	HYD1 vs HYD2
C-reactive protein	0.03	0.01	0.03
Interleukin-6	0.38	0.41	0.12
Viscosity	0.32	0.44	0.08
Pain at rest	0.20	0.09	0.29
Pain at walking	0.35	0.74	0.46
Degree of swelling	0.18	0.05	0.03
ROM deficit in flexion	0.09	0.08	0.05
ROM deficit in extension	0.27	0.14	0.31

[a]Effect sizes are indicated as small (d = 0.20–0.49), medium (d = 0.50–0.79), and large (d ≥ 0.80).

Abbreviations: CON, control group; HYD1, group supplemented with oral hydrogen; HYD2, group supplemented with oral hydrogen and topical hydrogen packs; ROM, range of motion.

on oxidative stress-mediated disorders and inflammatory diseases in humans.

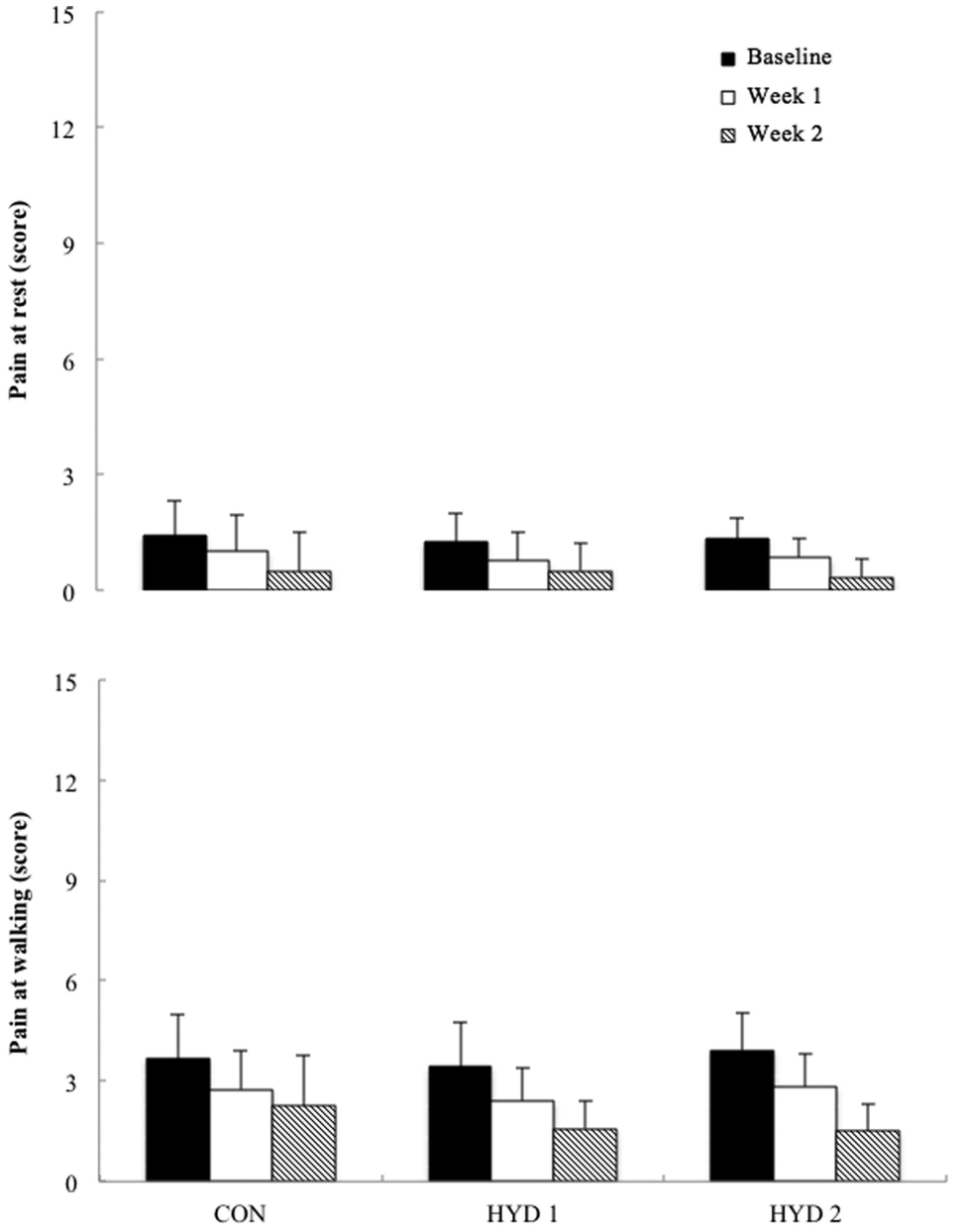
As has previously been reported, oxidative stress is a significant factor in cell damage arising from sports-related soft tissue injuries.[23] The acute response to trauma includes a drastic activation of immunocompetent cells and factors, interstitial edema, and reduction of the microvascular blood supply, when highly toxic ROS are released during the peroxidation of membrane lipids.[24] This results in cell destruction and subsequent pain, swelling, bruising, and loss of function.[25] The standard medical treatment involves the RICE protocol, which reduces associated swelling and pain, but rapid elimination of ROS and inflammation markers in athletes suffering soft tissue injuries may be beneficial for enhanced recovery in terms of clinical markers and functional abilities. Our results suggest that the addition of hydrogen to traditional soft tissue injury treatment positively affects selected clinical and biochemical indicators of postinjury recovery such as plasma viscosity and flexibility of the injured area.

Several inflammatory markers (eg, CRP, erythrocyte sedimentation rate, fibrinogen, ferritin, IL-6, and plasma viscosity) are monitored in musculoskeletal medicine after the injury and inflammation.[26] These biomarkers are elevated immediately after a soft tissue injury, with levels correlating to the clinical stages of the condition.[27] Evaluation of the time course of biomarkers after injury is relevant for monitoring management and recovery.[26] In the present study, a decrease in selected blood inflammatory markers was noted throughout, for all experimental protocols, indicating reduced inflammation during recovery. However, significant differences were found between groups for changes in plasma viscosity; athletes who supplemented with both topical and oral hydrogen experienced a much faster decline in plasma viscosity relative to the control group. Because plasma viscosity sensitivity and specificity are better than those of erythrocyte sedimentation rate or CRP in inflammation,[28] we can assume that the hydrogen treatment may have positively affected the inflammation process in injured athletes. Although oxidative stress is involved in the development of postinjury inflammation, the antioxidant effect of hydrogen may not be the only driving factor causing positive anti-inflammatory effects of administration; the possible impact of hydrogen on downregulation of proinflammatory citokines[12] after musculoskeletal injury requires further investigation.

Although most sports-related soft tissue injuries recover rapidly, different therapy protocols are implemented to accelerate the process of return to sport after injury.[29] With restoration of function of the injured limb as a main goal of injury treatment, aggressive acute and subacute treatment protocols during healing will facilitate recovery in athletes.[25] Traditional medical treatment of soft tissue injury is designed to decrease swelling and pain, and to regain the mobility of the injured limb. The present study demonstrates similar positive dynamics of recovery for limb swelling and pain among groups, both at rest and while walking. It seems that the addition of hydrogen to the traditional treatment protocol did not affect pain reduction or edema during recovery when compared with traditional treatment only. However, comparison of effect size for pain while walking revealed a moderate-to-large effect of treatment between the control group and the group supplemented with both oral and topical hydrogen (d = 0.74). Although the *P* value was insignificant, it seems that the hydrogen group clinically outperformed the control group in respect to pain control. This is meaningful in a clinical context, suggesting that adding hydrogen to traditional treatment methods may be more effective in reducing pain during recovery.

Interestingly, subjects supplemented with hydrogen showed statistically significant improvement in range of motion of the injured limb during recovery. Although some improvements were seen in both flexion and extension of injured limb after hydrogen administration, the effects were no more than small to moderate, indicating modest clinical relevance for health care providers. However, it seems that the use of a control group consisting of an active treatment (ie, RICE protocol) along with a limited number of participants recruited probably made it harder to show the beneficial effects of intervention. We attempted to improve on this active and effective treatment for soft tissue injuries with the addition of hydrogen, yet the use of a true control

Figure 1. Pain at rest and while walking during the study.



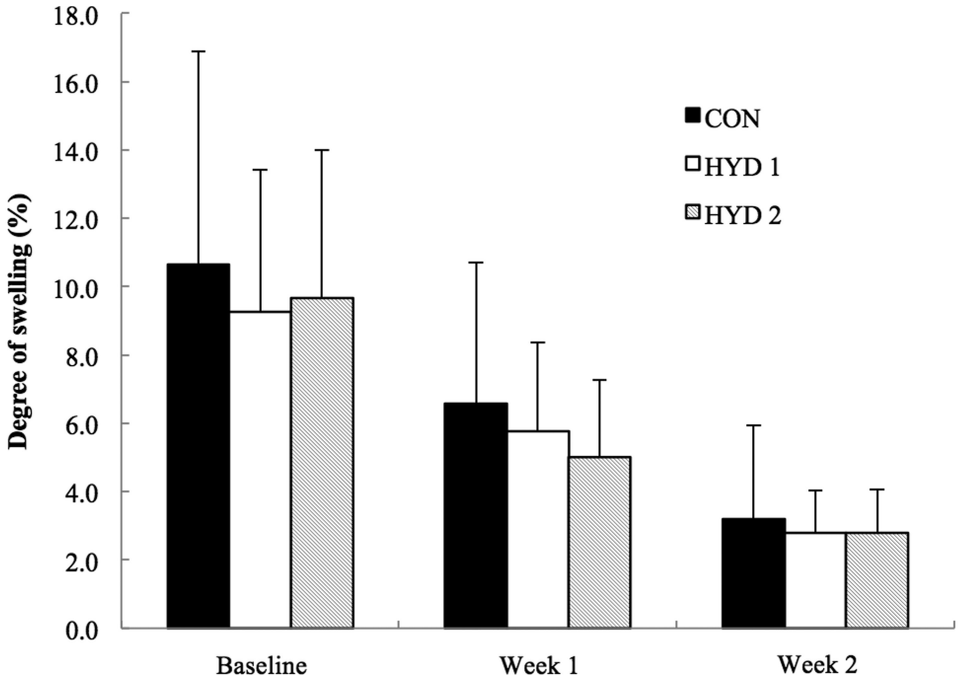
Abbreviations: CON, control group; HYD1, group supplemented with oral hydrogen; HYD2, group supplemented with oral hydrogen and administered with topical hydrogen-rich packs.

group without any treatment probably would augment the favorable effects of hydrogen, both statistical and clinical, which requires further research.

Previous studies found no serious adverse events of oral hydrogen administration, which is reported to be safe and easily applicable to humans.[10–16,30] This aligns with the results of the present study, in which there were no reports of severe side effects that might have limited participation, although 2 participants from the HYD1 group reported early diarrhea that was resolved after a few days of treatment. Although the diarrhea was reported to be mild in intensity, the abdominal side effects of hydrogen need further investigation.

Based on the data presented here, there is insufficient evidence to conclude that administration of hydrogen is a safe therapeutic strategy for soft tissue injuries. Furthermore, neither the long-term safety of hydrogen use in humans nor the pharmacokinetics of oral or topical hydrogen administration have as yet been studied in depth. It follows that phase II clinical studies are warranted on hydrogen biotransformation and removal, along with postmarketing

Figure 2. Degree of swelling during the study.



surveillance trials to determine its distribution, metabolism, and excretion, and evaluation of rare or long-term adverse effects of hydrogen over a much larger patient population and longer time period than was possible during the pilot trials. In the present study, poor compliance within treatment may be accounted for by participants' perceptions of the treatment as short-term, and by their improved sense of well-being before the end of the study, which would accord with earlier findings of low adherence.[31] However, these compliance issues need more clarification before any final conclusion can be reached on hydrogen efficacy and safety in sports medicine.

Despite the evidence presented here that hydrogen administration positively affects recovery from soft tissue injury in male professional athletes, the present study has several limitations. First, this early evaluation was conducted with single-blinded design, no placebo control, and exclusion of subjects with severe musculoskeletal injuries. Future studies should evaluate the efficacy of molecular hydrogen in sports medicine using double-blind, randomized trials with a placebo-controlled approach on large patient groups that include both moderate and severe sports-related injuries. Second, other possible confounding factors were not considered that might be responsible for variations in recovery outcomes between groups, such as the site, mechanism, and type of injury, the age and professional experience of participants, and previous history of injuries. Third, the small size of the experimental groups (n = 12) could be considered a limiting factor, not least because compliance with the protocol was not perfect, and as a consequence, observed differences between the groups on several outcomes (eg, pain at rest and while walking), although of small to moderate magnitude, did not reach statistically significant levels. The follow-up period of 2 weeks is also too short, and future studies should use long-term follow-up trials to evaluate the effectiveness and safety of molecular hydrogen administration for widespread clinical use.

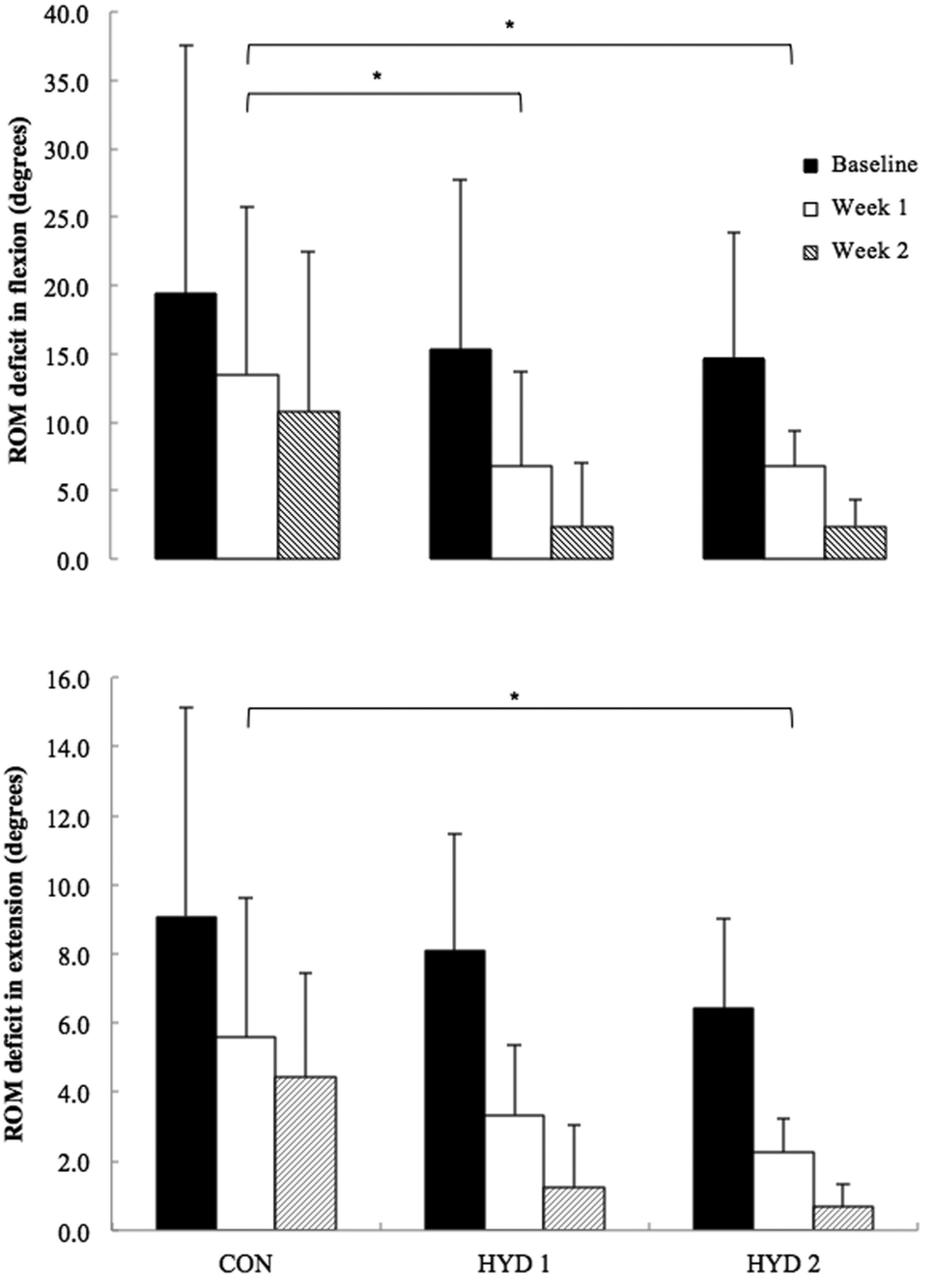
This study assessed only a few important biochemical components of soft tissue injury recovery, neglecting further parameters that might be directly or indirectly connected to hydrogen intake, such as creatine kinase, endothelial leukocyte adherence, and mean protein content. Because hydrogen affects derivatives of reactive oxidative metabolites, biological antioxidant power, and superoxide dismutase in healthy subjects,[32] it would be interesting for future studies to assess a range of antioxidant parameters during hydrogen administration in athletes who have suffered a soft tissue injury. Additional clinical outcome measures, such as being able to return to sports or to regain muscular strength, and the time it takes to do so, should also be explored in future research.

Conclusion

As an additional agent to supplement traditional conservative treatment of acute sports-related soft tissue injuries, 2-week

© Postgraduate Medicine, Volume 126, Issue 5, September 2014, ISSN – 0032-5481, e-ISSN – 1941-9260

Figure 3. ROM deficit in flexion and extension during the study.



*Significant difference between groups at $P < 0.05$.
Abbreviation: ROM, range-of-motion.

administration of hydrogen improved the level of plasma viscosity and boosted ROM recovery of the injured limb. The use of oral and topical hydrogen potentially represents a novel therapeutic strategy for the treatment of the soft tissue injury in male professional athletes. However, larger, long-term studies of the safety of hydrogen administration will be needed before any conclusion can be reached concerning the use of hydrogen as a safe therapeutic agent in a clinical environment.

Acknowledgments

This study was supported by the Serbian Ministry of Science (grant number 175037; principal investigator: Aleksandar Nedeljkovic, PhD; co-principal investigator:

Sergej M. Ostojic, MD, PhD), and grant no. 012–12C from the SevenPoint2 (Newport Beach, CA).

Conflict of Interest Statement

Sergej M. Ostojic, MD, PhD, Boris Vukomanovic, MD, Julio Calleja-Gonzalez, PhD, and Jay R. Hoffman, PhD, FACSM, FNSCA, have no conflicts of interest to declare.

References

1. Biundo JJ Jr, Mipro RC Jr, Fahey P. Sports-related and other soft-tissue injuries, tendinitis, bursitis, and occupation-related syndromes. *Curr Opin Rheumatol.* 1997;9(2):151–154.
2. Baarveld F, Visser CA, Kollen BJ, Backx FJ. Sports-related injuries in primary health care. *Fam Pract.* 2011;28(1):29–33.
3. Giannotti M, Al-Sahab B, McFaull S, Tamim H. Epidemiology of acute soccer injuries in Canadian children and youth. *Pediatr Emerg Care.* 2011;27(2):81–85.
4. Chen MR, Dragoo JL. The effect of nonsteroidal anti-inflammatory drugs on tissue healing. *Knee Surg Sports Traumatol Arthrosc.* 2013;21(3):540–549.
5. Leversedge FJ, Srinivasan RC. Management of soft-tissue injuries in distal radius fractures. *Hand Clin.* 2012;28(2):225–233.
6. Merrick MA. Secondary injury after musculoskeletal trauma: a review and update. *J Athl Train.* 2002;37(2):209–217.
7. Ohno K, Ito M, Ichihara M, Ito M. Molecular hydrogen as an emerging therapeutic medical gas for neurodegenerative and other diseases. *Oxid Med Cell Longev.* 2012;2012:353152.
8. Zhang JY, Liu C, Zhou L, et al. A review of hydrogen as a new medical therapy. *Hepatogastroenterology.* 2012;59(116):1026–1032.
9. Zheng XF, Sun XJ, Xia ZF. Hydrogen resuscitation, a new cytoprotective approach. *Clin Exp Pharmacol Physiol.* 2011;38(3):155–163.
10. Ono H, Nishijima Y, Adachi N, et al. Improved brain MRI indices in the acute brain stem infarct sites treated with hydroxyl radical scavengers, Edaravone and hydrogen, as compared to Edaravone alone. A non-controlled study. *Med Gas Res* 2011;1(1):12.
11. Nakayama M, Nakano H, Hamada H, Itami N, Nakazawa R, Ito S. A novel bioactive haemodialysis system using dissolved dihydrogen (H2) produced by water electrolysis: a clinical trial. *Nephrol Dial Transplant.* 2010;25(9):3026–3033.
12. Ito M, Ibi T, Sahashi K, Ichihara M, Ito M, Ohno K. Open-label trial and randomized, double-blind, placebo-controlled, crossover trial of hydrogen-enriched water for mitochondrial and inflammatory myopathies. *Med Gas Res.* 2011;1(1):24.
13. Nakao A, Toyoda Y, Sharma P, Evans M, Guthrie N. Effectiveness of hydrogen rich water on antioxidant status of subjects with potential metabolic syndrome—an open label pilot study. *J Clin Biochem Nutr.* 2010;46(2):140–149.
14. Kajiyama S, Hasegawa G, Asano M, et al. Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. *Nutr Res.* 2008;28(3):137–143.
15. Yoritaka A, Takanashi M, Hirayama M, Nakahara T, Ohta S, Hattori N. Pilot study of H_2 therapy in Parkinson's disease: a randomized double-blind placebo-controlled trial. *Mov Disord.* 2013;28(6):836–839.
16. Ishibashi T, Sato B, Rikitake M, et al. 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. *Med Gas Res.* 2012;2(1):27.
17. Noda K, Shigemura N, Tanaka Y, et al. A novel method of preserving cardiac grafts using a hydrogen-rich water bath. *J Heart Lung Transplant.* 2013;32(2):241–250.
18. Järvinen TA, Järvinen TL, Kääriäinen M, et al. Muscle injuries: optimising recovery. *Best Pract Res Clin Rheumatol.* 2007;21(2):317–331.
19. Flaherty SA. Pain measurement tools for clinical practice and research. *AANA J.* 1996;64(2):133–140.
20. Jaki T, Wolfsegger MJ, Ploner M. Confidence intervals for ratios of AUCs in the case of serial sampling: a comparison of seven methods. *Pharm Stat*. 2009;8(1):12–24.
21. Dole, M, Wilson FR, Fife WP. Hyperbaric hydrogen therapy: a possible treatment for cancer. *Science.* 1975;190(4210):152–154.
22. Hong Y, Chen S, Zhang JM. Hydrogen as a selective antioxidant: a review of clinical and experimental studies. *J Int Med Res*. 2010;38(6):1893–1903.
23. McGinley C, Shafat A, Donnelly AE. Does antioxidant vitamin supplementation protect against muscle damage? *Sports Med*. 2009;39(12):1011–1032.
24. van der Vusse GJ, van Bilsen M, Reneman RS. Ischemia and reperfusion induced alterations in membrane phospholipids: an overview. *Ann N Y Acad Sci.* 1994;723:1–14.
25. Kannus P, Parkkari J, Järvinen TL, Järvinen TA, Järvinen M. Basic science and clinical studies coincide: active treatment approach is needed after a sports injury. *Scand J Med Sci Sports*. 2003;13(3):150–154.
26. Kumbhare D, Parkinson W, Dunlop RB, Adili A. Biochemical measurement of injury and inflammation in musculoskeletal surgeries. In: Al-Aubaidi Z, ed. *Orthopedic Surgery*. Split, Croatia: InTech; 2012:165–182.
27. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med.* 1999;340(6):448–454.
28. Watson J, Round A, Hamilton W. Raised inflammatory markers. *BMJ.* 2012;344:e454.
29. Nepple JJ, Matava MJ. Soft tissue injections in the athlete. *Sports Health.* 2009;1(5):396–404.
30. Ostojic SM. Serum alkalinization and hydrogen-rich water in healthy men. *Mayo Clin Proc.* 2012;87(5):501–502.
31. Edwards V, Wilson AA, Harwood HF, et al. A multicentre comparison of piroxicam and indomethacin in acute soft tissue sports injuries. *J Int Med Res.* 1984;12(1):46–50.
32. Aoki K, Nakao A, Adachi T, Matsui Y, Miyakawa S. Pilot study: effects of drinking hydrogen-rich water on muscle fatigue caused by acute exercise in elite athletes. *Med Gas Res.* 2012;2(1):12.