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Surface-Dependent Adaptations in Cardiac Function, Aerobic Power, MicroRNA-1, and Hand-2 Expression following Tabata Training in Elite Athletes

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A B S T R A C T

Objective: Tabata training has the potential to induce favorable adaptations in elite athletes. However, the underlying mechanisms remain incompletely understood. Furthermore, varying the training surface can contribute to improvements in athletes' fitness by introducing diversity into the training program. This study compared the effects of Tabata training conducted on an indoor hardcourt vs. sand on maximum oxygen consumption (VO_{2max}), left ventricular morphology, and the expression of microRNA-1 (miR-1) and Hand-2 in elite beach soccer players.

Methods: Sixteen players were randomly assigned to either a soft (SS) or hard (HS) surface group. The training variables were similar between the groups and only the type of training surface was different. The protocol (4-min sets of 20 s of exercise, 10 s of rest) was performed three sessions/week for six weeks. Shuttle-run test and two-dimensional echocardiography were performed 48 h and 72 h, respectively, before and after the intervention. Additionally, venous blood samples were collected before and 6 h after the first and last session.

Findings: Repeated measures ANOVA revealed significant time × group interactions for increases in stroke volume (SV; P= 0.039), left ventricular end-diastolic volume ($p = 0.013$) and dimension ($p = 0.029$), ejection fraction ($p = 0.017$), and reductions in relative posterior wall thickness ($p = 0.036$). The SS group exhibited greater post-training increases in miR-1 and Hand-2 protein ($p < 0.0001$). Moreover, both groups experienced similar increases in VO_{2max} values ($p < 0.0001$).

Conclusion: Tabata training on sand can produce greater improvements in SV values compared to indoor hardcourt, despite similar improvements in relative VO_{2max}. These cardiac improvements may partly relate to enhanced venous return, and the molecular findings suggest a potential contribution of miR-1 upregulation, whereas the role of Hand-2 remains less clear due to limitations in serum-based assessment.

Keywords: *sand, hard surface, high-intensity interval training, echocardiography, VO_{2max}*

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1. Introduction

Tabata training, a form of high-intensity intermittent training (HIIT), involves four minutes of continuous 20-second bouts of exercise at an intensity equivalent to 170% of maximum oxygen uptake (VO_{2max}), followed by 10 s of rest. It has been reported that Tabata training produced similar improvements in aerobic fitness among young men compared to training at 70% VO_{2max}, while also resulting in greater increases in their anaerobic capacity by 28% (1).

Research has demonstrated that the incorporation of HIIT during the preparatory phase of team sport athletes, such as soccer players, can lead to further enhancements in certain physical fitness parameters (2). Furthermore, HIIT has been shown to produce similar improvements in the endurance capacity of these athletes when compared to traditional soccer training methods (3). Beach soccer is a team sport that has received relatively limited attention from researchers, and there is a paucity of information regarding the physiological aspects of this discipline. Studies have revealed that players spend approximately 60% of the match duration working at more than 90% of their maximum heart rate (HR_{max}). Consequently, beach soccer can be categorized as a high-intensity interval sport with substantial energy demands, wherein the anaerobic system plays a crucial role in dealing these energy requirements (4).

Among the physiological adaptations attributed to HIIT protocols is the enhancement of heart structure and functions, as well as aerobic power (5). The potential of HIIT to augment VO_{2max} in soccer players has been documented (6-8), suggesting that part of this improvement in aerobic power may be attributed to positive cardiac adaptations. This training modality has been shown to elicit comparable, and in some instances, superior cardiac adaptations among both endurance athletes (9) and healthy untrained individuals (10) when compared with moderate-intensity endurance training. However, the cellular mechanisms underpinning these adaptations remain insufficiently understood.

In recent years, research on microRNAs (miRs) has expanded, aiming to elucidate these mechanisms. miRs are small RNA molecules, comprising a sequence of 18 to 25 nucleotides, whose expression level alterations may be associated with the pathogenesis of numerous problems, including cardiovascular diseases (11). In this context, miR-1 has been identified as a pivotal regulator of myocardial hypertrophy (12, 13). For example, Fathi et al. (2020) demonstrated that 14 weeks of endurance training at an intensity of 75% of VO_{2max} resulted in improved cardiac structure and functions in Wistar rats, concomitantly with a significant increase in miR-1 expression (12). Conversely, Care et al. (2007) concluded that myocardial hypertrophy induced by HIIT (eight weeks of running at 85-90% VO_{2max}) was associated with a reduction in miR-1 expression (13). Despite these divergent findings, circulating miR-1 levels in humans show a significant positive correlation with aerobic capacity (14), and Denham et al. (2016) proposed that miR-1 may serve as an independent predictor of VO_{2max} (15).

On the other hand, miR-1 may augment the expression of several target proteins, one of which is heart and neural crest derivative expressed 2 (Hand-2). This protein plays a role in cardiac hypertrophy and is expressed in the heart and nervous tissues (16). The Hand-2 protein plays a pivotal role in the formation of the ventricular wall, heart chambers, and aortic arch, and its suppression leads to defects in the development of ventricular myocytes (17). Maintaining accurate levels of Hand-2 is essential for the proliferation and normal growth of cardiac myocytes, and miR-1 plays a crucial role in the growth and development of ventricular myocytes by regulating the levels of this protein (18). The role of miR-1 in controlling the balance between differentiation and proliferation of cardiac myocyte progenitors through Hand-2 has been suggested (19). Therefore, the expression of the Hand-2 gene, as a target protein of miR-1, appears to play a key role in the process of myocardial hypertrophy. In this regard, it has been observed that long-term endurance training can induce physiological hypertrophy of the myocardium in healthy male rats through a significant increase in the expression of miR-1 and Hand-2 (12). Moreover, evidence suggests that a period of HIIT exercises can increase the weight of the heart and left ventricle (LV) of healthy rats compared to the control group through the increase of Hand-2 (20).

Regarding athletic adaptations, training surface is an additional factor that may influence the quality of the preparation phase (21-24). Exercise on sand requires greater mechanical work and higher energy expenditure due to its instability (25). Studies suggest that energy expenditure during comparable activity can be 1.3 to 2.7 times higher on sand than on hard surfaces (24-26). This increased demand may lead to greater motor unit recruitment and higher muscle activation (22, 25, 27). Given that training variation is essential to prevent monotony and overtraining, sand and hard-surface training can serve as complementary modalities that provide distinct mechanical and physiological stimuli (28). However, no study has directly compared the effects of training on sand versus hard surfaces in beach soccer

players. Therefore, the aim of the present study was to compare the effects of Tabata training performed on sand versus a hard surface on aerobic power, left ventricular structure and function, and serum levels of miR-1 and Hand-2 in elite beach soccer players.

2. Methods

2.1 Study design and participants

This quasi-experimental study employed a pretest-posttest design. Sixteen elite beach soccer players with at least four years of experience in the youth and adult leagues participated in this research (Table 1). All players were in the off-season phase and regularly participated in 2-3 training sessions per week, including specific training with the ball and resistance training. The main inclusion criteria for participation were the absence of any injury or disease affecting performance, and a negative COVID-19 test result before attending the training protocol. All players received a clear explanation of the study purpose and procedures and provided written informed consent.

Table 1. Descriptive characteristics of the players (n = 16).

Variables	Sand (n = 8)	Hard (n = 8)	All
Age (years)	23.5 ± 2.7	22.9 ± 2.5	23.2 ± 2.5
Height (cm)	176 ± 4	179 ± 7.8	177.5 ± 6.1
Body mass (kg)	75.2 ± 8.5	76.7 ± 4.4	75.9 ± 6.5
Body mass index (kg/m^2)	24.2 ± 1.7	24.1 ± 1	24.1 ± 1.4

2.2 Research procedure

The players were randomly assigned to either the soft surface (SS, n = 8) or hard surface (HS, n = 8) group. All training variables were similar between the groups, with the exception that the SS group performed the training protocol on beach sand, while the HS group completed the protocol on a standard indoor hardcourt. All players were instructed to maintain their usual dietary habits throughout the training period. Additionally, the breakfast and lunch provided on the testing days were standardized between the groups. Breakfast consisted of two medium slices of bread, about three teaspoons of white cheese, two walnuts, two teaspoons of honey, and two boiled eggs. Lunch included one medium roast chicken breast and 12 spoons of cooked rice. Pre- and post-test assessments were performed at the same time of day to minimize circadian rhythm effects.

2.3 Training protocol

All training sessions were conducted in the morning. The protocol consisted of three sessions per week for six weeks, incorporating selected exercises based on the method proposed by Tabata et al. (1996) (1). Each training set was performed for 4 min, with a work-to-rest ratio of 2:1 (20 s of exercise followed by 10 s of rest). Each set comprised 2 exercises that were alternated in 8 repetitions, with a one-minute rest period between sets. Total session duration ranged from 34 to 39 minutes, aligned with the official duration of a beach soccer match. Before beginning the training period, the players were thoroughly familiarized with the exercises and the 6-20-point Borg rating of perceived exertion (RPE, 6-20) scale within a separate session. Additional details of the training protocol are provided in the table 2.

Table 2. Tabata training protocol.

Week	Exercises	Duration (min)	Intensity (RPE, 6-20)
1-2	1- Forward and Backward Running + Heel Touch Crunch 2- Sumo Squat + Plank Jacks 3- Lateral Barrier Jump + Flutter Kicks 4- Sprint + Jumping Lunges 5- Squat Jump with Rotation + Knee to Squat 6- Burpee whit Long Jump + Toe Touches Sit up 7- Side to Side Shuffles + Cross-Crunches	34	17-18
3-4	Sets 1 to 7 were similar to the exercises of week 1-2 8- Seal Jack + Shoulder tap	39	19-20
5-6	All exercises were similar to the exercises of week 1-2	34	19-20

2.4 Aerobic power measurement

All measurements performed in the present study were carried out under similar conditions across the groups. To assess aerobic power, the 20m multistage fitness test (Beep test) was conducted in the evening, 48 h before and after the training period. Prior to starting the test, each player completed a 10-min warm-up. The Beep test is a widely used maximal running aerobic fitness test that involves continuous running between two lines 20 m apart, in time with recorded beeps. VO_{2max} was estimated using the standard table provided by Ramsbottom et al. (1988) (29).

2.5 Echocardiography

Two-dimensional transthoracic echocardiography was conducted 72 h before and after the training period by an expert cardiologist using a cardiovascular ultrasound system (Affiniti 50, Philips, Netherlands) in a standard room. The examinations were conducted in a standard room with players in the left lateral decubitus position. All morphological and functional LV measurements followed the guidelines of the American Association for Echocardiography and the European Association for Cardiovascular Imaging (30).

2.6 Blood sampling

Venous blood samples were collected before and after the first and last training sessions. Players arrived at the laboratory at 6:00 am, where 5 mL of blood was drawn from the brachial vein under standardized conditions. A second sample was obtained 6 h after both the first and last training sessions (31). Blood was collected into tubes without anticoagulant to allow serum separation. After clotting at room temperature, samples were centrifuged using a ScanSpeed 1730R device (Labogene, Denmark). The separated serum was stored at −70°C until analysis.

2.7 miR-1 and Hand-2 measurements

Total RNA was extracted using Trizol reagent (Kiazist Company) according to the manufacturer's protocol. cDNA was synthesized from 1 µg of extracted RNA. The 20 µL reaction mixture contained 1.25 µM random hexamer, 1.25 µM oligo-dT, 0.25 mM of each dNTP, 200 U reverse transcriptase, reverse transcriptase buffer (Invitrogen, Paisley, UK), 10 mM DTT, and 40 U RNase inhibitor. Random hexamers, RNA, oligo-dT, dNTPs, and RNase inhibitor were incubated at 65°C for 5 min and then cooled on ice for 5 min. Reverse transcriptase buffer and DTT were added, followed by incubation at 37°C for 2 min, a 50-min incubation at 37°C, and a final 15-min incubation at 70°C. Real-time PCR was performed using the Rotor-Gene 6000 system (QIAGEN, Hilden, Germany). Relative miR-1 expression was calculated using the comparative CT method and normalized to U6 as the internal control.

Hand-2 protein levels were assessed using western blotting. Serum samples were mixed with Tris buffer (pH 6) and heated at 100°C for 5–10 min. Gel electrophoresis was conducted at 120 V for 45 min using 10% SDS-PAGE, and proteins were transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% fat-free milk for 1 h at room temperature and incubated with primary antibodies for 1 h and secondary antibodies (1:1000) for 1 h. Protein bands were visualized using the Invitrogen detection kit (Lot Number: WB319614).

2.8 Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.4.3, with significance set at $p < 0.05$. Normality was assessed using the Shapiro–Wilk test. A repeated measures ANOVA (2 × 2 design: two time points × two groups) with Tukey's post-hoc test was used to compare VO_{2max} and echocardiographic parameters. A 2 × 4 repeated measures ANOVA design (two groups × four measurements) was used for miR-1 and Hand-2. Mauchly's test assessed sphericity, and Greenhouse–Geisser correction was applied when assumptions were violated. Pearson's correlation coefficient was also used to analyze relationships between variables.

3. Results

3.1 Aerobic power

The interaction between time and group was not statistically significant with regards to changes in VO_{2max} values among the athletes ($p = 0.464$). However, the increase in VO_{2max} values was statistically significant for both the SS ($p < 0.0001$) and HS ($p < 0.0001$) groups (Figure 1).

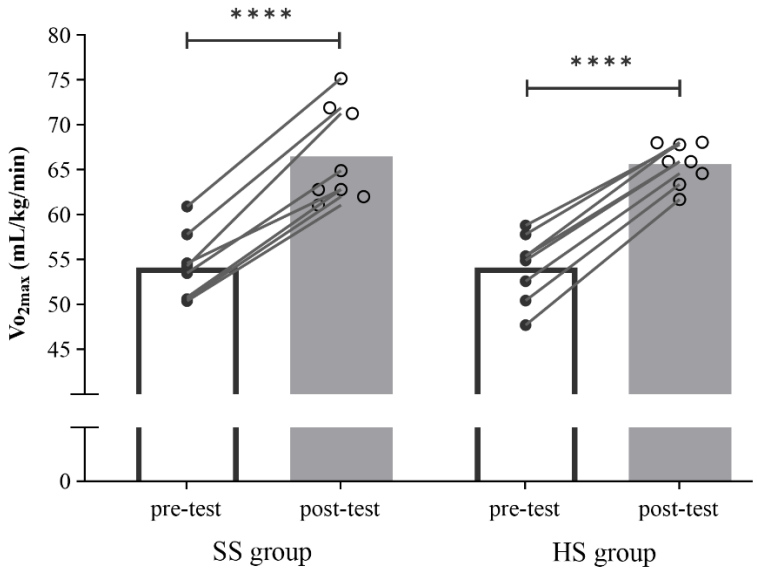

Figure 1. Changes in aerobic power from pre- to post-test between the sand (SS) and hard (HS) surface groups. $^{****}p < 0.0001$

3.2 Changes in the LV parameters

The interaction between time and group was significant for several structural and functional parameters of the left ventricle (Table 3). These included increases in stroke volume (SV; p=0.039), left ventricular end-diastolic volume (LVEDV; $p = 0.013$), left ventricular end-diastolic dimensions (LVEDD; $p = 0.029$), and left ventricular ejection fraction (EF; $p = 0.017$), as well as a reduction in relative thickness of the left ventricular posterior wall (RWT; $p = 0.036$). Furthermore, Tukey's post-hoc test revealed significant differences between the groups at the end of the study period for left ventricular mass index (LVMI; $p = 0.018$) and left ventricular end-systolic dimensions (LVESD; $p = 0.030$).

Table 3. Changes in structural and functional parameters of the left ventricle.

	SS group	HS group	Time × Group
LVMI (g/m^2)			
pre	73.04 ± 4.15	69.11 ± 3.04	
post	88.32 ± 6.4	80.87 ± 6.82	
change (%)	20.85 ± 2.5	17 ± 7.3	p=0.089
pre-post p value	<0.0001	<0.0001	
RWT (g/m^2)			
pre	0.3 ± 0.01	0.3 ± 0.02	
post	0.29 ± 0.01	0.3 ± 0.01	
change (%)	-3.23 ± 5.04	0.89 ± 3.45	p=0.036
pre-post p value	0.035	0.812	
PW (mm)			
pre	7.69 ± 0.5	7.61 ± 0.52	
post	8 ± 0.35	8.08 ± 0.4	
change (%)	4.3 ± 3.64	6.1 ± 3.3	p=0.185
pre-post p value	0.001	<0.0001	
LVEDV (mL)			
pre	104.53 ± 14.37	102.77 ± 11.45	
post	114.15 ± 11.25	108.39 ± 10.15	
change (%)	9.73 ± 4.9	5.63 ± 2.5	p=0.013
pre-post p value	<0.0001	0.0001	
LVESV (mL)			

pre	33.2 ± 3.15	31.13 ± 2.2	
post	30.13 ± 4.49	28.43 ± 2.89	p=0.685
change (%)	-9.5 ± 6.2	-8.7 ± 6.7	
pre-post p value	<0.001	0.001	
LVEDD (mm)			
pre	51.1 ± 0.99	50.64 ± 1.02	
post	55.13 ± 2.07	53.29 ± 2.13	p=0.029
change (%)	7.84 ± 2	5.2 ± 2.2	
pre-post p value	<0.0001	<0.0001	
LVESD (mm)			
pre	31.9 ± 1.83	30.43 ± 0.96	
post	33.18 ± 3.38	30.53 ± 1.08	p=0.080
change (%)	3.76 ± 5	0.35 ± 2.55	
pre-post p value	0.023	0.968	
SV (mL)			
pre	71.33 ± 13.69	71.63 ± 11.78	
post	84.02 ± 9.63	79.95 ± 8.58	p=0.039
change (%)	19.4 ± 10.4	12.4 ± 6	
pre-post p value	<0.0001	<0.0001	
FS (%)			
pre	37.58 ± 2.52	39.89 ± 1.38	
post	40.45 ± 3.8	42.63 ± 1.5	p=0.880
change (%)	7.6 ± 4.12	7 ± 5.5	
pre-post p value	<0.001	<0.001	
EF (%)			
pre	67.4 ± 3.62	69.83 ± 3.07	
post	73.15 ± 3.02	73.33 ± 1.75	p=0.017
change (%)	8.63 ± 3.4	5.1 ± 2.62	
pre-post p value	<0.0001	<0.0001	

SS: sand surface, HS: hard surface, LVMI: left ventricular mass index, RWT: relative wall thickness, PW: posterior wall, LVEDV: left ventricular end diastolic volume, LVESV: left ventricular end systolic volume, LVEDD: left ventricular end diastolic dimension, LVESD: left ventricular end systolic dimension, SV: stroke volume, FS: fraction of shortening, EF: ejection fraction.

3.3 Changes in miR-1 and Hand-2

A repeated measures ANOVA test revealed significant time × group interactions for both miR-1 ($p < 0.0001$) and Hand-2 protein ($p = 0.0002$). As illustrated in figure 2, miR-1 values were significantly increased in the SS group compared to the HS group following the final training session ($p < 0.0001$). Additionally, Hand-2 protein expression was significantly higher in the SS group than the HS group both before ($p = 0.0002$) and after ($p < 0.0001$) the last session (Figure 3).

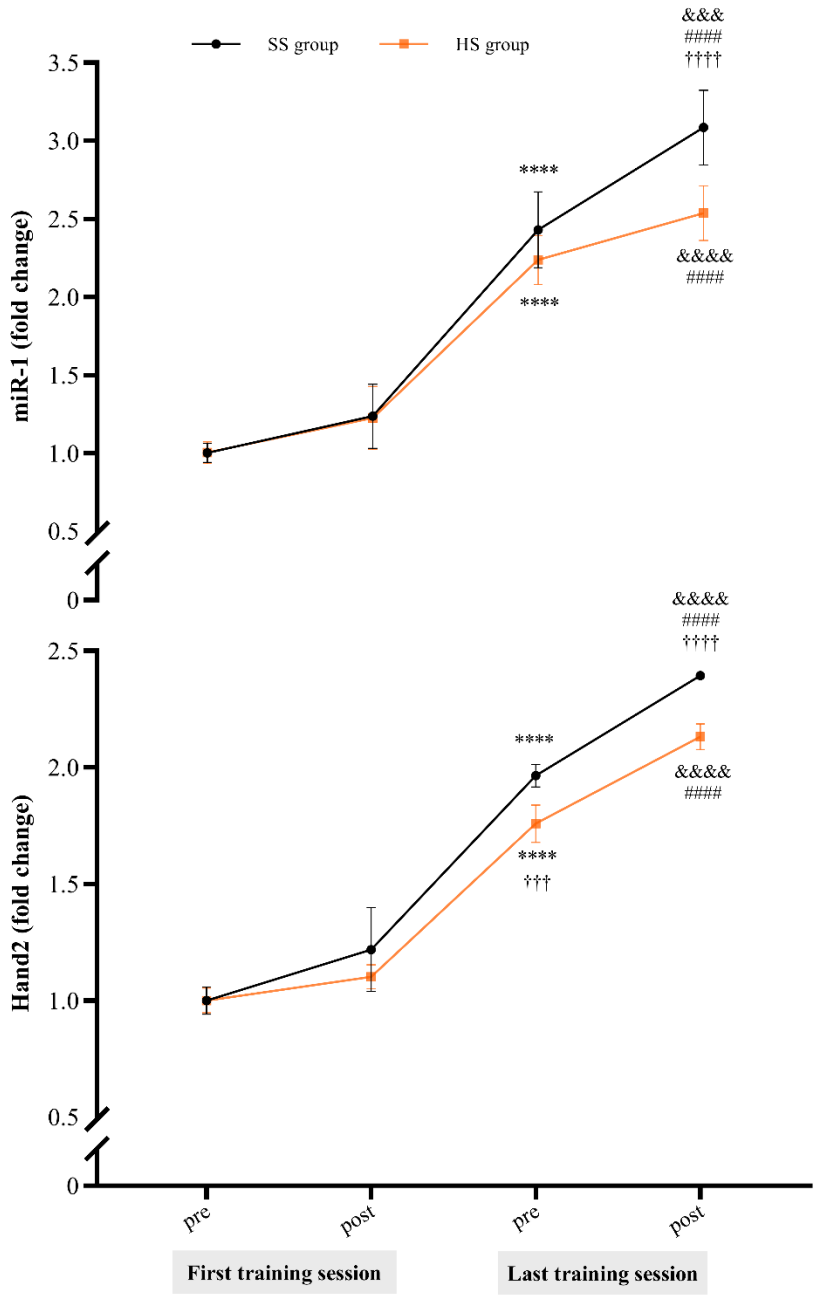

Figure 2. Changes in the relative expression level of microRNA-1 (miR-1) and heart and neural crest derivative expressed 2 (Hand-2) protein from pre- to post-test among the sand (SS) and hard (HS) surface groups. $^{****}p < 0.0001$ vs. before the first session; $^{\&\&\&}p < 0.001$, and $^{\&\&\&\&}p < 0.0001$ vs. before the last session; $^{\#\#\#\#}p<0.0001$ vs. after the first session; $^{\dagger\dagger\dagger}p < 0.001$, and $^{\dagger\dagger\dagger\dagger}p < 0.0001$ vs. the HS group.

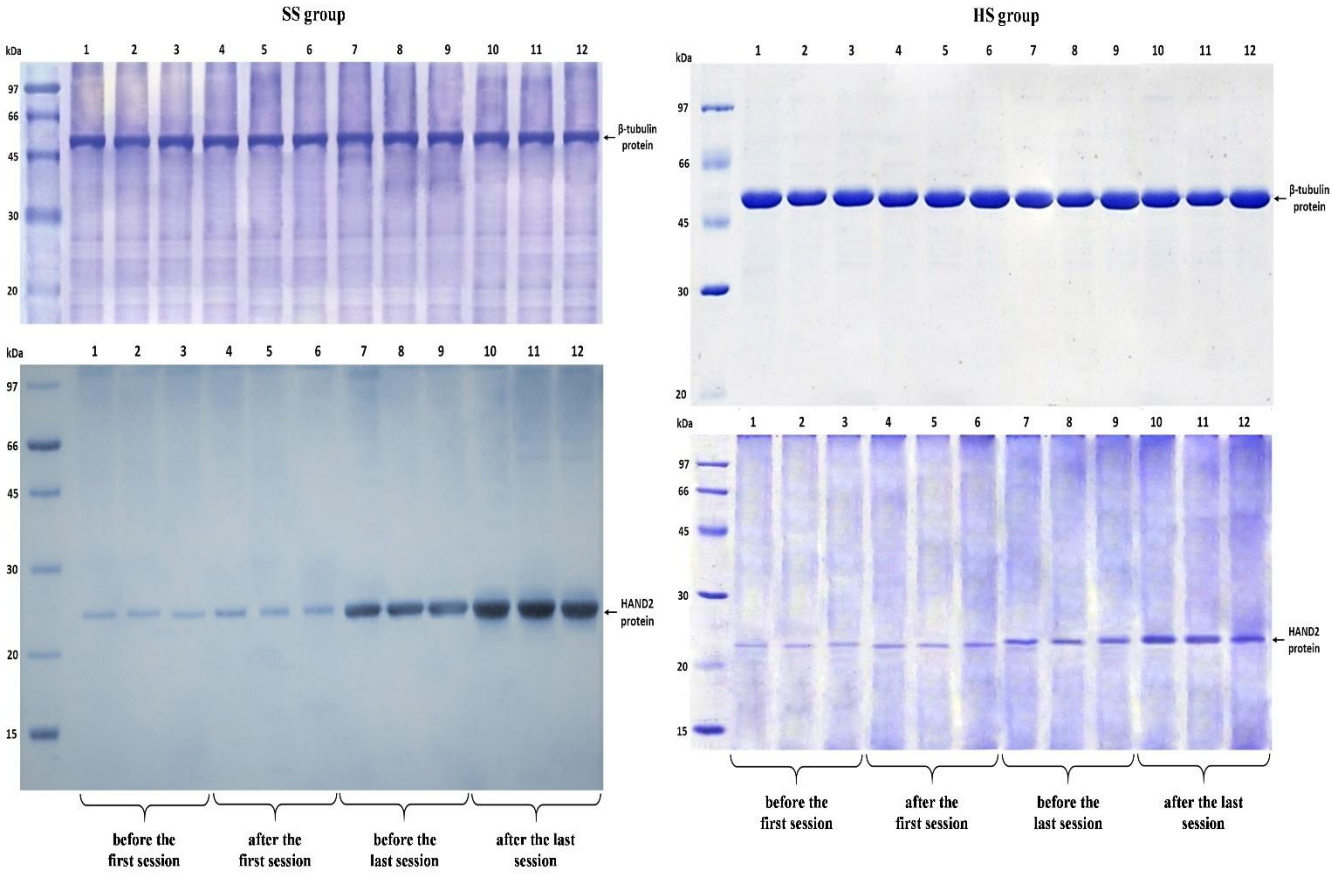

Figure 3. Summary of findings of protein expression assessed by Western blot analysis between the sand (SS) and hard (HS) surface groups. β-tubulin was used as loading control. Hand-2: heart and neural crest derivative expressed 2.

3.4 Correlations between the parameters

The SS group exhibited significant correlations between post-test miR-1 levels and several parameters, including VO_{2max} (R = 0.89, p = 0.003), LVMI (R = 0.84, p = 0.009), left ventricular posterior wall thickness (PW; R = 0.88, p = 0.004), LVEDV (R = 0.98, $p < 0.0001$), left ventricular end-systolic volume (LVESV; R = 0.76, p = 0.027), LVEDD (R = 0.98, $p < 0.0001$), LVESD (R = 0.96, $p < 0.0001$), and SV (R = 0.87, p = 0.005). However, no significant correlations were found between changes in Hand-2 protein and the LV parameters in the SS group. In contrast, the HS group showed significant correlations between post-test Hand-2 protein levels and VO_{2max} (R = 0.76, p = 0.028) and LVMI (R = 0.73, p = 0.039), as well as significant correlations between post-test miR-1 levels and LVESV (R = 0.88, p = 0.004) and fractional shortening (FS; R = 0.93, p = 0.001).

4. Discussion and Conclusion

The present study examined the impact of a six-week Tabata training protocol performed on either an indoor hardcourt or sand surface on the aerobic power, LV structure and function, and serum levels of miR-1 and Hand-2 protein in a cohort of elite beach soccer players. The findings demonstrated that the sand-based training protocol led to significant improvements across all measured LV parameters, while the HS group exhibited no statistically significant changes in specific indices such as RWT and LVESD. Furthermore, the sand-based training elicited a significantly greater average increase in LVMI (20.8% vs. 17%) and LVESD (3.8% vs. 0.3%) compared to the HS training group.

Comparison of the post-intervention LVEDD values between the two groups revealed a significant increase in the sand-based training cohort relative to the HS group (55.1 mm vs. 53.3 mm). However, the average LVEDD for both

groups remained within the normal range for men (≤ 56 mm) (32). Furthermore, the established criteria for left ventricular morphology categorize normal structure as RWT < 0.42 and LVMI ≤ 115 g/m^2, concentric hypertrophy as RWT ≥ 0.42 and LVMI > 115 g/m^2, and eccentric hypertrophy as RWT < 0.42 and LVMI > 115 g/m^2 (33). Based on this classification system, the left ventricular morphology of the athletes in both training groups fell within the normal range, with no significant between-group differences observed. Overall, these findings suggest that Tabata training performed on either an indoor hardcourt or sand surface does not have a detrimental impact on left ventricular morphology.

Despite a 3.2% greater decrease in RWT in the SS training group compared to the HS cohort, these changes did not reach the threshold for eccentric hypertrophy. While no significant intergroup difference was detected in the post-intervention LVESV values (SS: 9.5%; HS: 8.7%), the time × group interaction was significant for LVEDV. Specifically, the percent increase in LVEDV was higher in the SS group (9.7%) relative to the HS group (5.6%). These results indicate that Tabata training on sand may elicit greater venous return to the heart during the diastole phase.

Correspondingly, the significant time × group interaction for the SV changes suggests that the enhanced venous return associated with sand-based training led to a greater increase in SV in this group (19.4%) compared to the HS cohort (12.4%). Collectively, these findings imply that a 6-week Tabata training program performed on sand, as opposed to a more stable surface, may confer positive effects on SV, likely mediated through increased venous return. This may be partially attributable to the higher mechanical demands of exercising on an unstable SS, which has been shown to require 1.15 times more mechanical work and 1.4 times greater energy expenditure compared to a firmer surface at a given speed (25).

Furthermore, based on the EF equation (i.e., SV / LVEDV), the SS group appeared to exhibit greater improvements in LV diastolic function compared to the HS group. Enhancements in LV systolic function were also more pronounced in the SS group. The significance of the time × group interaction for EF, together with the larger percentage increase observed in the SS group (8.6% vs. 5.1% in the HS group), suggests that not only did a greater volume of venous blood return to the hearts of athletes in the SS group, but they also ejected more blood with each contraction, thereby contributing more effectively to systemic circulation. These findings are consistent with the Frank–Starling mechanism, which states that increased venous return leads to greater myocardial stretch and thus stronger contractions, ultimately increasing SV. These results align with Sheykhlouvand et al. (2022), who reported significant increases in SV (~12%), LVEDV (~9.5%), and EF (~5%) in well-trained male kayak sprint athletes after eight weeks of HIIT (34).

Another notable finding of this study was the increase in the relative expression of miR-1 in both groups following the intervention. However, the average increase observed after the final training session was greater in the SS group compared to the HS group (27.7% versus 13.4%). Regarding Hand-2, serum levels of this protein were found to be significantly higher in the SS group than in the HS group, both before and after the final training session. Although circulating Hand-2 may reflect broader regulatory activity, it does not directly represent myocardial expression because the protein is predominantly localized within cardiac tissue. Thus, the serum-based findings should be interpreted with caution, and future studies incorporating tissue-specific measurements are needed to clarify the underlying cardiac adaptations.

Taken together, these patterns suggest that the distinct cardiac responses observed in the SS group may be partially influenced by the differential regulation of these two factors. To further investigate this hypothesis, Pearson's correlation coefficient was employed, revealing a strong significant correlation between the post-test expression of miR-1 and various parameters in the SS group, including VO_{2max} (R = 0.89), LVMI (R = 0.84), PW (R = 0.88), LVEDV (R = 0.98), LVESV (R = 0.76), LVEDD (R = 0.98), LVESD (R = 0.96) and SV (R = 0.87). Furthermore, in the HS group, a significant correlation was observed between the post-test expression of Hand-2 and VO_{2max} (R = 0.76) and LVMI (R = 0.73), as well as between the post-test expression of miR-1 and LVESV (R = 0.88) and FS (R = 0.93). Collectively, these observations suggest that the differences in the aforementioned cardiac parameters following the training protocol (particularly on the sand surface) may be attributable in part to increased expression of miR-1. This increase likely facilitates positive morphological and structural adaptations of the LV (within the normal range defined for humans) through mechanisms involving other target proteins, aside from Hand-2.

In this context, Alamdari and Armanfar (2019) demonstrated that an eight-week regimen of HIIT, consisting of 6 to 8 bouts of 30 to 60 s of running, was associated with an increase in the expression of miR-1 and VO_{2max} values in young male athletes (35). Furthermore, the findings of the

present study align with those of Ghorbani et al. (2017) and Vakili et al. (2001), who reported that 6 to 8 weeks of HIIT performed at an intensity of 85-90% of VO_{2max} can enhance the relative expression of miR-1 in both patient (36) and healthy (37) rat models. These results suggest that alterations in miR-1 expression within myocardial tissue are likely linked to the signaling pathways associated with adaptations induced by HIIT (37). Recently, Fathi et al. (2020) concluded that the increased expression of miR-1 and its target genes, including SRF, HDAC-4, and Hand-2, may play a critical role in facilitating beneficial structural changes in the hearts of rats following 14 weeks of endurance training (12).

In the present study, a six-week program of Tabata training conducted on both indoor hardcourt (21.6%) and sand (22.9%) resulted in a significant enhancement of the VO_{2max} values among the participants. The observed alterations in the structure and function of LV, particularly the improvement in SV, may account for this increase in aerobic power. However, despite the higher SV values recorded in the SS group compared to the HS group, no significant difference was noted between the two groups regarding the increase in VO_{2max} following the training period. This suggests that improvements in aerobic power was not solely driven by SV and other cardiac adaptations; additional mechanisms (e.g., improvements in the oxidative and buffering capacity of the muscles, mitochondrial adaptations, and other biochemical changes) likely contributed as well (38). Given that these variables were not measured, future research should investigate these potential mechanisms in response to Tabata training. Moreover, our findings indicated a strong correlation between the post-test expression of miR-1 and the VO_{2max} values of the SS group ($R = 0.89$). In contrast, a strong correlation was observed between the post-test expression of Hand-2 protein and the VO_{2max} values of the HS group ($R = 0.76$). These findings suggest that the elevation of these signaling factors may play a significant role in the improvement of VO_{2max} values among athletes as a result of engaging in HIIT protocols. Therefore, further research is necessary to determine the precise mechanisms through which miR-1 and Hand-2 influence aerobic power.

Consistent with the present findings, several studies have reported similar results. Tabata et al. (1996) demonstrated a significant increase (7 mL/kg/min) in VO2max after a six-week Tabata regimen (1). Safania et al. (2011) and Impellizzeri et al. (2006, 2008) also showed significant VO_{2max} increases in soccer players following HIIT (6-8). Additionally, Sperlich et al. (2011) and Helgerud et al. (2001) reported improvements in VO_{2max} ranging from 7% to 11% after 5-8 weeks of HIIT in soccer players (39, 40).

In contrast, some authors have reported inconsistent findings. For example, Binnie et al. (2014) compared sand and grass surfaces during an eight-week conditioning program in female athletes and found that the improvement in relative VO_{2max} was greater in the sand group (22). These discrepancies may reflect differences in training duration (8 vs. 6 weeks) and participant sex (female vs. male athletes in the present study). In a study comparing HIIT responses between trained men and women, the authors concluded that HIIT protocols should be tailored by gender due to anthropometric and physiological differences that affect real-world exercise performance (41).

This study has several limitations. First, the relatively small sample size, inherent to research involving elite athletes, may have reduced the statistical power of the findings. Second, the absence of a non-training control group restricts the ability to isolate the pure training effect from potential time-related adaptations. Finally, the study did not incorporate complementary muscular or metabolic assessments (e.g., indicators of mitochondrial function, buffering capacity, or lactate kinetics) which limits the depth of physiological interpretation.

In conclusion, the present findings indicate that six weeks of Tabata training on sand, compared with an indoor hardcourt, may lead to a greater increase in SV in elite beach soccer players, while producing similar improvements in relative VO_{2max}. This increase in SV is likely attributable to enhanced venous return. Additionally, some of the positive cardiac adaptations observed in the sand group may be related to a greater increase in miR-1 expression and, to a lesser extent, Hand-2 expression. These two factors may contribute to improved morphological and functional indicators of cardiac performance through intracellular mechanisms.

Authors' Contributions

The conceptualization of the study was conducted by H.M.B., A.D., and H.A. The methodology was developed by H.M.B. and A.D., while data curation and software development were handled by H.M.B. Validation was performed by A.D. and H.A., and the formal analysis was carried out by H.M.B. Resources were provided by H.M.B. and H.A. The original draft of the manuscript was prepared by H.M.B., and the review and editing processes were

completed collaboratively by H.M.B., A.D., and H.A. Visualization of the findings was performed by H.M.B., under the supervision and project administration of A.D. and H.A. All authors have read and approved the final version of the manuscript.

Declaration

We wish to disclose that artificial intelligence tool (i.e., ChatGPT-4o) was utilized to enhance the manuscript's wording, readability, and language quality.

Transparency Statement

Data are available for research purposes upon reasonable request to the corresponding author.

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Declaration of Interest

The authors report no conflict of interest.

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Ethical Considerations

The present research protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the University of Guilan (IR.GUILAN.REC.1400.037).

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