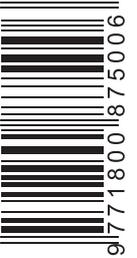




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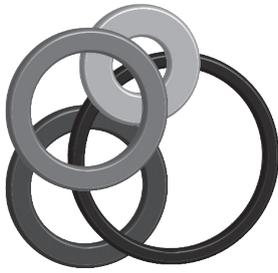


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Dear Readers,



It gives us great pleasure to introduce the second issue of this year's volume of Montenegrin Journal of Sports Science and Medicine (MJSSM). We will standardly introduce to you the main achievements since last issue in March and add some additional personal insight into the reasons why MJSSM is such a great journal and you should work with us.



Since the CARS-CoV-2 virus is still with us and continue to attack our daily routine, the science has to be the best answer to disrupting many regular aspects of our life, including sport and physical activity. As scientific activities were not compromised in pandemic time, as most scientists were forced to spend most of their time at home, in front of their computers, this fact reflect the growth of the MJSSM that has continued enhancing its own quality from day to day. Our office has been receiving a large number of quality manuscripts and the journal officers as well as editors and reviewers have much more work to do. The most prestigious databases also recognized the mentioned fact (Web of Science and Scopus). One of these databases (Scopus) continue recognizing the development of our journal that is proved by reaching high impact scores for the fourth year (CiteScore 2020: 3.0, SJR 2020: 0.302; SNIP 2020: 0.633), while the ongoing tracker is promising better CiteScore calculation in 2022 (CiteScoreTracker 2021: 1.9; updated on 04 June, 2021) which is scheduled for Spring 2022. On the other hand, we are still preparing our journal to be evaluated again by Web of Science to reach a long-lasting and eager impact factor and inclusion in SCIE (Science Citation Index Expanded) and SSCI (Social Science Citation Index) databases. Therefore, we believe that 2022 will be the year of our highest reach, mostly because we have worked hard and that we deserve further progress and visibility at the international level as current Web of Science Citation Report is promising us this kind of success (Total Publications: 115; h-index: 9; Average citations per item: 3.13; Sum of Times Cited: 360; Without self-citations: 341; Citing articles: 251; Without self-citations: 237; updated on 03 July, 2021). Nevertheless, we must keep in mind that this success has not only been achieved by the management of the journal, our editors, reviewers and authors, as well as readers, have contributed equally. So, we want to thank all the participants in the rapid development of our journal again and again, and to invite all those who have not participated before, to join us in the future, to continue in the same rhythm to the same direction.

We have to remind our editors, reviewers and authors, as well as readers that our journal will continue working on growing academic publication in the fields of sports science and medicine; all clinical aspects of exercise, health, and sport; exercise physiology and biophysical investigation of sports performance; sport biomechanics; sports nutrition; rehabilitation, physiotherapy; sports psychology; sport pedagogy, sport history, sport philosophy, sport sociology, sport management; and all aspects of scientific support of the sports coaches from the natural, social and humanistic side, in various formats: original papers, review papers, editorials, short reports, peer review - fair review, as well as invited papers and award papers, as well as promote all other academic activities of Montenegrin Sports Academy and Faculty for Sport and Physical Education at University of Montenegro, such as publishing of academic books, conference proceedings, brochures etc.

Lastly, we would thank our authors one more time, who have chosen precisely our Journal to publish their manuscripts, and we would like to invite them to continue our cooperation to our mutual satisfaction. Thank you all of you for reading us and we hope you will find this issue of MJSSM informative enough.

Editors-in-Chief,  
Prof Dusko Bjelica, PhD  
Assoc. Prof Stevo Popovic, PhD





# Effect of Oral Supplementation with L-Carnitine on Performance Time in a 5000 m Race and Responses of Free Fatty Acid and Carnitine Concentrations in Trained-Endurance Athletes

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## Abstract

This study was designed to determine the effect of oral supplementation with L-carnitine on the performance time in a 5000 m race. In addition, free fatty acid, blood carnitine, lactate, and glucose responses to the race following the supplementation period were measured. Twenty male trained-endurance athletes were randomly divided into two groups (L-carnitine, n = 10 (22.13 ± 2.66 yrs) or placebo, n = 10 (21.63 ± 2.23 yrs)). The study was performed with a randomized, double-blind, placebo-controlled parallel-group, in which participants ingested an L-carnitine supplement or a placebo 2 × 1.5 g/day for 3 weeks. Athletes completed a 5000 m race before and after the supplementation period. Blood samples were collected from each athlete before and after the race, pre- and post-supplementation to measure the physiological responses. Data showed that there were no differences in performance time before (p=0.624) and after (p=0.407) supplementation period between groups and within a group (p>0.05). No differences existed in physiological responses between groups after supplementation before beginning the race (p>0.05), except for the blood carnitine level, which was significantly higher in the L-carnitine than the placebo (P=0.001) group. After the finish of the race, however, data showed better physiological responses in response to L-carnitine supplementation compared to the placebo group (p<0.05). In conclusion, although L-carnitine supplementation increases blood carnitine concentration, it has no beneficial effect on performance time of 5000 m race probably due to the short duration of the race; it might also have no ergogenic effect.

**Keywords:** *β-oxidation, mitochondria, neuropathies, nitric oxide, running economy*



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## Introduction

Despite the obvious cardiopulmonary benefits associated with distance running in a physically active population, several

potential limiting factors have been observed in endurance athletes (Abbas & Laursen, 2005). Metabolic factors seem to play a central role in fatigue during prolonged endurance exercise

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Conflict of interest: None declared.

in elite runners (Petersen, Hansen, Aagaard, & Madsen, 2007). Muscle and liver glycogen depletion is a major cause of fatigue in prolonged exercise events (Abbias & Laursen, 2005; Hanon, Thépaut-Mathieu, & Vandewalle, 2005). Elite runners require from 5,600 to over 40,000 kcal of energy during competitive endurance events (Brouns et al., 1989; Tarnopolsky, 2004). Carbohydrates and fats represent the predominant fuel sources during prolonged endurance exercise (Belcastro, Albisser, & Litteljohn, 1996; Tarnopolsky, 2004). However, muscle and liver glycogen is the primary source of energy during the beginning and the late stages of an endurance race (Petersen et al., 2007). Higher glycogen stores can maintain muscle contractile properties, excitation-contraction coupling, and delay muscular fatigue (Abbias & Laursen, 2005; Gandevia, 2001; Petersen et al., 2007). Several factors can improve running economy by using more fatty acids and sparing glycogen, including L-carnitine.

L-Carnitine (3-hydroxy-4-N-trimethylaminobutirate) is a hydrophilic (Bene, Csiky, Komlosi, Sulyok, & Melegh, 2011; Miklos et al., 2016) amino acid derivative presented throughout the central and peripheral nervous system (Bavari, Tabandeh, Varzi, & Bahramzadeh, 2016) and is predominantly found in cardiac and skeletal muscle (Broad, Bolger, & Galloway, 2006). L-Carnitine is biosynthesized from the amino acids lysine and methionine (Bavari et al., 2016; Mojtaba, Laleh, Mohsen, & Zohreh, 2011) in the kidney, liver, and brain (Delaš, Dražić, Čačić-Hribjan, & Sanković, 2008), in a process that requires the vitamins B<sub>6</sub>, B<sub>3</sub>, C, niacin, and iron (Broad et al., 2006; Delaš et al., 2008). The total estimated carnitine content is approximately 1.2 μmol/kg body mass (Rebouche, 1992). Red meat, fish and dairy products are reported to be the primary exogenous carnitine, which is estimated to be 2–12 μmol/kg body mass daily (Demarquory et al., 2004) or 20–300 mg per day (Delaš et al., 2008).

L-Carnitine plays a primary physiological role in mitochondrial β-oxidation (Bene et al., 2011; Zhang et al., 2012; Miklos et al., 2016) by the transportation of long-chain fatty acids from cytosole into the mitochondria (Bene et al., 2011; Siddiqui, Mughal, Siddiqui, & Hayat, 2015; Zhang et al., 2012). This process is mediated by the carnitine-palmitoyltransferase (CPT) enzymatic system (CPT I, carnitine-acylcarnitinetranslocase and CPT II) (Brass, & Hiatt, 1998; Delaš et al., 2008). In addition, L-carnitine modulates the ratio of acyl coenzyme A (CoA):CoA (Stumpf, Parker, & Angelini, 1985), serves as an energy source in the form of acetyl carnitine (Bene et al., 2011), and acts as an antioxidant (Pekala et al., 2011). All together, L-carnitine has a potential role in reducing intramuscular metabolic stress. Of relevance, L-carnitine decreases cytosolic iron concentration (Pekala et al., 2011), thereby reducing reactive oxygen species (ROS) production (Ba-

vari et al., 2016).

Oral supplementation with L-carnitine is used by endurance athletes to increase its content in skeletal muscle, increase fatty acid oxidation during exercise (Brass, & Hiatt, 1998), decrease toxic acyl groups (Peters et al., 2015; Stumpf et al., 1985), maintain the activity of pyruvate dehydrogenase (Brass, & Hiatt, 1998), preserve muscle glycogen, and delay muscular fatigue (Brass, & Hiatt, 1998; Smith, Fry, Tschume, & Bloomer, 2008; Wall et al., 2013). The increased use of fatty acids for energy production during prolonged exercise is beneficial to runners because it reduces muscle glycogen and thus increases aerobic capacity.

To the best knowledge of the author of the present paper, several studies have studied the effect of L-carnitine with acute ingestion (Eizadi, Pourvaghar, Nazem, Eghdami, & Khorshidi, 2009; Kashef & Saei, 2017; Mojtaba et al., 2011; Vecchiet et al., 1990) and different supplementation periods (Greig et al., 1987; Smith et al., 2008; Wächter, Vogt, & Kreis, 2002); most of those studies focused on maximal oxygen consumption (VO<sub>2max</sub>) measurements on a cycle ergometer and conducted on healthy untrained subjects. However, no study has investigated the concentration of carnitine following a middle-distance race, such as 5000 m in endurance athletes. Consequently, the present study aimed to determine the effect of L-carnitine supplementation on performance time of 5000 m race. In addition, free fatty acid, blood carnitine, lactate and glucose responses to the race following L-carnitine supplementation period were measured. This study hypothesized that L-carnitine may enhance performance by using more fatty acids that produce more than 100 adenosine triphosphate (ATP) in each molecule and reducing glycogen utilization.

## Methods

### Participants

The participants were 20 male trained-endurance athletes who used no medical drugs, dietary supplements, or doping. Demographic data of the participants are shown in Table 1. All participants trained once a day (approximately 90 min), three times per week. The participants were informed about the potential risks and benefits involved in participation. Each participant voluntarily provided written informed consent before participation in the present study. This study was approved in advance by the local scientific research committee (protocol SS 100-2020).

### Study design

In a randomized, double-blind, and parallel-group approach, the participants were divided into two groups: L-carnitine (*n*=10) and placebo-control (*n*=10). Participants ingested

**Table 1.** Participants' demographic data

Variables	LC	Pla	P value
Age (years)	22.13± 2.66	21.63± 2.23	0.522
Height (cm)	176.30 ± 3.86	174.12 ± 4.06	0.278
Mass (kg)	66.07 ± 4.30	64.90 ± 3.30	0.141
BMI (kg/m <sup>2</sup> )	21.89± 0.57	22.19± 0.47	0.304
resting HR (bpm)	64.70 ± 3.52	66.42± 4.22	0.232
Training volume (min/week)	460.00± 26.42	400.00± 37.35	0.095
Training experience (years)	3.80 ± 1.89	4.32 ± 2.16	0.615

Note. All data are expressed as mean ± SD. No differences existed between groups for any demographic variable, ensuring homogeneity between groups. Significance level was set at *P* < 0.05. LC: L-Carnitine, Pla: Placebo.

no coffee, energy drink, or other substances that could affect the results 24 hours prior to the beginning of the trials. Each trial in both groups consisted of a 5000 m race following a 10 min warm-up (jogging, joint mobilization, and stretching).

#### Study protocol

All athletes in both groups completed the 5000 m race on a track before supplementation of L-carnitine or placebo. On the following day, athletes were instructed to ingest L-carnitine in the L-carnitine group and maltodextrin in the placebo group for three weeks. After one day of the completion of supplementation, the race was performed in the same order for all athletes in both groups. Both pre- and post-supplementation trials for both groups were performed at the same time of the day (08.20 AM) to control for the circadian rhythm effect. All athletes were instructed to fast three hours prior to the trial, except for 500 ml water (21 °C) taken 90 min prior to the race to avoid possible dehydration. Randomization was equalized by speed and experience of training to ensure the homogeneity between groups. The homogeneity of the demographic variables of the participants between groups was equal ( $P > 0.05$ ) (see Table 1).

#### Control of pre-experimental status

Athletes were asked to maintain their routine training sessions and were instructed to refrain from strenuous exercise 48 hours prior to each trial. None of the athletes had ever consumed L-carnitine supplement before this study. They were not permitted to ingest any nutritional supplement throughout the supplementation period. They were also requested to maintain their normal diet throughout the supplementation period. Athletes wore the same attire for each test; a T-shirt, shorts, and shoes that they normally train in.

#### Supplementation protocol

For three weeks,  $2 \times 1.5$  g of L-carnitine capsule (L-carnitine, Capsule, Arazo Nutrition, USA) and maltodextrin per day were provided to experimental and placebo participants, respectively. The selected dose of L-carnitine was used depending on the literature that stated that the normal dosage of L-carnitine is 1–5 g/day (Wall et al., 2013).

#### Blood sample analysis

Blood samples were collected before and immediately after the race pre- and post-supplementation in both groups

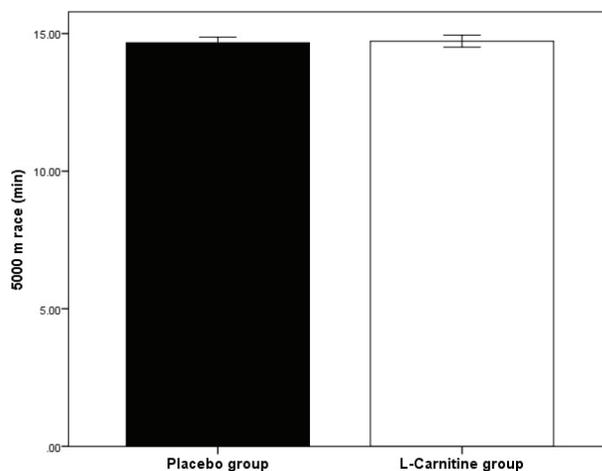
to measure blood carnitine, lactate, glucose, and free fatty acid. Venepuncture was used from the median vein to obtain blood samples; 2 ml of blood was dispensed into a plain tube containing clot activator to measure blood glucose using (Integral 400, Switzerland); 2 ml was dispensed into an anticoagulant EDTA tube to measure plasma lactate and free fatty acids, and 6 ml was frozen ( $-4$  °C) for 1 hour to measure carnitine. The blood tubes were centrifuged at 5000 rpm for 5 min. The plain blood tube was centrifuged at  $-4$  °C and 3500 rev/min for 5 min to allow for the extraction of serum, which was used to measure carnitine with a spectrometry device (Molecular Analysis, Spectrometry, Iceland). Plasma-free fatty acid was analysed with an Elecsys device (RIA, 2010, Switzerland), and plasma lactate was similarly analysed (Integral 400, Switzerland). The reference ranges of variables were as follows: 33.0–71.0  $\mu\text{mol/L}$  for carnitine, 0.63– 2.44 mmol/L for lactate,  $< 0.7$  for free fatty acid, 3.9–6.2 for blood glucose.

#### Statistical analysis

Because the normal distribution was verified ( $p > 0.05$ ) using Shapiro-Wilk test, a paired sample t-test was used to analyse the possible differences in carnitine, free fatty acid, glucose, and lactate within a group (between before and after the race, and between before and after supplementation). An independent t-test was utilized to analyse the differences in these variables between groups. Two-way ANOVA with repeated measures on (pre vs post) was used to determine if any significant main effects were present for the performance times of the 5000 m race between groups (experimental and placebo). Statistical analyses were carried out using SPSS version 23.0. All data are presented as mean  $\pm$  SD. The level of statistical significance was set at  $P < 0.05$ .

#### Results

Figure 1 illustrates the performance times for the 5000 m race after the supplementation period in both groups. There was no significant difference in 5000 m times between groups before ( $p=0.624$ ) and after ( $p=0.407$ ) the supplementation period. In addition, data also revealed that the time of the race was not changed from pre- ( $14.76 \pm 3.01$  min) to post- ( $14.66 \pm 1.21$  min) L-carnitine supplementation, and from pre- ( $14.81 \pm 2.05$  min) to post- ( $14.72 \pm 1.05$  min) placebo supplementation ( $p=0.694$ ;  $p=0.701$ , respectively).



**Figure 1.** Time of 5000 m race. Mean values  $\pm$  standard deviation (SD) are shown in the figure. No differences existed between groups ( $p=0.407$ ). Significance level was set at  $p < 0.05$ .

At baseline, there were no differences in the physiological parameters between the pre- and post-supplementation periods within a group ( $p>0.05$ ), except for blood carnitine, which was significantly higher after than before L-carnitine supple-

mentation (Table 2). All parameters were at similar levels between the L-carnitine and placebo groups, except carnitine was higher in the L-carnitine group than in the placebo group (Table 3).

**Table 2.** Physiological parameters before start of the race within a group before and after supplementation period

Parameters	LC group			Pla group		
	Pre-suppl (Pre-race)	Post-suppl (Pre-race)	P value	Pre-suppl (Pre-race)	Post-suppl (Pre-race)	P value
Carnitine ( $\mu\text{mol/L}$ )	51.81 $\pm$ 4.36	62.31 $\pm$ 4.12	0.002*	51.64 $\pm$ 3.17	51.97 $\pm$ 2.47	0.346
Free fatty acid (mmol/L)	0.57 $\pm$ 0.03	0.56 $\pm$ 0.06	0.053	0.58 $\pm$ 0.04	0.58 $\pm$ 0.05	0.162
Blood glucose (mmol/L)	5.67 $\pm$ 1.30	5.72 $\pm$ 2.27	0.181	5.68 $\pm$ 0.96	5.66 $\pm$ 1.33	0.529
Blood lactate (mmol/L)	2.11 $\pm$ 1.22	2.01 $\pm$ 2.86	0.631	2.05 $\pm$ 1.67	1.98 $\pm$ 2.05	0.425

Note. All data are expressed as mean  $\pm$  SD. \*Significant differences between before and after supplementation (before starting the race) within a group. Significance level was set at  $p<0.05$ . LC: L-Carnitine, Pla: Placebo, Pre: before, Post: after, suppl: supplementation.

**Table 3.** Physiological parameters before start of the race between groups before and after supplementation period

Parameters	Pre-suppl		P value	Post-suppl		P value
	LC (Pre-race)	Pla (Pre-race)		LC (Pre-race)	Pla (Pre-race)	
Carnitine ( $\mu\text{mol/L}$ )	51.81 $\pm$ 4.36	51.64 $\pm$ 3.17	0.637	62.31 $\pm$ 4.12	51.97 $\pm$ 2.47	0.001*
Free fatty acid (mmol/L)	0.57 $\pm$ 0.03	0.58 $\pm$ 0.04	0.067	0.56 $\pm$ 0.06	0.58 $\pm$ 0.05	0.052
Blood glucose (mmol/L)	5.67 $\pm$ 1.30	5.68 $\pm$ 0.96	0.468	5.72 $\pm$ 2.27	5.66 $\pm$ 1.33	0.318
Blood lactate (mmol/L)	2.11 $\pm$ 1.22	2.05 $\pm$ 1.67	0.492	2.01 $\pm$ 2.86	1.98 $\pm$ 2.05	0.546

Note. All data are expressed as mean  $\pm$  SD. \*Significant differences between before and after supplementation (before starting the race) between groups. Significance level was set at  $p<0.05$ . LC: L-Carnitine, Pla: Placebo, Pre: before, Post: after, suppl: supplementation

After completion of the race, the data revealed significantly ( $p<0.05$ ) physiological changes between pre- and post-L-carnitine supplementation but there were no differences noted in

the placebo group (Table 4). Additionally, the data indicated better responses after L-carnitine supplementation compared to the placebo group (Table 5).

**Table 4.** Physiological responses to the race within a group before and after supplementation

Parameters	LC group			Pla group		
	Pre-suppl (Post-race)	Post-suppl (Post-race)	P value	Pre-suppl (Post-race)	Post-suppl (Post-race)	P value
Carnitine ( $\mu\text{mol/L}$ )	49.88 $\pm$ 4.36	57.61 $\pm$ 3.44	0.001*	50.91 $\pm$ 3.11	50.87 $\pm$ 3.12	0.846
Free fatty acid (mmol/L)	0.59 $\pm$ 0.05	0.49 $\pm$ 0.07	0.003*	0.59 $\pm$ 0.03	0.60 $\pm$ 0.03	0.102
Blood glucose (mmol/L)	4.72 $\pm$ 2.24	5.34 $\pm$ 1.41	0.031*	4.85 $\pm$ 2.36	4.69 $\pm$ 0.21	0.711
Blood lactate (mmol/L)	6.85 $\pm$ 1.92	4.66 $\pm$ 0.92	0.002*	6.94 $\pm$ 2.18	7.21 $\pm$ 0.74	0.335

Note. All data are expressed as mean  $\pm$  SD. \*Significant differences between before and after supplementation, immediately after the race, within a group. Significance level was set at  $p<0.05$ . LC: L-Carnitine, Pla: Placebo, Pre: before, Post: after, suppl: supplementation.

**Table 5.** Physiological responses to the race between groups before and after supplementation

Parameters	Pre-suppl		P value	Post-suppl		P value
	LC (Post-race)	Pla (Post-race)		LC (Post-race)	Pla (Post-race)	
Carnitine ( $\mu\text{mol/L}$ )	49.88 $\pm$ 4.36	50.91 $\pm$ 3.11	0.362	57.61 $\pm$ 3.44	50.87 $\pm$ 3.12	0.002*
Free fatty acid (mmol/L)	0.59 $\pm$ 0.05	0.59 $\pm$ 0.03	0.335	0.49 $\pm$ 0.07	0.60 $\pm$ 0.03	0.001*
Blood glucose (mmol/L)	4.72 $\pm$ 2.24	4.85 $\pm$ 2.36	0.462	5.34 $\pm$ 1.41	4.69 $\pm$ 0.21	0.002*
Blood lactate (mmol/L)	6.85 $\pm$ 1.92	6.94 $\pm$ 2.18	0.277	4.66 $\pm$ 0.92	7.21 $\pm$ 0.74	0.001*

Note. All data are expressed as mean  $\pm$  SD. \*Significant differences between before and after supplementation between groups. Significance level was set at  $p<0.05$ . LC: L-Carnitine, Pla: Placebo, Pre: before, Post: after, suppl: supplementation

## Discussion

This study was designed to allow the assessment of physiological parameters during a 5000 m race and performance time following L-carnitine supplementation in endurance-trained

athletes.

The main finding was that athletes completed the 5000 m race in approximately similar times regardless of the supplementation ( $p=0.701$ ). This finding was associated with the ele-

vated blood carnitine in the L-carnitine group compared to the placebo group. It might be suggested that L-carnitine should not be classified as an ergogenic aid (Mojtaba et al., 2011). In addition, this finding might be attributed to the short distance of the race, leading to the inability of athletes in the L-carnitine group to complete the race in a shorter time. Thus, we suggest a further protocol, such as an incremental exercise protocol on (time until volitional fatigue) or a 10,000 m race, which determine the real potential effect of L-carnitine supplementation. Greig et al. (1987) showed no significant changes in  $VO_{2max}$  between L-carnitine supplementation (2 g/day for 2–4 weeks) and placebo in healthy untrained subjects. Smith et al. (2008) showed no differences between or within groups in muscle (Vastus lateralis) carnitine content, time to fatigue, and anaerobic power in untrained men and women after eight weeks of L-carnitine supplementation (1 g/day, 3 g/day, or placebo). Kashef & Saei (2017) observed an increased  $VO_{2max}$  during testing to exhaustion (Bruce incremental exercise) following acute ingestion of 3 g of L-carnitine 90 min prior to testing compared to the placebo group in students. Vecchiet et al. (1990) demonstrated enhanced incremental cycle ergometer performance following a single dose of L-carnitine (2 g) 1 h prior to exercise compared to placebo in collegiate students. Eizadi et al. (2009) demonstrated that a single dose of 3 g of L-carnitine did not improve exercise performance during submaximal cycle ergometer in healthy people. Wächter et al. (2002) reported that long-term supplementation with L-carnitine (4 g/day for 3 months) had no positive effect on  $VO_{2max}$ . The failure of long term L-carnitine supplementation to improve performance might be because of the greater excretion of L-carnitine with prolonged supplementation (Eizadi et al., 2009).

A normal plasma concentration range of total carnitine (TC) is 30–90  $\mu\text{mol}$ , free carnitine (FC) 26–52  $\mu\text{mol}$ , and acyl-carnitine esters (AC) 2–10  $\mu\text{mol}$  (Rebouche, 1992). L-Carnitine deficiency occurs when its concentrations below 20  $\mu\text{mol}$  or when the AC:FC ratio is higher than 0.4 (Lennon, Shrago, Madden, Nagle, & Hanson, 1986). Usually, the range of daily urinary carnitine excretion is 22–291  $\mu\text{mol}$  (Broad et al., 2006). The reduction in FC could result in reduced performance, particularly endurance exercise. Broad et al., (2006) investigated the concentration of plasma carnitine and urinary carnitine excretion following a seven-day weighed food program (3 meals/day of carbohydrate, fats and proteins) in 14 non-vegetarian endurance-trained adult males. They found that total plasma carnitine was 44  $\mu\text{mol/L}$ , urinary carnitine excretion was 437  $\mu\text{mol/day}$ , and the acyl free carnitine was 0.28, indicating athletes were not at risk of carnitine deficiency. Bene et al. (2011) showed that L-carnitine supplementation (1 g intravenously for 12 weeks) increased acylcarnitine to 1.6–4.8-fold in haemodialysis patients aged 39–85 years, and decreased by 11–74% three months after the cessation of supplementation. They suggested that the elevation of carnitine or acylcarnitine return to its normal range following carnitine discontinuation.

L-Carnitine supplementation has a beneficial effect in the L-carnitine group in the present study. For instance, the physiological parameters were better in response to L-carnitine supplementation.

Although the plasma FFA value measured for all athletes in both groups did not exceed reference range values by means, data analysis revealed a significant decrease in its value three

weeks after L-carnitine supplementation compared to the Placebo group. This might indicate an increased cellular uptake of fatty acids from blood and subsequent enhanced transportation of fatty acids into mitochondria for  $\beta$ -oxidation (Kashef & Saei, 2017). Delaš et al. (2008) demonstrated that L-carnitine supplementation (2 g/day for 2 weeks) only in sedentary healthy subjects did not induce changes in blood glucose, triacylglycerols, total cholesterol, high-density lipoprotein (HDL), creatine kinase; however, the FFA level was decreased from 0.439 mmol/dry muscle to 0.279 mmol/dry muscle. Mojtaba et al. (2011) demonstrated that FFA,  $VO_{2max}$ , HR, HDL, and low-density lipoprotein (LDL) were similar either after a single dose of 3 g of L-carnitine-L-tartrate or placebo in healthy non-active subjects. Eizadi et al. (2009) reported no differences in FFA levels between pre ( $0.69 \pm 0.24$  mg/dl) and post ( $0.72 \pm 0.14$  mg/dl) supplementation. Thus, athletes can perform an exercise with high energy expenditure following L-carnitine supplementation when the time or distance of exercise is not determined.

It has been reported that elevation in plasma fatty acid availability prior to exercise could reduce utilization of muscle glycogen (Brass & Hiatt, 1998; Smith et al., 2008), which promotes an important pathway to spare glycogen stores (Wall et al., 2013; Brass & Hiatt, 1998; Kashef & Saei, 2017) and subsequent improvement in endurance capacity. However, the fatty acid oxidation ratio is decreased when an exercise intensity exceeds 70%  $VO_{2max}$  (Wall et al., 2013), and glycolysis is increased (Peters et al., 2015; Wall et al., 2013). This results in depletion of glycogen stores and subsequent exercise cessation. In-line, L-carnitine is an indispensable compound for mitochondrial energy source by transporting long-chain fatty acids across the mitochondrial inner membrane as acyl-carnitine esters (Peters et al., 2015; Siddiqui et al., 2015), by regulating COA homeostasis (Miklos et al., 2016), by buffering toxic acyl-COA (Broad et al., 2006; Peters et al., 2015), by serving as a source of acetyl-L-carnitine, acetylcholine, and L-glutamate, in which they contribute to energy-producing reactions (Zhang et al., 2012), and by stimulating nitric oxide production (Miklos et al., 2016), in which it enhances pulmonary gas exchange and acts as a vasodilator and therefore increased blood flow (Verges, Flore, Favri-Juvin, Lévy, & Wuyam, 2005).

Our findings showed higher blood glucose with L-carnitine supplementation than with maltodextrin in the placebo group, which might indicate the role of L-carnitine supplementation in the utilization of FFAs for a fuel source instead of glycogen. This supported by our finding that showed lower blood lactate concentration in the L-carnitine group than the placebo group. The explanation of this result might be attributed to the lesser glucose utilization as an energy source during the race following L-carnitine supplementation and therefore decreased glucose cellular uptake. Another explanation by which L-carnitine supplementation elevated glucose levels and decreased lactate concentrations in the working skeletal muscle is that L-carnitine can reduce lactic acid accumulation by decreasing the utilization of liver and muscle glycogen (Siddiqui et al. 2015). Blood lactate in the study of Greig et al. (1987) was higher in placebo ( $11.7 \pm 2.1$  mmol/L) than L-carnitine ( $10.1 \pm 2.6$  mmol/L) after an incremental cycle ergometer, although the difference was not statistically significant.

Furthermore, slow-twitch fibres are the predominant working fibres during endurance running, such as the 5000 m race, ensuring increased ATP production by beta-oxidation.

In an animal study, Siddiqui et al. (2015) demonstrated that the rabbit soleus muscle exhibited a decline in muscle contraction force of about 57% in an L-carnitine group (5 mg/day for 2 weeks) with lactate levels of 23 mmol/kg compared with about 70% in a control group with lactate levels of 23 mmol/kg. They suggested that L-carnitine supplementation could delay the onset of fatigue, specifically in Type II muscle fibres.

### Conclusion

The finding of this study revealed that 2 x 1.5 g/day of L-carnitine for three weeks had no effect on the performance time of 5000 m in endurance athletes, although blood carnitine was significantly higher following the supplementation period compared to the placebo group. L-carnitine supplementation decreased free fatty acids and blood lactate and maintained blood glucose levels following the endurance trial.

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# Factors that Differentiate Winning and Losing in Men's University Basketball

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## Abstract

The objective of this study is to analyse the game-related statistics that differentiate winning and losing teams, according to the finale game scores in a men's university basketball league. Samples were gathered from the archival data of the 2019–2020 regular season of the league. Sixteen game-related statistics were analysed: two- and three-point field-goals (both successful and unsuccessful), free-throws (both successful and unsuccessful), defensive and offensive rebounds, assists, steals, turnover, blocks, second-chance points, fast break points, fouls committed and received. The data were clustered into different game types based on the final outcome point differences: all games, balanced games (11 points and below) and unbalanced games (12 points and above). Discriminant function analysis was conducted to identify the performance indicators that classify winning and losing games. The results revealed that winning and losing in balanced games were discriminated by successful two-point field goals, unsuccessful two-point field goals, unsuccessful three-point field goals, successful free-throws, assists, steals, blocks, second-chance points, fast-break points, fouls committed, and fouls received. For unbalanced games, winning and losing were distinguished by successful two-point field goals, successful three-point field goals, successful free-throws, unsuccessful free-throws, defensive rebounds, blocks, fast-break points, and fouls received. In conclusion, offensive and defensive indices are critical to winning and losing in university-level basketball.

**Keywords:** *sports performance, game-related statistics, notational analysis, males*



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WINNING AND LOSING IN MEN'S UNIVERSITY BASKETBALL

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## Introduction

Notational analysis is the process of recording, treatment, and diagnostics of events taking place in a competition (Drust, 2010). In basketball, notational analysis plays an essential role in formulating strategies and optimizing training load (Sampaio et al., 2004; Lorenzo et al., 2010; Sampaio et al., 2015).

Also, basketball-related statistics help improve the efficiency of players during the season (Sampaio et al., 2015) and predict final team rankings (Ziv et al., 2010).

Indeed, basketball-related statistics provide useful information in winning and losing games. Ibáñez et al. (2008) demonstrated that field goal attempts and defensive rebounds

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contribute to winning a game in men's basketball. Similarly, Gómez et al. (2008) found that defensive rebounds determined success in balanced and unbalanced games in men's basketball. In another study, Ibáñez et al. (2008) found that two-point field goal attempts and defensive rebounds differentiated winning and losing in balanced games while only successful two-point field goals significantly affected the result in unbalanced games. Csátlajay et al. (2009) revealed that three-point performance, free throws, and defensive rebounding were decisive in winning and losing.

Recent trends in basketball notational analysis include fast breaks and second-chance points. Evangelos et al. (2005) suggested that the fast break is an important factor in winning games. It presents a higher scoring opportunity by delaying defensive set-up. In contrast, second-chance points reflect success in scoring after an offensive rebound. Conte and Lukonaitiene (2018) noted that winning teams have a higher number of second-chance points compared to the losing team during unbalanced games. Both fast break and second-chance points increase field goal attempts, leading to higher chances of scoring (Sampaio & Janeira, 2003).

In the university basketball settings in the Philippines, there seems to be a paucity in the literature that distinguish factors that affect the outcome of a basketball game. Such information will allow better interpretation of the game and logically increase the applicability to improve training plans (Sampaio & Janeira, 2003; Gómez et al., 2008). Furthermore, basketball game-related statistics can aid in the long-term development of university basketball programmes. Therefore, this study aims to analyse the game-related statistics that differentiate winning and losing teams in a university basketball league in the Philippines.

## Methods

This study covered fifty-six regular-season games from the 2019-2020 university basketball league in the Philippines. The following game-related statistics were used: two-point field goals (successful and unsuccessful), three-point field goals (successful and unsuccessful), free throws (successful and unsuccessful), defensive rebounds, offensive rebounds, assists, steals, turnovers, second-chance points, fast-break points, fouls (committed and received), blocks, recovered ball possessions, and ball possessions.

The collected game statistics were normalized to ball possessions (BP) multiplied by 100. Ball possession was calculated as  $BP = (\text{field-goals attempted}) - (\text{offensive rebounds}) + (\text{turnovers}) - 0.4 \times (\text{free-throws attempted})$  (Oliver, 2004).

To control for the effects of the situational variables, the game types were obtained using k-cluster analysis, allowing a cut-off value of point differences in the outcome of a given game. The cluster analysis classified 67.0% ( $n = 75$ ) of the games with final score differences of 12 points and above (unbalanced games), while 33.0% ( $n = 37$ ) of the games have score differences of 11 points and below (balanced games).

An independent samples T-test was conducted to differentiate game-related statistics between winning and losing teams. A discriminant function analysis was conducted to identify the separators in winning (Group 1) and losing teams (Group 2). A structural coefficient (SC) of above 0.30 was used as the criteria for differentiating variables in winning and losing (Gómez et al., 2008). The discriminant score (D) was derived from the unstandardized discriminant function coef-

ficients ( $\beta$ ) to predict the game outcome computed as  $D = \beta_0 + \beta_1 \times V_1 + \dots + \beta_{15} \times V_{15}$ , where  $\beta_0$  is the constant value,  $\beta_1$ – $\beta_{15}$  are the unstandardized coefficients for each variable,  $V_1$ – $V_{15}$  are the values of game-related statistics (Gómez et al., 2014). The group prediction was made from the cutting scores of the centroids, calculated as:  $\text{cutting score} = (\text{mean centroid 1} + \text{mean centroid 2}) / 2$ . Values with  $D > \text{cutting point}$  will be in Group 1.  $D$  values  $< \text{cutting point}$  will be assigned to Group 2. Statistical analyses were carried out using the commercial statistical package (IBM SPSS ver 25, Armonk, NY) with alpha set at 0.05 level.

## Results

### All Games

The result showed that both successful and unsuccessful two-point field goals were significantly different in winning and losing games at  $t(110) = 3.13$ ,  $p = 0.00$  and  $t(110) = -2.70$ ,  $p = 0.00$ , respectively. Successful three-point field goals were also statistically significant at  $t(110) = 3.83$ ,  $p = 0.00$ . Another parameter that showed significant difference were defensive rebounds at  $t(110) = 3.91$ ,  $p = 0.00$ . Assists were also statistically significant between winning and losing games at  $t(110) = 6.56$ ,  $p = 0.00$ . Blocks were statistically different between winning and losing teams at  $t(110) = 4.80$ ,  $p = 0.00$ . There was also a significant difference between winning and losing teams for second chance points at  $t(110) = 4.16$ ,  $p = 0.00$ .

### Balanced Games

There was a significant difference in successful three-point field-goals between winning and losing games,  $t(35) = 2.25$ ,  $p = 0.03$ . Assists were significantly different in winning and losing games at  $t(35) = 3.48$ ,  $p = 0.00$ . A significant difference in blocks also existed between winning and losing games,  $t(35) = 3.30$ ,  $p = 0.00$ .

### Unbalanced Games

There was a significant difference in successful two-point field goals between and losing games,  $t(73) = 3.92$ ,  $p = 0.00$  and in unsuccessful two-point field goals,  $t(73) = -2.99$ ,  $p = 0.00$ . Successful three-point field goals showed a significant difference at  $t(73) = 3.11$ ,  $p = 0.00$ . A significant difference is also found in defensive rebound at  $t(73) = 2.940$ ,  $p = 0.004$ . A significant difference in assists also existed between winning and losing games at  $t(73) = 5.09$ ,  $p = 0.00$ . Blocks showed significant difference between winning and losing games at  $t(73) = 2.88$ ,  $p = 0.00$ . Lastly, fast break points were also statistically significant in winning and losing games,  $t(73) = 3.61$ ,  $p = 0.00$ .

### Discriminant Analysis for All Games

The discriminant analysis cross-validation percentage in winning and losing in all games was 88.4%. The most powerful discriminators in winning and losing in men's university basketball were the successful two-point field-goals (SC = 0.32), successful three-point field goals (SC = 0.57), unsuccessful three-point field-goals, successful free-throws (SC = -0.47), defensive rebounds (SC = 0.62), assists (SC = 0.31), steals (SC = 0.33), and blocks (SC = 0.43). The cutting score for all games is 0.00, and  $D = -6.80 + 0.05 \times \text{successful two-point field goals} \times -0.02 \times \text{unsuccessful two-point field goals} + 0.13 \times \text{successful three-point field goals} - 0.04 \times \text{unsuccessful three-point field goals} - 0.04 \times \text{successful free-throws} - 0.04 \times \text{unsuccessful free-throws} + 0.07 \times \text{defensive rebounds} + 0.02 \times \text{offensive}$

**Table 1.** Differences in basketball-related statistics

	All Games (n = 112)		Balanced (n = 37)		Unbalanced (n = 75)	
	Winners	Losers	Winners	Losers	Winners	Losers
Successful 2-point field goals*†	32.4 ± 6.60	28.5 ± 6.40	33.4 ± 7.94	35.0 ± 4.82	31.5 ± 5.36	26.5 ± 5.48
Unsuccessful 2-point field goals†	36.9 ± 9.68	41.5 ± 8.46	40.2 ± 9.95	46.0 ± 8.25	34.2 ± 8.74	40.1 ± 8.10
Successful 3-point field goals*##	13.2 ± 5.01	9.90 ± 3.91	13.4 ± 6.56	9.03 ± 3.32	12.9 ± 3.54	10.1 ± 4.07
Unsuccessful 3-point field goals	29.4 ± 9.33	28.9 ± 8.74	33.9 ± 9.59	34.8 ± 8.97	25.9 ± 7.57	27.2 ± 7.95
Successful free-throws	16.6 ± 12.2	16.1 ± 9.56	28.8 ± 11.6	27.3 ± 8.36	11.5 ± 5.76	8.09 ± 4.23
Unsuccessful free-throws	10.5 ± 6.06	9.30 ± 5.15	13.4 ± 6.83	13.1 ± 4.86	8.09 ± 4.23	8.12 ± 4.69
Defensive rebounds*†	50.7 ± 9.18	44.5 ± 8.81	55.1 ± 8.63	50.6 ± 9.69	47.9 ± 8.45	42.4 ± 7.6
Offensive rebounds	24.7 ± 8.18	23.6 ± 8.48	31.0 ± 7.00	31.5 ± 9.40	20.0 ± 5.30	21.2 ± 6.29
Assists*##	27.5 ± 6.87	18.9 ± 5.20	28.4 ± 7.78	19.8 ± 5.83	25.1 ± 5.82	18.7 ± 5.04
Steals	8.20 ± 4.11	7.80 ± 3.87	9.48 ± 5.27	8.51 ± 4.17	8.23 ± 2.93	7.54 ± 3.80
Turnovers	24.0 ± 8.04	24.9 ± 6.38	26.8 ± 9.61	22.8 ± 7.15	23.1 ± 6.34	25.5 ± 6.07
Blocks*##	7.10 ± 3.90	4.45 ± 2.66	8.99 ± 3.62	5.26 ± 2.48	6.36 ± 3.76	4.21 ± 2.69
2 <sup>nd</sup> chance points*	26.4 ± 11.5	20.1 ± 8.39	32.2 ± 11.5	27.5 ± 7.65	19.9 ± 8.32	17.8 ± 7.29
Fast break points†	18.2 ± 6.87	13.1 ± 6.37	20.2 ± 6.71	17.5 ± 7.27	16.8 ± 6.70	11.7 ± 5.47
Fouls committed	25.5 ± 7.20	28.4 ± 7.26	28.9 ± 8.75	31.1 ± 8.00	25.6 ± 5.48	27.5 ± 6.90
Fouls received	28.2 ± 10.79	27.0 ± 8.92	38.2 ± 10.0	36.7 ± 6.36	23.6 ± 6.12	24.1 ± 7.42
Recovered Ball Possession	15.7 ± 5.72	12.2 ± 4.75	15.5 ± 5.06	13.2 ± 4.95	18.0 ± 6.26	10.9 ± 4.23
Ball Possession	65.6 ± 7.73	66.3 ± 7.12	64.3 ± 8.55	67.3 ± 7.27	65.5 ± 6.67	65.0 ± 6.86

Note: \*significant in all games at p < 0.05; #significant in balanced games at p < 0.05; †significant in unbalanced games at p < 0.05.

rebounds + 0.05 × assists + 0.08 × steals + 0.13 × blocks + 0.00 × second-chance points + 0.04 × fast-break points - 0.02 × fouls committed + 0.01 × fouls received.

*Discriminant Analysis for Balanced Games*

There was a 86.5% cross-validation percentage in winning and losing in balanced games. Powerful discriminators in win-

**Table 2.** Discriminant analysis structure coefficients for game-related statistics of winning and losing games

Game-related statistics	All Games	Balanced	Unbalanced
Successful 2-point field goals <sup>abc</sup>	0.32	-0.50	0.63
Unsuccessful 2-point field goals <sup>b</sup>	-0.17	-0.30	-0.09
Successful 3-point field goals <sup>ac</sup>	0.57	0.05	0.59
Unsuccessful 3-point field goals <sup>ab</sup>	-0.38	-0.52	-0.27
Successful free-throws <sup>abc</sup>	-0.47	0.38	-0.67
Unsuccessful free-throws <sup>c</sup>	-0.20	0.19	-0.42
Defensive rebounds <sup>ac</sup>	0.62	0.18	0.61
Offensive rebounds	0.15	-0.14	0.06
Assists <sup>ab</sup>	0.31	0.91	0.21
Steals <sup>ab</sup>	0.33	0.65	0.24
Blocks <sup>abc</sup>	0.43	0.84	0.36
2 <sup>nd</sup> chance points <sup>b</sup>	0.03	0.64	-0.06
Fast break points <sup>bc</sup>	0.24	-0.30	0.31
Fouls committed <sup>b</sup>	-0.11	0.30	-0.17
Fouls received <sup>bc</sup>	0.11	-1.11	0.45
Eigenvalue	1.14	1.66	1.50
Wilks Lambda	0.47	0.38	0.40
Canonical Correlation	0.73	0.79	0.77
Chi-square	77.9	26.9	60.0
DF	15	15	15
P	0.00	0.03	0.00
Reclassification %	88.4	86.5	93.3

Note: <sup>a</sup>Structure coefficient discriminant values ≥ 0.30 in all games; <sup>b</sup>Structure coefficient discriminant values ≥ 0.30 in balanced games; <sup>c</sup>Structure coefficient discriminant values ≥ 0.30 in unbalanced games.

ning and losing in balance games were successful two-point field goals (SC = -0.50), unsuccessful two-point field goals (SC = -0.30), unsuccessful three-point field goals (SC = -0.52), successful free-throws (SC = 0.38), assists (SC = 0.91), steals (SC = 0.65), blocks (SC = 0.84), second-chance points (SC = 0.64), fast-break points (SC = -0.30), fouls committed (SC = 0.30), and fouls received (SC = -1.11). The cutting score for balanced games is -0.39, and  $D = -0.21 - 0.07 \times \text{successful two-point field goals} + 0.03 \times \text{unsuccessful two-point field goals} + 0.01 \times \text{successful three-point field goals} - 0.06 \times \text{unsuccessful three-point field goals} + 0.04 \times \text{successful free-throws} + 0.03 \times \text{unsuccessful free-throws} + 0.02 \times \text{defensive rebounds} - 0.02 \times \text{offensive rebounds} + 0.13 \times \text{assists} + 0.13 \times \text{steals} + 0.26 \times \text{blocks} + 0.06 \times \text{second-chance points} - 0.04 \times \text{fast-break points} + 0.04 \times \text{fouls committed} - 0.12 \times \text{fouls received}$ .

#### *Discriminant Analysis for Unbalanced Games*

For unbalanced games, there was a 93.3% cross-validation percentage in winning and losing games. Powerful discriminators include successful two-point field goals (SC = 0.63), successful three-point field goals (SC = 0.59), successful free-throws (SC = -0.67), unsuccessful free-throws (SC = -0.42), defensive rebounds (SC = 0.61), blocks (SC = 0.36), fast-break points (SC = 0.31) and fouls received (SC = 0.45). The cutting score for balanced games is 0.179, and  $D = -8.72 + 0.12 \times \text{successful two-point field goals} + 0.15 \times \text{successful three-point field goals} - 0.04 \times \text{unsuccessful three-point field goals} - 0.10 \times \text{successful free-throws} - 0.09 \times \text{unsuccessful free-throws} + 0.08 \times \text{defensive rebounds} - 0.01 \times \text{offensive rebounds} + 0.04 \times \text{assists} + 0.07 \times \text{steals} + 0.11 \times \text{blocks} - 0.01 \times \text{second chance points} + 0.05 \times \text{fast-break points} - 0.03 \times \text{fouls committed} + 0.07 \times \text{fouls received}$ .

## **Discussion**

The purpose of this study was to identify the factors that differentiate winning and losing teams in a university basketball league in the Philippines. In the present study, successful two-point field goals, successful free throws and blocks were the most powerful variables in differentiating between winning and losing teams in all three analyses: all games, balanced games, and unbalanced games. Field goal shooting is the most fundamental skill of the game as it shows the offensive quality of the winning team (Sampaio et al., 2006). Additionally, successful field-goal shooting reflects an efficient offensive system that contributes to the higher possibility of winning the game. Successful free throws are also a crucial performance indicator as they contribute to achieving a higher number of scored points (Csátraljay et al., 2009). The development of fundamental sequences in field-goal and free-throw shooting, with progression to game-like conditions lead to shooting efficiency, thereby providing a better opportunity for positive game outcome. Blocks, in contrast, prevent the opponent from scoring (Ibáñez et al., 2008). Blocking entails timing and anticipation that add to a good team defensive system.

In balanced games, winning and losing games were differentiated by successful two-point field goals, unsuccessful two-point field goals, unsuccessful three-point field goals, successful free-throws, assists, second-chance points, fast-break points, steals, blocks, and fouls committed. Winning teams demonstrated better field goal efficiency through higher successful two-point field goals and free throws while exhibiting lower unsuccessful two-point and three-point field goals. Winning

teams also posted more assists made than losing teams did in balanced games. Assists indicate teamwork and better selection of moment to pass the ball (Sampaio et al., 2004; Garcia et al., 2013). The selection of field goal opportunities results in higher field goal percentages (Sampaio & Janeira, 2003). Winning teams executed more fast-break occurrences. This finding coincides with the results presented by Conte et al. (2017), which showed that more fast breaks increased winning percentage in both elite and sub-elite basketball teams. The fast break is an important element in the offensive basketball system because it reduces the transition time from defence to offence (Krause et al., 2008). Some coaches prefer fast breaks as the first option in any offensive attack due to the advantages it creates for the team (Wootten & Wootten, 2012). Taking advantage of the open court with fast breaks shifts the momentum to the offensive team with minimal or no defensive set-up in such situations.

Winning teams also presented more second-chance points than losing teams did in balanced games. More scoring opportunities are made available with second-chance points (Ibáñez et al., 2008). The second-chance points reflect the persistence and creativity to score from spontaneous conditions. In this study, winning teams received more fouls from the opponents. This situation increases scoring momentum by allowing more time for offensive set-up or scoring unguarded via free throws. On parameters related to defence, winning teams displayed more steals and blocks.

Additionally, winning teams committed fewer fouls. Steals, blocks, and fewer fouls represent defensive effectiveness, reducing the scoring chances of opponents (Ibáñez et al., 2008). Thus, winning teams in balanced games created more opportunities for scoring and were successful in executing the offensive moments. The offensive advantage in winning games was accompanied by greater defensive abilities in steals, blocks, and lower fouls.

In unbalanced games, winning and losing teams were differentiated by successful two-point field goals, successful three-point field goals, successful free-throws, unsuccessful free-throws, fast breaks, fouls received, defensive rebounds, and blocks. In this study, winning teams demonstrated better offensive execution from successful two-point field goals, successful three-point field goals, successful free-throws, and unsuccessful free-throws. Higher two-point field goals in winning teams signify better shot selection, leading to an increased shooting percentage (Trninić et al., 2002).

Furthermore, higher success from the three-point area reflects an additional offensive threat to the opponents. The outcomes in two-point and three-point field goal success in winning games in this study decrease the defensive ability of the opposing team by creating a large area for defensive coverage. Successful free throws and unsuccessful free-throws also differentiated winning and losing teams in unbalanced games. Winning teams converted more free throws with minimal failure. The free throw allows an offensive player to shoot in a stable position without a defender (Pakosz & Konieczny, 2016). Winning and losing during unbalanced games was also differentiated by fast breaks. Winning teams displayed more fast-break points than losing teams did. This situation exhibits the ability of the winning team to score quickly from defensive to offensive transition (Trninić et al., 2002). Another variable that discriminated winning and losing is the total fouls received. Winning teams received fewer fouls than the losing teams did, which translates to fewer interruptions in the of-

fensive rhythm of winning teams. In regard to defensive skills, winning teams in unbalanced situations posted more defensive rebounds, blocks, and steals than losing teams did. Defensive rebounds indicate aggressive defence, which disrupts offensive play (Sampaio & Janeira, 2003; Gomez et al., 2008). Blocks and steals stop a team from creating moments for scoring (Trninić et al., 2000). Thus, winning in unbalanced games is discriminated by offensive competence across scoring areas (free throws, two-point field goals and three-point field goals).

Limitations of the current study are acknowledged. First, a generalization of results should be avoided as the findings are only applicable to the teams involved in the league, specific to the 2019-2020 season. Second, the 1st to 4th quarter game-related statistics were analysed in this study. Inclusion of variables in shorter periods (per quarter or halves) can help determine trends in winning and losing in relation to phases of the game. Lastly, only regular-season games were included in this study. Future studies should include a comparison of the regular-season and postseason games to help establish the critical factors in higher competitive stages of the season.

This study highlighted the game-related statistics differentiating winning and losing in a men's university basketball league in the Philippines. Winning and losing games in balanced and unbalanced games are differentiated by offensive and defensive variables that are crucial for gaining an advantage against the opponents. The findings of this study can help coaches design and implement a basketball training programme that develops appropriate offensive and defensive skills essential to game success.

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# Efficacy of the WATERinMOTION Aquatics Exercise Programme on the Body Weight and Composition of Sedentary Older Women with Overweight/Obesity

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## Abstract

Some controversy remains regarding the effects of aquatic exercise on body weight and composition. The purpose of this study was to determine the short-term impacts of the WATERinMOTION aquatics exercise program on body weight and composition without nutritional intervention in sedentary older women with overweight/obesity. The study was developed as a quasi-experimental project (pre/post-study). Forty-four inactive women were volunteers from a convenience sample with a mean age of  $71.1 \pm 5.7$  years (Ukraine, 2019). Participants were allocated randomly into two groups: WATERinMOTION ( $n=22$ ) or a control group ( $n=22$ ). Meanwhile, the WATERinMOTION group performed the WATERinMOTION exercise programme with two weekly sessions of 55 minutes each. The control subjects did not participate in any physical exercises. They were asked to perform their routine activities during the study. Both programmes lasted one month. Height, body weight, body mass index (BMI), waist circumference (WC), fat mass (FM), total body water (TBW), and free fat mass (FFM) were the anthropometric variables that were measured pre/post-study. The analysis found statistically significant differences in body weight ( $-0.7$ ,  $P=0.004$ ), BMI ( $-0.3$ ,  $P=0.002$ ), and FM ( $-0.6$ ,  $P=0.03$ ) between the pre and post measurements of the WATERinMOTION group. Moreover, the comparison of groups at post revealed a significant difference in body weight ( $P<0.001$ ), BMI ( $P<0.001$ ), TBW ( $P=0.005$ ), FM ( $P<0.001$ ), FFM ( $P=0.003$ ), and WC ( $P=0.007$ ). The WATERinMOTION programme, which is not associated with nutritional monitoring, showed significant benefits for losing weight and the body composition of sedentary older women who are overweight and/or obese.

**Keywords:** *body composition, exercise therapy, resistance training, waist circumference, obesity, weight loss*



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## Introduction

The global obesity epidemic is becoming worse, and reduced physical activity or increased energy consumption are

two effective factors in this regard (Church & Martin, 2018). Atherosclerosis, stroke, hyperlipidemia, certain malignancies, and sleep disturbances are some of the serious health risks

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associated with obesity (Kivimäki et al., 2017). Ageing is a natural occurrence, but the incidence of obesity in the elderly has doubled in recent years (Vuittonet et al., 2020). Along with serious risks for health, obesity can lead to chronic painful states which are comorbid during ageing (Vuittonet et al., 2020). Physical exercise, in addition to increasing the number of calories consumed by the body to gain energy (Rezaeipour, 2019; Rezaeipour & Apanasenko, 2020), is also important for those suffering from chronic pain (Geneen et al., 2017). Currently, body mass index (BMI) is used to determine the normal weight and recognition of underweight, overweight, and obese people (World Health Organization, 2000). However, BMI measurements cannot separately quantify the composition of body fat (Nowak et al., 2019). Some people may have a BMI that places them in the obese category but have no excess body fat. Accordingly, BMI along with the measuring of body fat instead of merely measuring body weight is preferable to determine the possible health risks to an individual due to various adverse health outcomes (Nowak et al., 2019). Bioelectrical impedance analysis (BIA) is a quick, non-invasive, simple, and inexpensive method, as well as a good tool to evaluate body fat (Rezaeipour & Apanasenko, 2019).

When a person attempts to reduce body weight or keep healthy body weight, exercise is especially important. However, some persons have restrictions that may limit their ability to take part in land-based exercise programs; for example, those who are obese or have low levels of fitness, as well as people with mobility difficulties due to senility, neuro-skeletal dysfunctions, and pulmonary disorders (Neiva et al., 2018). Raffaelli et al. (2010) found that aquatic exercise compared to land-based exercise led to noticeable rises in physical activity. Aquatic exercise is defined as a low-impact activity that occurs in water (Sanders et al., 2016), which, due to greater resistance than air, increases heart rate and energy flow and includes movements of the whole body (Tamin & Loekito, 2018).

At the same time, literature reviews confirm the usage of aquatic exercise to promote body weight and composition (Penaforde et al., 2015; Pereira et al., 2018; Rezaeipour & Apanasenko, 2019). Other researchers do not accept the effect of water exercises on body weight (Charmas & Gromisz, 2019) and composition (Charmas & Gromisz, 2019; Rica et al., 2013; Siqueira et al., 2017), and there is still controversy over this issue (Rezaeipour, 2020). Also, no literature has been found that would specifically assess the efficacy of the WATERinMOTION aquatic exercise programme on body weight and composition. The WATERinMOTION programme is a standardized aquatic exercise program with music that contains a warm-up, linear and lateral movements, team formation, dynamic group workouts, upper and lower body suspension, strengthening the core body, and flexibility (Darley, 2020). This music, in particular, is adapted so that people of any age can take part in it and the choreography fits the music. While other aquatic exercise studies have had at least a six-week (Ferrigan et al., 2017) or longer programmes (Neiva et al., 2018; Rezaeipour, 2020; Rezaeipour & Apanasenko, 2019; Rezaeipour & Nychporuk, 2019), this study investigated whether two weekly sessions per month would be enough to result in significant health benefits.

The purpose of this study was to define whether the short-term WATERinMOTION aquatics exercise programme is effective in promoting participants' body weight, waist circumference (WC), and altering body composition. It was hy-

pothesized that the WATERinMOTION, which is designed to be used for approximately one month, may be a good option for promoting weight reduction, WC, and body composition of sedentary older women who are overweight and/or obese.

## Methods

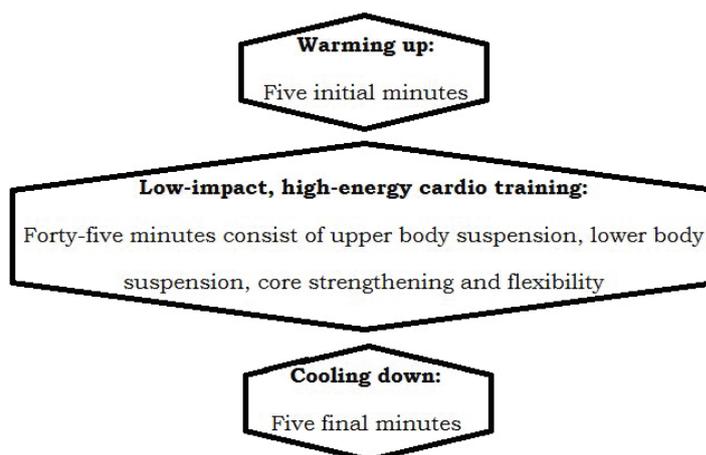
### *Participants and study design*

Forty-four participants (mean age of 71.1±5.7 years) of this quasi-experimental study were taken from a convenience sample of women volunteered to study at the Ukrainian Centre for Sports Medicine, Kyiv, in 2019. A call from a website was used to inform potential participants. With the probability of a population of 49 people, a 95% confidence level, and a five-confidence interval, a sample size of at least 44 would have been desirable, according to Creative Research Systems (2012). The research team, which included two physicians and two trainers, explained the study to the participants. A medical examination was carried out, and participants presented a physician's permit, which confirmed that the study was not prohibited for participants.

The participants were allocated randomly into two groups: WATERinMOTION (n=22) or a control group (n=22). The WATERinMOTION group performed the WATERinMOTION exercise programme with two weekly sessions of 55 minutes each. The control subjects did not participate in any physical exercises; they were asked to perform their routine activities during the study. The random distribution was made by a manual lottery in which individuals cast their lotteries (Rezaeipour & Apanasenko, 2019). Both programs lasted one month. Participants voluntarily consented and participate based on the following inclusion criteria: sedentary lifestyle as physical exercise frequency from one to two times a month or less (Rezaeipour & Nychporuk, 2019), women aged 65 and over, BMI of  $\geq 25 \text{ kg.m}^{-2}$  (Rezaeipour, 2020; Rezaeipour & Apanasenko, 2019), living self-sufficiently and classified with the level of the functional status of 3, 4 or 5 on the Basic Activities of Daily Living scale (the average status was three in this study) (Spirdus et al., 1995). The assessment of the cognitive status of more than 18 on the Mini-Mental State Examination was another inclusion criterion (Ogden et al., 2006). Exclusion criteria included weight instability ( $\pm 2\text{kg}$ ) in the previous three months, blood pressure greater than 140/85 mmHg, missing three consecutive exercise sessions or more than a third of sessions, and a history of weight control supplements or medications (such as hormone replacement therapy). Blood pressure was assessed based on recommendations of the European Society of Hypertension (O'Brien et al., 2005) using a validated Omron M10-IT (HEM-7080IT-E, Kyoto, Japan) sphygmomanometer. The protocol of the study with all relevant strategies and institutional policies was drawn up under the Helsinki Declaration. The Institutional Research Ethics Committee approved this study and its informed consent forms.

### *WATERinMOTION program*

The typical WATERinMOTION program consisted of five minutes of warm-up and 45-minute of low-impact, high-energy cardio training, which consisted of linear and lateral suspensions, upper and lower body movements, strengthening the core body, flexibility, and five final minutes of cooling down (Darley, 2020). Supplementary Materials (Figure 1 and Table 1) are provided for a more detailed explanation of WATERinMOTION.



**Figure 1.** The organizational structure of the aquatic exercise program of WATERinMOTION

Additionally, the music used for the WATERinMOTION is specifically adapted so that people of any age can take part in it, and the choreography fit the music (Darley, 2020). The conditioning of intensity was considered moderate to vigorous or 70–85% of the maximum heart rate (Rezaeipour, 2020). Participants'

heart rates were checked with a waterproof heart rate monitor (Polar Electro Oy, Kempele, Finland) at all the training sessions. The typical water fitness temperature is 28–30 °Celsius (Bure, 2019), and the pool depth was 1/30 m. The sessions of the WATERinMOTION program were carried out in an indoor pool.

**Table 1.** The aquatics exercise program of WATERinMOTION

Warm-up (5 min)		
Gradual training of the body and mind with cardio moves to enter the main phase of the exercise program		
Main exercise (45 min)		
Stage 1	Upper-body suspension	Focusing on the upper body muscles with different levels of exercise using the next song.
	Lower-body suspension	Improving the range of motion, increasing the heart rate slowly, strengthening the cardiovascular system and balance using aqua dumbbells, and exercising the muscles of the buttocks, thighs, hips, ABS, and back through resistance training.
	Core strengthening	Exercising the abdominal and back muscles.
Stage 2	Upper-body suspension	Increasing the strength, tone, and endurance in the muscles of the chest, back, biceps, triceps, and shoulders using one or both aqua dumbbells.
	Lower-body suspension	Continuing the resistance training and increasing the heart rate and core temperature again, using the aqua dumbbells and high-intensity, low-impact movements.
	Core strengthening	Returning to the power centre and enhancing tone and tighten the middle section of the body with exercise.
Stage 3	Flexibility	Enhancing the mobility and flexibility of the whole body with the last sports music track.
Cool-down (5 min)		
Easy stretching and relaxation exercises		

#### Body measurements

Participants' anthropometric measures of body weight in kilogram (kg), height in centimetres (cm), and body composition obtained before starting the first aquatic exercise session by the research teams, and their BMI was computed from this data. Both pre/post measures of participants' weight (with a Scale-Tronix model 5002, Wheaton, IL, USA) and height (with a stadiometer) were conducted with the accuracy of 0.1 kg, and 0.1 cm, respectively (Rezaeipour & Apanasenko, 2019). These measures were taken by participants dressed in light clothing and without footwear. The BMI formula is "the weight of a person in kilogram, which is divided into the height squares in meter" (CDC, 2017). A BMI of 25.0 or higher is defined as overweight, and a BMI of 30 or more is defined as obese (CDC, 2017). The WC measurement was carried out in the horizontal plane at the middle point between the crest of the ilium and the last rib at the

end of a normal exhalation (Rezaeipour & Nychyporuk, 2019).

#### Analysis of body composition

The composition of the body was estimated by tetrapolar BIA (BIA 310A, Biodynamics, USA), which gives data on total body water (BW), fat-free mass (FFM), and fat mass (FM) (Rezaeipour, 2020; Rezaeipour & Apanasenko, 2019). The reliability of these data are accepted (Mainenti et al., 2011). Before the BIA test, these recommendations were emphasized for each person: do not drink alcohol or caffeine for 48-hour before the test; do not participate in physical exercise within 24-hour before the test; do not consume food or beverages before the test; do not take diuretics for seven days before the beginning of the evaluation (Pereira et al., 2018). The FFM prediction was conducted using data provided by the BIA based on the equation of Gray et al. (Gray et al., 2018) for the elderly, which is

as follows:

FFM (in Kg) = 0.00151 (height<sup>2</sup> in cm) - 0.0344 (resistance in ohms) + 0.14 (body weight in Kg) - 0.158 (age in full years) + 20.387; FM (in kg) = body weight - FFM.

To control eating habits and drink intake, a diary was used, which had previously been approved for use in households (Rezaeipour, 2020; Rezaeipour & Apanasenko, 2019). Participants were instructed to complete their dietary records within 24-hours of four days (three days a week and weekend day), and the same method was carried out immediately following the study to report altered eating habits and drink intake during the study period (Rezaeipour, 2020; Rezaeipour & Apanasenko, 2019). All participants were asked to maintain their dietary habits, drink intake, and leisure-time physical activities throughout the study. Each of the participants was tracked every two-week.

#### Statistical analysis

Data entry and statistical analysis were utilizing IBM SPSS 21.0 (SPSS Inc. USA) for Windows. Normally distributed of

data was verified using the Shapiro-Wilk test and expressed in the form of mean values  $\pm$ SD. Comparing the pre- and post-study was carried out using research data analysis with paired samples t-test and a Bonferroni correction. Comparisons between groups were carried out using research data analysis by Analysis of Covariance (ANCOVA). The results were corrected using the Bonferroni correction. The mean differences were statistically significant if the P-values were less than 0.05. Bonferroni correction was computed by dividing alpha by the number of analyses performed. The statistically significant levels decreased from 0.05 to 0.008. Linear regression analysis was applied to study a relation between all significant (dependent) variables and changes in body weight (independent).

#### Results

No side effects and dropouts were reported during the study. Forty-four participants who met the study criteria completed the study. Table 2 shows the variables evaluated in the study groups pre- and post-study.

**Table 2.** Mean of the Studied Groups' Variables Pre- and Post-Study

Variables	WATERinMOTION Group		Control Group		P ANCOVA#
	Group Mean Pre-Study	Group Mean Post-Study	Group Mean Pre-Study	Group Mean Post-Study	
Body weight (kg)	85.5 $\pm$ 13.8	84.8 $\pm$ 14.3*	85.9 $\pm$ 15.3	86.1 $\pm$ 15.1	< 0.001
BMI (kg.m <sup>-2</sup> )	33.4 $\pm$ 5.5	33.1 $\pm$ 5.8*	33.5 $\pm$ 5.8	33.6 $\pm$ 5.7	< 0.001
TBW (L)	35.5 $\pm$ 6.4	35.3 $\pm$ 6.4	35.6 $\pm$ 6.9	35.7 $\pm$ 6.8	0.005
FM (kg)	35 $\pm$ 7.8	34.4 $\pm$ 8.1*	35.2 $\pm$ 8.5	35.3 $\pm$ 8.4	< 0.001
FFM (kg)	48.5 $\pm$ 8.9	48.5 $\pm$ 9.1	48.7 $\pm$ 9.6	48.9 $\pm$ 9.4	0.003
WC (cm)	87.2 $\pm$ 9.3	87 $\pm$ 8.79	87.6 $\pm$ 10.1	88 $\pm$ 10.1	0.007

Note. Data are reported as mean $\pm$ SD; BMI: body mass index; TBW: total body water; FM: fat mass; FFM: fat-free mass; WC: waist circumference; \*: P-value < 0.008 in comparison with pre-study; #: Differences between groups.

As can be seen from Table 2, differences between the groups of the studied variables were not significant at pre-study ( $P > 0.05$ ). From the point of view of time (pre- and post-study), significant interaction influences were found in body weight (-0.7,  $P = 0.004$ ), BMI (-0.3,  $P = 0.002$ ), and FM (-0.6,  $P = 0.003$ ) of the WATERinMOTION group. Moreover, the comparison of groups at post revealed a significant difference in all the variables studied (Table 2).

The regression analysis disclosed a significant relationship between BMI, FM, and changes in body weight. The regression coefficient in predicting the body weight variable was 0.342 ( $P = 0.001$ ) and 0.685 ( $P < 0.0001$ ) for BMI and FM, respectively.

#### Discussion

The present study examined the short-term effects of the WATERinMOTION aquatic exercise program without nutritional monitoring on body weight and composition in sedentary overweight and/or obese older women; it has shown that there is a link between the WATERinMOTION programme and significant alterations in body weight and composition.

Kirsten et al. (2017) carried out a six-week program of the water aerobics and concluded that aerobic exercise in water could reduce body weight and FM in the middle-aged population. Penaforte et al. (2015) carried out an aquatic exercise programme that lasted two months with three weekly sessions in obese older women and observed a significant decline in body weight, BMI, and FM. Pereira et al. (2018) conducted an aquatic exercise program (12 weeks) in older adults and

recorded reduced body weight and loss of FM. Rezaeipour and Nychyporuk (2019) conducted a 12-week water aerobics programme (three-session a week) in postmenopausal females and reported a significant reduction in weight loss parameters. Furthermore, other studies by Rezaeipour (2020) and Rezaeipour and Apanasenko (2019) on pool workouts for three months revealed a significant decline in body weight, BMI, and FM.

In contrast, Rica et al. (2013) conducted three sessions per week of a water aerobics programme for 12 weeks in obese older women. They did not see any changes in body composition parameters, but improvements in all function parameters such as arm flexion, walking time, and quality of life were reported. Siqueira et al. (2017) found no changes in the body composition of women with rheumatoid arthritis who participated in 16 weeks supervised programme comprising various types of water exercise in the form of three weekly sessions. Charmas et al. (2019) carried out swimming training, which lasted twelve weeks with three-time per week, in young women; no changes were found in body weight and FM.

As can be observed, disagreement about the influence of aquatic exercises on body weight and composition remains. The novel finding of our study is that body weight, BMI, and FM were decreased while surprisingly the duration of the WATERinMOTION program is about four weeks compared to the standard six, twelve, or more. The significant associations found in this study with reduced body weight, BMI, and FM support the study hypothesis and suggest that using a WATER-

inMOTION programme can yield some of the desired results on body weight and composition parameters for clinical use and show that it can be used clinically to achieve the desired results within a shorter time frame. Also, the WATERinMOTION program can be used as an alternative form of physical conditioning because of the benefits of water buoyancy and a reducer of obesity impacts.

Any type of exercise that leads to calorie-burning increases the likelihood of alterations in body composition. Water aerobics does this with internal resistance to water viscosity and creates pressure in any movement (Tamin & Loekito, 2018). Thus, the moderate weight loss experienced during the WATERinMOTION aquatic exercise was most likely induced by a lack of compensatory increase in calorie intake to match energy expenditure during training. In fact, from the findings of this study, it can be inferred that maintaining a calorie deficit with an aquatic exercise programme will promote a positive impact on the composition of the body.

Moreover, blood flow and bone density during muscle activity are affected by FMM and are directly related to the ability to exercise (Lee & Oh, 2015). This study showed that there was no statistically significant difference in FMM and the absence of a significant reduction in this parameter may be due to the shorter duration of the WATERinMOTION program than other studies.

Obese people, even when exercising in water, have problems with mobility and overcoming water resistance (Penaforte et al., 2015; Rezaei pour, 2020; Rezaei pour & Apanasenko, 2019). Thus, to obtain a more significant effect on body weight and on other variables under study, the intensity and speed of activity may not be enough, because both are directly connected to exercise outcomes. Increased WC due to visceral fat may be a risk factor that causes many disorders, such as arthritis and cardiometabolic (Carr et al., 2004). The result of WC was positive but insignificant for the WATERinMOTION group. Another study conducted on older people showed a greater reduction of the WC when water-walking and also of longer duration (24 to 48 weeks) (Naylor et al., 2020), which can explain its better result than the current one.

Although the results of the project seem promising, caution should be advised concerning various limitations. The study used a convenience sample of older women, and generalizations are thus limited, and these results may not be seen outside older women due to gender and age-related metabolic differences. The four-week time frame of the WATERinMOTION aquatics exercise programme and its isolated use without nutritional monitoring to assess changes in body weight and composition may have been other limiting factors for the programme's effectiveness. Also, this study was not able to provide a comparability analysis of ground exercises or other water exercise programs.

More research in the future may examine the WATERinMOTION aquatics exercise program to support its influences on body composition and other additional health benefits. Suggestions for future studies include the use of randomized controlled trial assignment and sampling in such a way that the population under study had the chance to be selected. It is also advisable to use a larger sample and a greater duration of exercise (more than one month). The study of the WATERinMOTION aquatics exercise without nutritional monitoring is also recommended in other sexes and ages with a sedentary lifestyle. Moreover, studying the diet-restricted WATERin-

MOTION is encouraged for overweight/obese sedentary older women and/or other genders and ages. Additional health data collection, such as resting heart rate and cardiometabolic risk factors before and after studying an aquatic exercise programme, may also be useful to examine.

In conclusion, despite the sessions being held only twice a week for one month, the WATERinMOTION aquatics program, which included no nutritional monitoring, showed significant benefits for losing weight and body composition of sedentary older women who are overweight and/or obese. The results of this study will enable health professionals, such as physicians and trainers, to recommend the WATERinMOTION to their patients who cannot easily exercise on the ground to help them achieve to their physical fitness and health goals.

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# The Effects of Aquatic Watsu Therapy on Gross Motor Performance and Quality of Life for Children with Cerebral Palsy

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## Abstract

The purpose of this study was to compare the effects of Immersion and Watsu<sup>®</sup> therapy protocols on children with cerebral palsy. Gross motor function (GMF), spasticity (MAS), and Quality of Life (QoL) parameters of twenty-three children (age: 7.5±2.8, BMI: 17±3.7) were measured. Subjects received Watsu<sup>®</sup> therapy and Immersion protocols in Watsu-Immersion (W-I, n=12) and Immersion-Watsu (I-W n=11) groups in different periods based on a cross-over design. The subjects received sessions for 30 minutes twice a week during 10-week experimental periods. The results were investigated with independent sample t-test and Wilcoxon rank-sum tests, which showed that there was no evidence of carryover effects in GMFM 88 and QoL. In contrast, Watsu<sup>®</sup> improved GMFM 88 ( $p<0.05$ ), Quality of Life ( $p<0.05$ ), and MAS-Upper spasticity ( $p<0.05$ ), scores significantly compared to immersion. The current results demonstrated the specific benefits of Watsu<sup>®</sup> therapy on children with CP, confirming the previous anecdotal reports. We recommend Watsu<sup>®</sup> as a safe and well-accepted complementary intervention for the management of CP.

**Keywords:** Watsu<sup>®</sup>, cerebral palsy, immersion, motor function, quality of life



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## Introduction

Cerebral palsy (CP) is the most common cause of childhood-onset, lifelong motor disability with the prevalence rate of about 2.1 per 1,000 live births in developed countries. The Center for Disease Control and Prevention stated that 1 in 321

children had been classified with CP, and it is found to be more common in boys than in girls (CDC, 2018).

As a result of non-progressive brain lesions, developmental neuromuscular and musculoskeletal disorders in CP are the result of neural impairments deteriorating the homeostat-

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ic state, the capacity for participation and the quality of life (QoL) (Hadders-Algra, 2014).

The constraints in CP are reduced joint mobility, contractures and bone deformities, abnormal motor control, and dependence on external support in daily life (Blair & Cans, 2018). Although physical and occupational therapy improves functional abilities at various stages of development, motor impairment, abnormal movement patterns, and postural deformities can deteriorate due to the lack of interventions over time (Blair & Cans, 2018). The coordinated and multidisciplinary management of CP focuses on the QoL to improve neuromuscular and musculoskeletal complications and prognosis (Hadders-Algra, 2014).

CP can cause a dramatic decrease in the physical activity level. Neuro-motor, socio-emotional, cognitive, and musculoskeletal conditions are the area of focus in the increasing number of aquatic studies (Carayannopoulos et al., 2020; Schitter et al., 2020). Many aquatic interventions are being investigated due to its feasibility and minimum adverse effects (Roostaei et al., 2017). In addition to the many other benefits observed, aquatic interventions have improved functional motor skills, mobility, strength, tension in the muscles, and inflammation (Choi & Cho, 2019; Lai, 2014).

Relatively new in the history of aquatic therapy, Watsu, is a hands-on, client-centred approach that seeks to improve relaxation, muscle tone in the sense of security, and promote trust in which the client can surrender him/herself to the water and the therapist. Watsu is recognized in rehabilitation for the variety of conditions (Brody & Geigle, 2009) including fibromyalgia, neurologic conditions, physical function, chronic disabilities, and lower back pain (Schitter et al., 2020). The gentle Watsu mobilization can enhance body awareness (Schitter & Fleckenstein, 2018). Although, its practicality, time and cost-efficiency benefits, Watsu is one of the least investigated forms of aquatic intervention; therefore, generalizing of the results for the variety of disabilities impossible.

The challenge in CP is the low level of QoL associated with gross motor dysfunction. QoL determines the physical and psychological wellbeing, self-perception, and pain levels. It can be improved with therapeutic and motor control interventions (Muñoz-Blanco et al., 2020).

Spasticity is expressed as a velocity-dependent increase in muscle tone, hyperreflexia, and the presence of primitive reflexes that can result in abnormal posture, delayed motor development, and atypical gait patterns (Li & Francisco, 2019). Neurological disorders can change the muscle tone dramatically. Holistic aquatic intervention studies on spasticity can provide insight for the aetiology and contribute to forming better multimodal treatment settings.

In brief, cost-effective modalities are essential in CP due to lifelong expensive health care. Therefore, we compared and evaluated the effectiveness of Watsu and Immersion on QoL, motor function and spasticity in CP.

## Methods

The study was conducted in two private rehabilitation facilities that provide health care and social services to disabled children in Istanbul, Turkey. A crossover design was conducted in comparison to the effect of Watsu and Immersion. All subjects were assigned to either Watsu-Immersion (W-I) or Immersion-Watsu (I-W) groups, according to aged-stratified randomization.

The standardized manual was used to perform gross motor function measurement (GMFM) 88, MAS and CP QoL, Version July 2, 2013, Primary Caregiver Questionnaire 4–12 years) tests at baseline and upon completion of the periods. We used CP QoL Child, an assessment to obtain insight into several aspects of children's lives (Waters et al., 2013) as a parent-reported QoL assessment correlating with the impairment (Arnaud et al., 2008). The GMFM 88 is an observational clinical tool designed to assess the level of impairments, motor skills and evaluate the improvement more accurately than GMFM 66 (Ko & Kim, 2013). The validity (Josenby et al., 2009) and reliability (Russell et al., 2000) of GMFM-88 were shown. The most common spasticity measure, modified Ashworth scale (MAS) was used. It is a six-point rating scale developed as a simple clinical classification to assess muscle tone (Ward et al., 2016).

The Deanship of Scientific Research at King Fahd University of Petroleum and Mineral, Dhahran, Saudi Arabia, approved the research protocol.

## Subjects

We assigned children with no seizures in the month before the study into two groups based on aged-stratified randomization with no significant differences in their descriptive data. There were 11 female and 12 male subjects aged 4–12 (N=23, age  $7.52 \pm 2.78$ , weight  $17.29 \pm 4.03$ , height  $94.83 \pm 11.26$ , BMI  $19.04 \pm 1.62$ ). Informed consent was obtained from all the subjects included in the study. Their parents provided informed consent forms and confirmed the participation of their children for the publishing of the outcomes. The subjects with no systemic condition finished their health check-up before the study. The gross motor function classification system (GMFCS) levels and type of CP (topographic distribution) were shown (Table 1). The subjects were able to understand basic verbal commands and report sensitivity to pain.

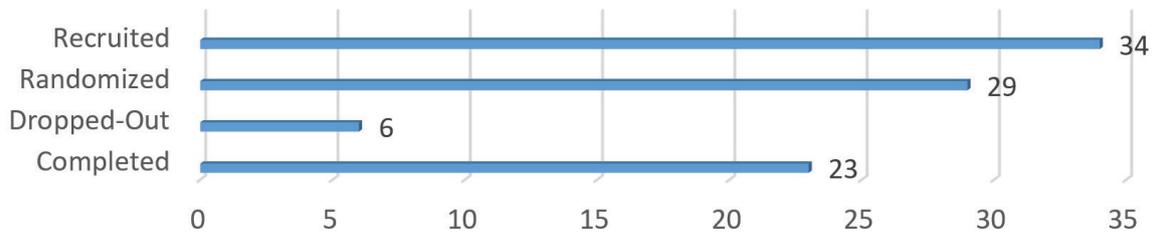
**Table 1.** The GMFCS levels and topographic distribution of the subjects

Groups	N	Gender		Age	Limb distribution		GMFCS	
		F	M		Diplegia	Hemiplegia	I	II
W-I	12	7	5	7.92 (3.11)	1	11	6	6
I-W	11	4	7	7.09 (2.42)	1	10	5	6

## Exclusion Criteria

The exclusion criteria were the previous surgical procedure for saliva control, drugs taken influencing saliva secretion and cardiac modulation at least 15 days before the test for the study. Subjects with pathologies associated with infectious or viral states,

inability to maintain an orthostatic position, medical history of cardiac diseases and any diseases known to affect the autonomic cardiac function (neurological, endocrine disorders) were not involved. Absenteeism and fatigue were the reasons for exclusion after the beginning of the experimental phase (Figure 1).



**Figure 1.** The total numbers of participants during the course of the study

*Experimental Design*

Experts used the standardized manual to perform GMFM 88, QoL and MAS, tests at baseline and upon completion of the periods. W-I group (n=12) received Watsu, whereas I-W group (n=11) received immersion in the first period (Figure 2). No aquatic intervention therapy, medications, or surgical procedures were applied in the six weeks of washout interval between the periods.

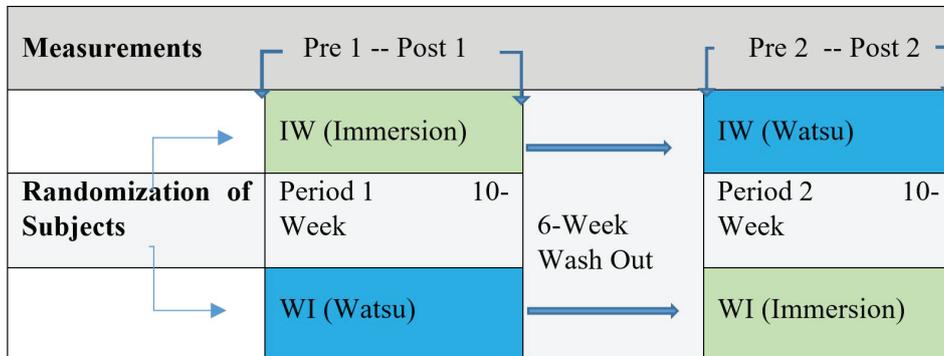
In the second period, the groups went from receiving Watsu to Immersion and from Immersion to Watsu therapy. Two certified practitioners experienced in the health care of children were in charge of the application of the adapted Watsu.

*Intervention Protocol*

In the Watsu therapy protocol, the bodies of the children were manipulated, stretched, and mobilized in the form of gentle twists, rocking and cradling movements together with massage and pressure point work. The 30-minute Watsu or immersion sessions were applied twice a week for ten weeks.

Both groups received Watsu and immersion in different periods. The sessions consisted of spine-rotating techniques, such as slow offering, leg offerings, spiral offering, free spine, accordion, rotating accordion, twist-over, corner-spread and chest-opening, seaweed. The Watsu therapists started with less impaired and less stiff side of the children with gentle support from under knees to keep the hips and knees flexed. They focused first on spinal movements progressing to the limbs and avoided stretching multiple joints into extension at the same time.

A constant intention was given to performing traction and rotational movements considering the limitations of children. In immersion, the back of the subject’s head and neck was supported by specific floating equipment allowing the rest of the body to remain in the water. No technique was applied while children were closely observed. The temperature of the pool water and air were 33°C and 28°C, respectively. The post-intervention data collection was identical to the baseline procedure.



**Figure 2.** The cross over design for this study

*Statistical Analysis*

The sum of the measured values in both periods for each subject was calculated and compared with the independent sample t-test to clarify the effectiveness of the washout period in eliminating any carryover effect. In the case of significant results, no comparison was performed between the therapy differences. The therapeutic effects were analysed using the first period values. Paired t-tests and Independent t-tests were used to compare the difference within and between the groups. In the case of no carryover effect, the effects were evaluated based on

the difference between the two therapies and the independent sample t-test was used. Wilcoxon rank-sum test and test procedures similar to those mentioned above were used in order to analyse ordinal data. The level of significance for all tests was set at 5% (p < 0.05).

**Results**

The subject’s descriptive data (whole sample) are presented in Table 2. None of their physical characteristics differences was significant (p>0.05).

**Table 2.** Physical characteristics of the groups - Mean (SD)

Groups	Age (year)	Weight (kg)	Height (cm)	BMI (kg/ m <sup>2</sup> )
W-I (n=12)	7.92 (3.12)	17.92 (4.56)	96.58 (12.52)	18.99 (1.77)
I-W (n=11)	7.09 (2.43)	16.61 (3.44)	92.91 (9.95)	19.09 (1.53)
Total (N=23)	7.52 (2.78)	17.29 (4.03)	94.83 (11.26)	19.04 (1.62)

Watsu improved GMFM 88 significantly compared to immersion ( $t=6.698, p=0.000$ ). No evidence of relevant carryover effects in GMFM 88 ( $t=-1.386, p=0.180$ ) was found, (Table 3).

Watsu improved QoL significantly compared to Immersion ( $t=2.212, p=0.038$ ). No relevant carryover effects were seen in QoL ( $t=1.555, p=0.135$ ), (Table 4). Upper Spasticity after Watsu was significantly better compared to Immersion ( $Z=-2.222, p=0.026$ ). No carryover effect was seen in Upper Spasticity ( $Z=-1.125, p=0.261$ ). Watsu improved the lower spasticity more than immersion did ( $Z=-1.025, p=0.305$ ). There were carryover effects ( $Z=-2.009, p=0.045$ ), (Table 5).

**Table 3.** GMFM 88 measurement results of the subjects - Mean (SD)

Groups	Gross Motor	Period 1		Period 2	
W-I	GMFM 88	0.037 (0.011)	0.039 (0.010)	0.037 (0.011)	0.038 (0.010)
I-W	GMFM 88	0.031 (0.010)	0.032 (0.009)	0.031 (0.010)	0.033 (0.010)

**Table 4.** Quality of life questionnaire results of the subjects - Mean (SD)

Groups	Quality of Life	Period 1		Period 2	
W-I	QoL	64.78 (2.81)	65.42 (2.57)	64.75 (2.83)	64.92 (2.84)
I-W	QoL	64.36 (3.61)	64.45 (3.45)	64.36 (3.44)	64.55 (3.45)

**Table 5.** Modified Ashworth Scale scores of the subjects - Mean (SD)

Groups	MAS	Period 1		Period 2	
W-I	Upper Spasticity	1.47 (1.35)	1.42(1.33)	1.47(1.35)	1.47 (1.35)
I-W		2.09 (.86)	2.06 (.89)	2.09 (.86)	1.97 (.91)
W-I	Lower Spasticity	2.15 (.84)	2.06 (.89)	2.12 (.88)	2.12 (.88)
I-W		2.57 (.91)	2.57 (.91)	2.54 (.90)	2.30 (.91)

**Discussion**

The initial evidence for the effect of Watsu on GMFM 88, QoL and MAS indices were provided by this current study (Table 3, 4, 5), in line with previous studies suggesting the benefits of aquatic therapies on various areas of functioning (Roostaei et al., 2017; Lai et al., 2014).

The varying intervention approaches ranging from passive to active and physical to psychological are needed to provide sufficient amounts of afferent input (Scott et al., 2020). Thus, the altered visual, tactile, proprioceptive and vestibular inputs in Watsu therapy may have played a critical role in the reorganization of deteriorated sensory, neuromotor skills, which form the basis for motor functioning and muscular tonus improvements (Tables 3, 5). Also, the activated proprioceptive track, neuromuscular and afferent C-tactile fibre stimulation in warm water can reinstate the earlier stages of ontogeny, reducing motor function constraints (Barassi et al., 2018). Vestibular input and activation of afferent C-tactile fibres can also alleviate hypertonia that results in higher GMFM 88.

Moreover, sensory-motor inputs due to the enriched environment and bodywork could have improved not only GMF but also emotional regulation in CP (Meireles et al., 2017; Lai et al., 2014). Thus, integrated Watsu movements used to improve the individualization of the sessions and the thermal conductive properties of water can meet the needs in specific areas, including muscular tonus reduction (Table 5). As tonus reduces, the therapist could perform techniques more efficiently that can provide a higher quality of tactile and proprioceptive input with increased range of motion. This increase in ROM and efficiency can optimize body structures and re-educate the internal reference systems (Novak, 2014).

The kinesthetic sensations in Watsu from stretches, flexion and extension of the limbs and the torso, traction and rotation

of the spine are critical for the control of movement and can improve conscious influences on motor function, thus, GMFM 88 (Meireles, 2017). Watsu may have enhanced the accordance between proprioception and kinesthesia. Moreover, the use of a single leg or arm can improve awareness of laterality, which in turn, can improve motor output.

The close presence of the trusted therapist can likely cause feelings of security and relaxation, which can result in the ease of expressions of the feelings. The reduced protective muscle guarding and tonus might be the reflection of this potential. Therefore, the increased vestibular, tactile, and proprioceptive input to the cortico-limbic system in the form of mobilization of joints and manipulation and elongation of muscles might have contributed to the reduction in upper spasticity (Table 5). We believe that altered cardiac afferent input has played a role in the reduced tonus (Amichai & Katz-Leurer, 2014). Thus, the majority of techniques provided while two torsos are in close contact could have enhanced the sensory-motor afferent input improving spasticity. Moreover, a specific Watsu session can be implemented with a focus on either upper or lower limbs, considering the need of each child. Therefore, better spasticity levels and independence, adaptive behaviours, social participation and QoL might be achieved.

Many variables determine QoL (Noor et al., 2015), which requires a multidisciplinary approach (Novak, 2014). However, improved QoL implies the multidimensional wellbeing efficacy of Watsu (Table 4). The activation of afferent sensory visceral inputs to the cortico-limbic system and regulation of somatic efferent neuronal motor pathways are linked to the regulation of emotional state (Kinner et al., 2014), thereby, QoL. In the current study, the increased QoL might be rooted in the altered sensorimotor experience in the form of gentle movements, stretching, and trigger point work in

Watsu (Dull, 2008).

Nevertheless, QoL is linked to the psychosocial development of children and psychological stress that require a proper parental attitude (Al-Dababneh & Al Zboon, 2018; Arnaud et al., 2008). Parental dysfunction and stress can lead to the severity of the disorder and additional problems including, parents' reduced psychological adjustment, the mothers' QoL, and the way in which parents report QoL of their children (Noor et al., 2015). Families need to direct their attention to their personal needs in order to maintain day-to-day management of their responsibilities. The family members can also join and receive the therapy with their children to release parental stress while contributing to a variety of socio-emotional gains for their children that may improve QoL.

Moreover, the desired results require parental support as much as therapists do. Family-centred intervention services in health care can contribute to greater independence at a low-cost and time-efficient way. Intensive training is recommended for providing a variety of relaxing and emotional quality of touch, considering Watsu applications in the home environment. However, the families can apply the basic form of the therapy after having entry-level training. Therefore, the more engaged the families are, the better the understanding of the children's capabilities the families have. As a result, the family-centred care environment in improving motor function and QoL are supported (Kokorelias et al., 2019).

The results illuminated the potential extrapolations for future studies to achieve functional and structural adaptations using Watsu. Therefore, we suggest Watsu, as a versatile therapy to be incorporated in the aquatic rehabilitation programs for CP, considering no common side effects and family engagement possibilities. Nevertheless, establishing well-rounded physiotherapy practises from our study is limited.

## Conclusion

The current study provides initial evidence in favour of Watsu over immersion and suggests it as a complementary method to improve motor functions, QoL and spasticity in CP. This initial evidence can form a basis for the future large-scale studies comparing the efficacy of Watsu and its subdivisions with other aquatic interventions to achieve a higher QoL and enhanced motor function. In this regard, despite the promising findings of the current study, further research is needed to investigate the potential of Watsu on more diverse samples in the form of isolated therapy for individuals and community-based group therapy. The respiratory deficiency, emotional regulation, and participation or community integration can also be investigated considering the potential influence on physiologic and psychological parameters. Thus, the confirmed versatility of Watsu can help health professionals to utilize Watsu in different pediatric aquatic therapy protocols more efficiently. Lastly, longer follow-up periods and randomized controlled design studies are needed in future studies to confirm the effect of Watsu across age categories and GMFCS levels.

There are several limitations to this study. First, the possible influences of the diet were not evaluated. Second, the Water Orientation Test Alyn2 for aquatic skills assessment was not used. Third, parental stress and QoL were not measured simultaneously. Fourth, follow-up measurements were not performed.

Finally, the holistic approach of Watsu can enrich inter-

disciplinary CP treatment in water. Participation of individualized Watsu sessions may be a more attainable and attractive goal than taking conventional aquatic therapy.

## Acknowledgement

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# Supplementation with Ziziphus Jujuba Suppresses Apoptosis Signals in Neutrophils after Acute Exercise

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## Abstract

It is suggested that jujube might benefit exercise-induced immune fluctuations, specifically on neutrophils' apoptosis regulation, but its cellular mechanism is unclear. This study aimed to investigate the effect of one-week supplementation with Ziziphus jujuba on pro- and anti-apoptotic protein levels of neutrophils in response to a session of circuit resistance exercise. Fourteen young, healthy male students completed a session of circuit resistance exercise (75% 1RM, nine exercises, three sets) in two groups (n=7). While one group received a placebo, the other group was supplemented with jujube (0.5 g/kg body weight suspended in 2.5 cc distilled water) started a weekday before the exercise session. Blood samples were collected 30 minutes before, immediately, and two hours after the exercise. Neutrophils were collected and pro- (Calpain-1, Bax, Caspase-3) and anti-apoptotic (Calpastatin, XIAP) proteins measured with ELISA. Intracellular calcium ([Ca<sup>2+</sup>]<sub>i</sub>) was assessed using the Atomic Absorption/ Flame Emission method. Repeated-Measures ANOVA was used for the interaction effects of TIME×GROUP (3×2) at the significance level (p) of 0.05. The SPSS software was used for analyses. Levels of ([Ca<sup>2+</sup>]<sub>i</sub>), expression of calpain-, and caspase-3 were increased in response to circuit resistance exercise (p<0.05). In contrast, supplementation with jujube suppressed these changes (p<0.01). The data indicate that a single session of intensive circuit resistance exercise elevated apoptosis signalling in human neutrophils with the involvement of [Ca<sup>2+</sup>]<sub>i</sub>-Calpastatin-Calpain axis upstream caspase-3. Supplementation with the jujube solution attenuated cell death signalling, possibly by providing energy for neutrophils. Otherwise, the improvement of the anti-oxidant status might be protective against ROS-induced apoptosis during exercise.

**Keywords:** exercise, resistance exercise, ziziphus jujuba, neutrophil cell, cell apoptosis, intracellular calcium



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ZIZIPHUS JUJUBA SUPPRESSES NEUTROPHILS' APOPTOSIS AFTER EXERCISE

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## Introduction

Neutrophils are an essential part of the innate immune system (Ermer et al., 2013) that are the first responders during the beginning of inflammation, mainly as a result of

bacterial infection, environmental stress (Jacobs et al., 2010), and exercise (Kruger & Mooren, 2014), which are mobilized and migrate towards the sites of inflammation. A single bout of high-intensity exercise causes a marked increase in blood

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leukocyte counts, mainly instigated by the mobilization of neutrophils from the marginal into the circulating pool (Lagranha et al., 2008).

Clinically, exhaustive exercise is followed by a transient impaired immune response called an "open window." During this time, it has been shown that the susceptibility to infections is increased (Lagranha et al., 2005). It is suggested that this phenomenon may result from temporary impaired neutrophil functions and apoptotic cell death (Lagranha et al., 2007). There are conflicting data about the effects of exercise on neutrophil apoptosis. Recently, it has been found that resistance exercise with moderate intensity did not affect neutrophil apoptosis, while intensive resistance exercise, as well as marathon running, decreased the rate of apoptotic neutrophils. Concentric and eccentric treadmill exercise also delayed neutrophil apoptosis during the recovery period (Mooren et al., 2012). In contrast, it has been demonstrated that acute severe exercise-induced an oxidative state in neutrophils resulted in the acceleration of spontaneous neutrophil apoptosis (Syu et al., 2011).

The mechanisms of neutrophil apoptosis during exercise are not completely understood. Previous studies have shown that a single session of exercise induces DNA fragmentation, while mitochondrial membrane depolarization elevates the expression of the pro-apoptotic gene (bax and bcl-xS) and depresses the expression of the anti-apoptotic genes (bcl-xL) in rat neutrophils (Lagranha et al., 2005; Lagranha et al., 2004). Similarly, increased p53, caspase-3 expression, and p38 MAPK/JNK phosphorylation were shown (Lagranha et al., 2007). However, more recent studies reported beneficial effects of various types of exercise and training on the neutrophils' function and apoptosis in sedentary men (Chen et al., 2018), diabetic patients (Borges et al., 2019) and review of the literature (Chuong et al., 2019); but they did not mention the Bcl-2 family of apoptosis-signalling proteins. A recent study that was modelling the balance between apoptosis and necrosis in neutrophils, but not in exercise and training (Presbitero et al., 2019) explains some mechanism of neutrophils' apoptosis, but there is no data about the Bcl-2 family and calpastatin-calpain-calcium axis.

Regarding anti-apoptotic proteins, not in exercise and training, spontaneous neutrophil apoptosis is known to be regulated by a decrease of calpastatin. Reducing calpastatin levels will cause the constantly active calpains to cleave Bax into an active fragment and deactivate XIAP (Geering & Simon, 2011). It was also reported that calpastatin and calpain-1 show critical proximal elements in a cascade of pro-apoptotic events, result in Bax, mitochondria, and caspase-3 activation. The changed expression affects the life span of Neutrophils under pathologic conditions (Altzner et al., 2004). To date, no data have been published about the regulation for calpastatin during exercise. As calpastatin and calpain are calcium-dependent proteins, the involvement of the calpastatin-calpain-calcium axis is likely. Therefore, it is an interesting area to be studied.

Several herbal supplements have been tested to blunt the detrimental effects of exercise on the immune system and neutrophil responses. While supplementation of hydrolysed whey protein enriched with glutamine dipeptide before exhaustive treadmill exercise had no effect (Cury-Boaventura et al., 2008), glutamine supplementation prevented exercise effects by reducing neutrophil apoptosis (Lagranha et al., 2005; Lagranha et al., 2007; Lagranha et al., 2004).

*Ziziphus jujuba* Mill. or the jujube plant, is widely culti-

vated, from southwest Europe to China, India, and the Middle East (Gao et al., 2012), especially in Iran (Rodríguez Villanueva & Rodríguez Villanueva, 2017). It may be considered a functional food, having both nutritional and medicinal uses (Gao et al., 2012). Phytochemical studies showed that jujube fruits have various constituents, such as triterpenic acids (Lee et al., 2003), cerebrosides, flavonoids (Chen et al., 2013), nucleosides (Guo et al., 2013), phenolic acids (Du et al., 2013; Wang et al., 2011), sugars (Li et al., 2013), and amino acids (Guo et al., 2013).

Several studies have confirmed that jujube displays many critical biological properties, including anti-oxidative, neuroprotective, anticarcinogenic, anti-inflammatory, immunomodulatory, cardio-, hepato-, and gastrointestinal protective activities, thus supporting the putative health benefits deriving from its consumption (Plastina, 2016). Accordingly, the jujube might be beneficial for exercise and its acute immunologic consequences, such as neutrophil apoptosis. In previous studies, we demonstrated that one-week supplementation of jujube solution delayed neutrophil apoptosis-induced by an intensive circuit resistance exercise (Tayebi et al., 2014). However, the underlying mechanisms remain unknown.

The current study aimed to investigate the effect of one week of supplementation with jujube solution before a single session of intensive circuit resistance exercise on protein expression of caspase-3, calpastatin, calpain-1, Bax, XIAP, and  $[Ca^{2+}]_i$  in human neutrophils.

## Methods

### Participants

The present study was approved by the Research Ethics Committee of the Tarbiat Modares University of Medical Sciences and was conducted by the policy statement of the Declaration of the Iranian Ministry of Health that is following the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from 14 healthy and young male students. All subjects were requested to complete a medical examination to ensure that they had not taken any regular medication, smoked, consumed alcohol, or taken any regular exercise in the past two months. They also had to state that they are free of cardiovascular or metabolic diseases or recent upper respiratory tract infection symptoms in the month before the start of these tests. The volunteers were randomly assigned to two groups (n=7), including a Circuit Resistance Exercise group with placebo (age: 25±3 years, height: 171±2 cm, weight: 67.5±4.9 kg) and a Circuit Resistance Exercise group (n=7) with jujube solution (age: 25±1 years, height: 180±4 cm, weight: 74.1±5.8 kg).

### Measurement of exercise capacity

Participants were weighed three times before the main trial. A strength test was performed at first and second visits to determine one repetition maximum (1-RM) of all participants for each of the nine resistance exercises employed in the study. 1-RM value was determined by adding or removing weights after each attempt. Subjects were permitted to take as much time as they felt necessary for each attempt. A practice session was completed to ensure that each participant could complete the whole exercise session on the third visit and confirm that weight lifting was inducing fatigue by the end of the session. This was confirmed by visual and verbal feedback from participants. Records of 1-RM are presented in Table 1.

**Table 1.** Exercise's 1-RM Records of Participants

Variables (lb)	groups	Median	Minimum	Maximum
crunch	placebo	134	90	154
	Jujube solution	118	104	171
back extension	placebo	155	139	292
	Jujube solution	178	130	660
biceps curl	placebo	48	38	78
	Jujube solution	65	41	72
triceps press	placebo	51	48	78
	Jujube solution	60.5	48	78
knee extension	placebo	152	98	213
	Jujube solution	169	147	218
knee curl	placebo	104	52	128
	Jujube solution	98	60	180
standing calf raise	placebo	162	85	211
	Jujube solution	169.5	123	199
chest press	placebo	69	65	90
	Jujube solution	65	58	105
seated row	placebo	124	101	161
	Jujube solution	149.5	101	183

*Jujube Preparation*

The semi-dried fruits of *Ziziphus Jujube* were washed, and seeds were extracted, and the soft parts were removed and were dried at 50°C and ground to a powder using a pounder (Vahedi, Fathi Najafi, & Bozari, 2008).

*Combination Assessment of jujube extraction by Gas Chromatography-Mass Spectrometry (GC-MS)*

GC-MS revealed compounds of jujube extraction by semi-quantitative methods. The contents of the jujube extraction compounds were quantified using an internal standard

**Table 2.** Combination Assessment of Jujube Extraction by Gas Chromatography-Mass Spectrometry (GC-MS)

Combination	The area under the Peak (%)	Retention time (min)
furfural	51.33	20.21
4-Pyrone	9.51	17.07
Oleic acid	6.31	39.00
Palmitic acid	4.15	35.70
Imidazole	3.03	23.59
Cyclononasiloxane	2.03	42.05
Cyclodecasiloxane	1.75	35.63
Oxantin	1.61	10.65
Guanine	1.58	27.20
gamma-Sitosterol	1.17	44.56
Niphimycin	1.16	26.89
Iron	1.10	45.65
Butanediol	1.07	27.00
Phthalic acid	1.02	45.48
Pentasiloxane	1.01	48.34
Dodecanoic acid	0.97	27.616
octadecamethyl	0.95	32.604
Methyl 2-furoate	0.92	14.28
1,4-dicarboxylic acid	0.86	51.997
Tetradecanoic acid	0.74	31.840
Total	92.27	

(3-octanol, 99%, Sigma-Aldrich).

Wine hydrophobic compounds were analysed using an Agilent 5975 Mass Spectrometer coupled to an Agilent 7890A Gas Chromatograph (Agilent, Santa Clara, USA). A DB-WAX column (60 m×0.25 mm ID and 0.25 µm film thickness) was used for separation. The working factors were as listed: injector temperature of 210°C, EI source of 230°C, MS Quad of 150°C, and transfer line of 210°C. The primary temperature was 30°C for 8 min, which was increased to 150°C at a rate of 3°C per minute. The injector port temperature was 290°C, and helium was used as carrier gas at a flow rate of 1.5 ml/min. A total of 15 compounds were positively or tentatively identified by GC-MS that contains 92.27% of the area under the total peak (Table 2).

#### Exercise Protocol

Recent studies have reported that appetite hormones, such as ghrelin, increase during fasting and before feeding (breakfast, lunch, and dinner), increasing absorption levels (Tayebi et al., 2012). Accordingly, participants were taken to the test location after a 12 h overnight fast. All participants performed a session of circuit resistance exercise in two cycles, simultaneously. Each cycle contained nine exercises (Sit Up, back extension, biceps curl, triceps press, knee extension, knee curl, standing calf raise, chest press, seated row, machines were used in all exercises). The test included three non-stop circuits with a 3-minute active rest period between circuits. Each exercise was performed for 30 s (about 10–14 repeats) with one repeat maximum (1RM) of 75% (Tayebi et al., 2016; Tayebi et al., 2015). The exercise protocol is shown in Fig.1.

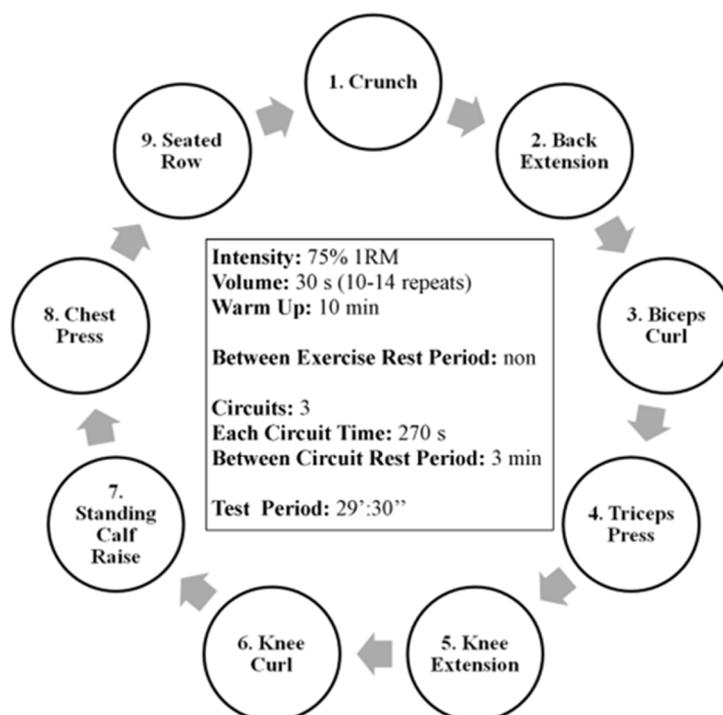


Figure 1. Exercise protocol

#### Supplement Protocol and blood collection

The groups received oral jujube solution (0.5 g/kg body weight in 2.5cc distilled water) and placebo (2.5cc/kg of body weight in distilled water sweetened by sugar with no calories and coloured by food dye); this supplement was taken each day for a week in a double-blind manner with no physical training within. The groups had a standard diet programme during this week for unification and healthy control.

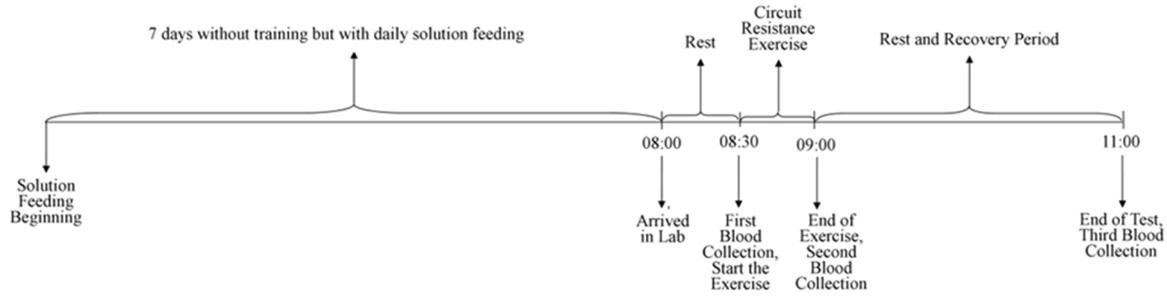
They received three meals per day: breakfast (10 kcal·kg<sup>-1</sup> BW, 70% carbohydrates, 18% protein, 12% fat), lunch (10 kcal·kg<sup>-1</sup> BW, 70% carbohydrates, 18% protein, 12% fat) and, dinner (18 kcal·kg<sup>-1</sup> BW, 70% carbohydrates, 15% protein, 15% fat). Subjects in both groups arrived at the test location at 08:00 and rested for about 30 minutes. All subjects performed circuit resistance exercise in two cycles at 08:30. On the 8<sup>th</sup> day and after 12 hours of overnight fasting, the first blood samples were taken at 08:30 AM. The second set of blood samples was taken immediately after exercise at 09:00; subjects then remained seated for 120 minutes. The third set of blood samples was taken at 11:00. The research design and blood collection of the second supplementation protocol is shown in Fig. 2.

#### Reagents

Anticoagulant ACD (a mixture of citric acid (Sigma Aldrich, Germany), dextrose (Sigma Aldrich, Germany), Dextran 6% (combination of dextran with at least 100000 MW (grade B, BDH lab, GPR™, England), 0.6 M KCl (a mixture of KCl (Sigma Aldrich, Germany), and dsdH<sub>2</sub>O), sodium citrate (Sigma Aldrich, Germany), and Deionized Sterile Distilled Water/dsdH<sub>2</sub>O (Baharafshan, B.I.R.D, Iran)), 0.9% NaCl (Sigma Aldrich, Germany), and dsdH<sub>2</sub>O), Phosphate buffered saline/PBS solution (combination of NaCl, KCl, Na<sub>2</sub>HPO<sub>4</sub> (Sigma Aldrich, Germany), glucose (Sigma Aldrich, Germany), KH<sub>2</sub>PO<sub>4</sub> (Sigma Aldrich, Germany), and dsdH<sub>2</sub>O) with pH 7.4 and Autoclaved, Hank's Balanced Salt Solution/HBSS without Ca & Mg (NaCl, KCl, Na<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, NaHCO<sub>3</sub> (Sigma Aldrich, Germany), and dsdH<sub>2</sub>O) with pH 7.4 and Autoclaved, Ficoll-Hypaque 10.77 (Baharafshan, B.I.R.D, Iran).

#### Neutrophil isolation

Neutrophils were purified from venous blood treated with ACD from healthy volunteers by three steps: Dextran sedimentation, Hypotonic lysis, and Ficoll sedimentation (Heit, 2001). Briefly, a solution of 6% Dextran & 0.9% NaCl



**Figure 2.** Research Design and Blood Collection

added into the mixture of ACD & blood, and after adequate mixing, it settled at room temperature until separation was complete. The yellowish supernatant was separated and centrifuged. After breaking the pellet by discarding the supernatant and re-suspension in dsdH<sub>2</sub>O at 20 s, it was mixed with 0.6M KCl and the solution diluted with PBS. After centrifugation, the supernatant was discarded and the pellet resuspended in PBS. The cell suspension was layered over Ficoll-Hypaque and centrifuged. Finally, the supernatant was discarded, and neutrophils resuspended in HBSS. Trypan Blue Viability Test showed that cell viability was ≥ 96%.

**Protein and [Ca<sup>2+</sup>]<sub>i</sub> Assessment**

Determination of caspase-3 (E20120710034, 0.05-10 ng/ml), calpain-1 (E20120710031, 13 -800 IU/L), calpastatin (E20120710032, 16 -1000 IU/L), Bax (E20120710035, 0.3-90 ng/ml), and XIAP (E20120710036, 0.05-20 ng/ml) is done by Human ELISA Kits (Glory Science Co., Ltd, China). [Ca<sup>2+</sup>]<sub>i</sub> assessed by Atomic Absorption/ Flame Emission method and SPECTROPHOTOMETER system (Shimadzu, AA-670).  
**Statistical Analysis**

Repeated measure (two-way) ANOVA was used to determine the interaction effects of TIME and SOLUTION by SPSS software at a significance level of p < 0.05. All data were presented as means with standard deviation (Mean ± SD).

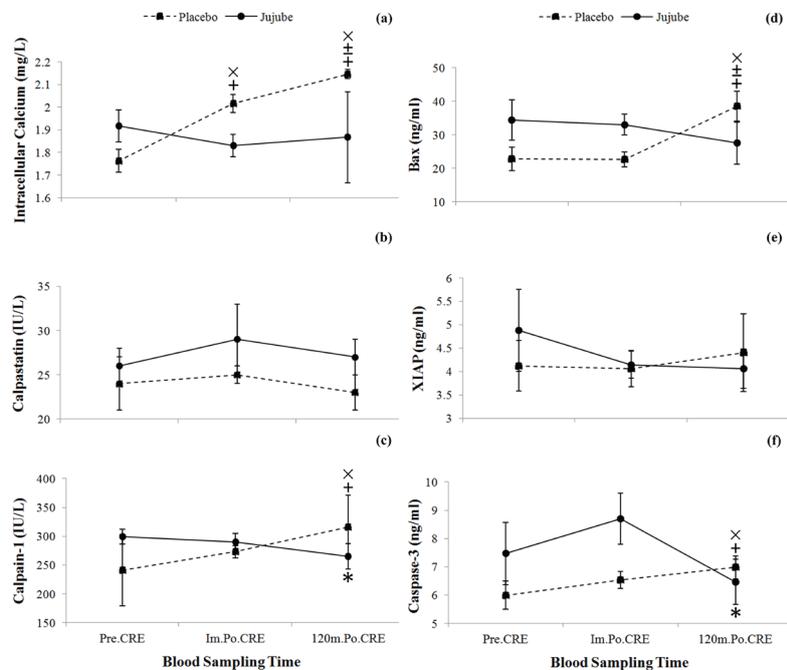
**Results**

**[Ca<sup>2+</sup>]<sub>i</sub>**

Sphericity assumption was met (Muchly's W=0.846; p=0.397). The interaction effect of TIME'GROUP was significant (F<sub>2,24</sub>= 6.252; p=0.007; Partial Eta Squared=0.343) and this effect was Linear (F<sub>1,12</sub>= 8.731; p=0.012; Partial Eta Squared=0.421). Accordingly, [Ca<sup>2+</sup>]<sub>i</sub> levels increased linearly in the placebo group during circuit resistance exercise and recovery period (Pre.CRE=1.76±0.05, Im.Po.CRE=1.83±0.04, 120m.Po.CRE=1.86±0.02mg/L). In contrast, in the jujube group [Ca<sup>2+</sup>]<sub>i</sub> levels remained unchanged over time (Pre.CRE=1.91±0.07, Im.Po.CRE=2.01±0.05, 120m.Po.CRE=2.14±0.02mg/L) [(Fig.3-a)].

**Calpastatin**

Sphericity assumption was met (Muchly's W=0.91; p=0.597). The interaction effect of TIME'GROUP was no



**Figure 3.** The Effect of One Week Supplementation with Ziziphus jujuba on some Pro- and Anti-Apoptotic Protein Levels of Human Neutrophils in Response to a Session of Circuit Resistance Exercise (a) Effect on intracellular calcium levels (b) Effect on Calpastatin expression (c) Effect on Calpain-1 expression (d) Effect on Bax expression (e) Effects on XIAP levels (f) Effects on Caspase-3 levels Pre: previous CRE: circuit resistance exercise Im.po: Immediately Post 120m: 120 minutes ; interaction effect of GROUP'TIME is significant at p<0.05 +: Significant difference with Pre.CRE in Placebo at p<0.05 ±: Significant difference with Im.Po.CRE in Placebo at p<0.05 \*: Significant difference with Pre.CRE in Jujube at p<0.05 (Error Bars present as SD)

significant ( $F_{2,24} = 2.003$ ;  $p = 0.157$ ; Partial Eta Squared = 0.143). Accordingly, calpastatin levels remained unchanged after exercise in both placebo (Pre.CRE =  $24 \pm 3$ , Im.Po.CRE =  $25 \pm 1$ , 120m.Po.CRE =  $27 \pm 2$  IU/L) as well as jujube (Pre.CRE =  $26 \pm 2$ , Im.Po.CRE =  $29 \pm 4$ , 120m.Po.CRE =  $27 \pm 2$  IU/L) groups (Fig.3-b).

#### Calpain-1

Sphericity assumption was met (Muchly's  $W = 0.619$ ;  $p = 0.071$ ). The interaction effect of TIME'GROUP was significant ( $F_{2,24} = 6.531$ ;  $p = 0.005$ ; Partial Eta Squared = 0.352) and this effect was Linear ( $F_{1,12} = 6.531$ ;  $p = 0.016$ ; Partial Eta Squared = 0.397). Although levels remained unchanged immediately after exercise in both placebo (Pre.CRE =  $241 \pm 62$ , Im.Po.CRE =  $273 \pm 11$  IU/L) as well as jujube (Pre.CRE =  $299 \pm 13$ , Im.Po.CRE =  $290 \pm 15$  IU/L) groups, a significant elevation and placebo (120m.Po.CRE =  $316 \pm 55$  IU/L) and decline in the jujube (120m.Po.CRE =  $265 \pm 22$  IU/L) group was found at 120 after exercise (Fig.3-c).

#### Bax

Sphericity assumption was met (Muchly's  $W = 0.636$ ;  $p = 0.083$ ). The interaction effect of TIME'GROUP was significant ( $F_{2,24} = 24.509$ ;  $p < 0.001$ ; Partial Eta Squared = 0.671) and this effect was both Linear ( $F_{1,12} = 24.742$ ;  $p < 0.001$ ; Partial Eta Squared = 0.673) and Quadratic ( $F_{1,12} = 23.666$ ;  $p < 0.001$ ; Partial Eta Squared = 0.664). Accordingly, in the placebo group, there were no changes during exercise (Pre.CRE =  $22.8 \pm 3.5$ , Im.Po.CRE =  $22.7 \pm 2.2$  ng/ml), but levels were significantly elevated during recovery period (120m.Po.CRE =  $38.5 \pm 3.4$  ng/ml). In contrast, no changes were observed at all time points in the jujube group (Pre.CRE =  $34.4 \pm 6.1$ , Im.Po.CRE =  $33 \pm 3.1$ , 120m.Po.CRE =  $27.5 \pm 6.2$  ng/ml) [(Fig.3-d)].

#### XIAP

Sphericity assumption was met (Muchly's  $W = 0.835$ ;  $p = 0.371$ ). The interaction effect of TIME'GROUP was significant ( $F_{2,24} = 3.262$ ;  $p = 0.056$ ; Partial Eta Squared = 0.214). Accordingly, levels remained unchanged in both placebo (Pre.CRE =  $4.12 \pm 0.54$ , Im.Po.CRE =  $4.06 \pm 0.39$ , 120m.Po.CRE =  $4.40 \pm 0.83$  ng/ml) as well as jujube (Pre.CRE =  $4.88 \pm 0.88$ , Im.Po.CRE =  $4.14 \pm 0.29$ , 120m.Po.CRE =  $4.06 \pm 0.29$  ng/ml) groups over time (Fig.3-e).

#### Caspase-3

Sphericity assumption was met (Muchly's  $W = 0.959$ ;  $p = 0.794$ ). The interaction effect of TIME'GROUP was significant ( $F_{2,24} = 12.053$ ;  $p < 0.0015$ ; Partial Eta Squared = 0.501) and this effect was both Linear ( $F_{1,12} = 14.623$ ;  $p = 0.002$ ; Partial Eta Squared = 0.549) and Quadratic ( $F_{1,12} = 10.189$ ;  $p = 0.008$ ; Partial Eta Squared = 0.459). Although caspase-3 levels remained unchanged during exercise in both placebo (Pre.CRE =  $6.0 \pm 0.5$ , Im.Po.CRE =  $6.5 \pm 0.3$  ng/ml) as well as jujube (Pre.CRE =  $7.4 \pm 1.1$ , Im.Po.CRE =  $8.7 \pm 0.9$  ng/ml) groups, concentrations significantly differ in the post-exercise period placebo (120m.Po.CRE =  $6.9 \pm 0.4$  ng/ml), jujube (120m.Po.CRE =  $6.4 \pm 0.8$  ng/ml) groups (Fig.3-f).

## Discussion

The results of the current study showed that neutrophils  $[Ca^{2+}]_i$  levels increased linearly during and after circuit resistance exercise. The jujube supplementation attenuated the increase of  $[Ca^{2+}]_i$ . Although calpastatin and XIAP

concentrations were unchanged in both groups and at all times, the calpain-1 levels of neutrophils increased linearly during and after exercise. However, levels of calpain-1 decreased in the jujube group and showed a similar regulation as  $[Ca^{2+}]_i$  levels. During recovery, Bax and caspase-3 increased in the placebo group, while it attenuated and decreased due to jujube supplementation. These results suggest that intensive circuit resistance exercise increases the pro-apoptotic proteins in human neutrophils, effectively suppressed by one-week supplementation with a jujube solution.

It was previously shown that intensive bouts of exercise could modulate neutrophil apoptosis. Mechanistically, this process can result either from a reduction in anti-apoptotic protein levels and/or elevation of pro-apoptotic protein levels (Kroemer et al., 1998). In neutrophils, it was reported that the cytosolic non-caspase cysteine protease calpain mediates apoptosis. Calpains are constitutively active in neutrophils but are kept in check by the endogenous inhibitor calpastatin. During neutrophil apoptosis, calpastatin is degraded, and calpains were shown to cleave Bax and to deactivate XIAP, thus causing neutrophil apoptosis (Geering & Simon, 2011). However, the neutrophils'  $[Ca^{2+}]_i$ -calpastatin-calpain axis had not previously been investigated during exercise conditions. Regarding apoptosis regulation in neutrophils during exercise, it is found that resistance exercise (90 min, three sets, 2 min rest period between exercises, 3 min rest between sets) with moderate intensity (60% 1RM) had no effect on neutrophils apoptosis. As we know, calpastatin with calpain-1 are critical elements in the proximal location of the pro-apoptotic cascade, which cause the activation of Bax, mitochondria, and caspase-3, and so it seems that their expression affect the neutrophils' life span under pathologic situations (Altznauer et al., 2004). However, the intracellular protein expression of calpastatin did not change significantly in this study, but based on its changed behaviour, it could make the calpain-1 active, because the low decrease of protein levels can cause enzymatic activity.

In contrast, both intensive resistance exercise and marathon running delayed neutrophils' apoptosis during the 3h recovery period. In parallel, it was noted that apoptosis modulation was accompanied by enhanced intracellular calcium ( $[Ca^{2+}]_i$ ) transients (Mooren et al., 2012). Mechanistically, it was shown that the expression of Granulocyte Colony-Stimulating Factor (G-CSF) during exercise might affect the delay of neutrophils' apoptosis by inhibition of caspase-3 and -9. Similarly, it strongly inhibited the activation of calcium-dependent cysteine proteases calpains (upstream of caspase-3) through the control of calcium permeation. Calpain inhibition resulted in the stability of XIAP and thus the inhibition of caspase-3 and -9 activity (van Raam et al., 2008). In this study, however we saw no significant changes in XIAP, but its behaviour changes in the jujube group were in line with calpain-1 changes and confirm the findings of the literature. However, other studies found contrasting results. An increased neutrophil apoptosis after exercise was found, which was attenuated by administration of glutamine. It was suggested that glutamine exhibits protective effects on mitochondrial integrity (Lagranha et al., 2004). In another study, it is reported that one session of exercise could increase caspase-3 gene expression in rat neutrophils by 52%. In contrast, exercise with supplementation of glutamine reduced the expression of caspase-3 by 63% in rested rats and by 75%

in exercised rats (Lagranha et al., 2007).

Based on the modulation of neutrophil apoptosis by nutritional supplements like jujube, some ideas for possible mechanisms can be derived. Jujube consists of 77% carbohydrates, 19% fat, 4.5-5.6% protein, various amino acids, such as glycine, histidine, leucine, isoleucine, phenylalanine, proline, serine, threonine, and three precursors of glutamine, such as alanine, aspartic acid, glutamic acid (Ghanbari Niaki et al., 2013; Ghanbari Niaki et al., 2013). Accordingly, it is suggested that glutamine and glucose might affect jujube effects on neutrophil apoptosis modulation during exercise. Both contents are the main energy sources of neutrophils (Lagranha et al., 2008). Generally, neutrophils are cells that use glutamine intensively for metabolism (Curi et al., 1997). Accordingly, jujube supplementation for one week may provide additional energy sources for neutrophils, which helps to stabilize cell metabolism during exercise conditions. In this regard, it is reported that 30 days of administration with jujube extraction reduced Bax expression and increased Bcl-2 expression in rats' heart muscle in response to two 15-min swimming bouts on two different days (Liang & Juan, 2011).

Besides providing energy, jujube fruit contains various minerals and vitamins, which might affect neutrophils' oxidative statuses; these include iron (involved in catalase), zinc (involved in superoxide dismutase), manganese (involved in superoxide dismutase), and vitamins C and A. In addition, also other jujube contents, such as glycoside complexes, including phenols (Quercetin and Kaempferol) as well as flavonoids and triterpenes, are known to have anti-oxidative properties (Afroz et al., 2014; Cheng et al., 2012; Choi et al., 2011; Gao et al., 2011; Gao et al., 2012). During exercise, it is known that increased oxygen turnover, tissue damage, and ischemia-reperfusion processes are followed by increased production of reactive oxygen species (ROS) (Afzalpour, et al., 2015; Afzalpour et al., 2014). In this regard, it was shown that repeated high-intensity acute exercise increased apoptosis in neutrophils (Syu et al., 2011; Tayebi et al., 2014), which were related to an increased oxidative state (Syu et al., 2011). Three weeks of jujube administration (0.4 g/kg body weight) reduced apoptosis and affected total antioxidant capacity (TAC) (Afzalpour et al., 2014) and glutathione peroxidase (GPX) levels (Afzalpour et al., 2015). It is found that 30 days of administration with jujube extraction reduced Bax expression and increased Bcl-2 expression in rats' heart muscle. It is assumed that the extraction reduced levels of lipid peroxidation and increased antioxidant enzyme activities in the heart muscle (Liang & Juan, 2011). Accordingly, the antioxidant effect of jujube might be an additional primary mechanism for reducing pro-apoptotic protein concentrations in neutrophils in response to an intensive circuit resistance exercise.

In summary, the present data indicate that a single session of circuit resistance exercise increased the levels of pro-apoptotic proteins (calpain-1, Bax, caspase-3) in human neutrophils. It is assumed that the  $[Ca^{2+}]_i$ -Calpastatin-Calpai mechanistically n axis upstream affects caspase-3. Supplementation with a jujube solution effectively suppressed these effects and might inhibit neutrophils' apoptosis. It is supposed that jujube might be an additional energy source for neutrophils or might protect neutrophils from the detrimental effects of ROS. However, clinical relevance for jujube supplementation during exercise has to be proved in future studies.

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# Participation in Physical Activity is Associated with Well-being in European University Students

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## Abstract

This study's objective was to analyse the associations between physical activity (PA) and well-being in a representative sample of European university students. This cross-sectional study was based on data from the European Social Survey round 6, 2012, comprising 3,143 European university students (1456 men and 1687 women) from 27 countries, with a mean age of 21.3±2.9 years. Socio-demographic data, PA, health perception, and the six dimensions of well-being were self-reported. Men reported practising PA more often than women did (4.4±2.2 versus 4.1 ± 2.2,  $p<0.001$ ) and had better health perception (4.2±0.7 versus 4.1 ± 0.8,  $p<0.001$ ) and total well-being score (5.5±1.2 versus 5.2 ± 1.3,  $p<0.001$ ). PA frequency was linearly associated with health perception ( $p<0.001$ ) as well as the overall well-being score ( $p<0.001$ ). There is a positive association between PA and the well-being of European university students. This emphasizes the importance of PA in the university students' lifestyle and the need to develop programmes that reduce physical inactivity.

**Keywords:** exercise, active lifestyle, health, epidemiology, young adults



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## Introduction

The regular practice of physical activity (PA) is known to function as a preventive factor for several diseases, such as obesity, diabetes, anxiety, and depression, thus contributing to the improvement of health (Rhodes et al., 2017). However, despite its benefits, a large part of the population does not practice enough PA to impact their health (Baptista et al., 2012; Hallal et al., 2012; Marques et al., 2015). Late

adolescence and the beginning of adulthood are critical age groups concerning participation in PA (Baptista et al., 2012; Hallal et al., 2012; Marques & Gaspar de Matos, 2014). Between the ages of 18 and 24, the prevalence of achieving PA guidelines recommendations decreases rapidly (Grim et al., 2011). Recently, a research study comprising 23 countries showed that 40% of university students were physically inactive (Pengpid et al., 2015).

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The transition from high school to university and the beginning of university life is a defining moment in the student's life and is composed of several changes at different levels, including physical and psychological well-being (Arnett, 2000; Bray & Born, 2004; Deforche et al., 2015; Gall et al., 2000). Although aware of the benefits of PA and the consequences of physical inactivity (Poobalan et al., 2012), university students have significantly high levels of physical inactivity (Pengpid et al., 2015; Poobalan et al., 2012), which seems to indicate that, in most cases, they are not prepared for the transition from a system in which practising PA is mandatory, as in secondary school, to one in which PA participation becomes a voluntary and individual responsibility, as in the university (Poobalan et al., 2012).

For adults, well-being is associated with attaining PA recommendations (Marques et al., 2016). Furthermore, among university students, regular PA is associated with higher levels of happiness and well-being (Dias et al., 2008), and those who are insufficiently active have higher levels of fatigue and lower levels of vigour (Bray & Born, 2004). Even though it is an important aspect of life, well-being has not been consistently explored (Engberg et al., 2015), and little is known about its association with PA in university students. Additionally, monitoring PA trends in younger adults becomes relevant in understanding factors such as attitudes and knowledge about health benefits, which may be associated with significantly low PA levels. Thus, the objective of this study was to analyse the associations between PA and well-being in a representative sample of European university students.

## Methods

### *Participants and Procedures*

The sample was obtained from the European Social Survey (ESS) round 6 database, which includes data from 28 European countries (Albania, Germany, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Hungary, Iceland, Ireland, Italy, Kosovo, Lithuania, Norway, Netherlands, Poland, Portugal, Russian Federation, Sweden, Switzerland, United Kingdom) and Israel. The ESS measures the attitudes, beliefs, and behaviours of the European population; its essential purpose is to raise the standards of rigour in transnational research.

The initial sample consisted of 54,673 participants aged 15 years or over. The following were excluded: (1) participants less than 18 and over 65 years of age, since the recommendations were different for these age groups; (2) participants who were not students and were older than 30 years old due to the target population to be studied; residents of Israel, because it is not a European country; (4) participants who did not report information on more than two socio-demographic variables. The final sample consisted of 3,143 participants (1456 men and 1687 women) with a mean age of  $21.3 \pm 2.9$  years old.

ESS uses proportional and representative samples from European countries, and participants were selected by means of postcode, population records, social security record data, or telephone directories. In the sampling procedure, statistical precision was maintained for all countries. Data was collected through a questionnaire, completed during a face-to-face interview lasting approximately one hour, which included items related to health, citizenship,

socio-demographic issues, socioeconomic issues, and PA. The questionnaire was translated by experienced translators into the corresponding language of each country. The study protocol subscribes to the Declaration on Professional Ethics of the International Statistical Institute.

## Measures

### *Well-being*

The personal and social well-being model of ESS round 6 uses 32 items that comprise six distinct dimensions, already described elsewhere (ESS, 2015; Michaelson et al., 2009). Using this model, the ESS enables collecting more detailed information on personal and social well-being through a set of response items, which allows for more detailed policy implications to be made within an area that remains relevant in debates throughout Europe (Jeffrey et al., 2015). These items and their response ranges are present in Table 1, and the aggregation of the items is described in the data analysis section.

### *Physical Activity and health perception*

Participation in PA was assessed using the question "On how many of the last 7 days you were physically active continuously for 20 minutes or longer?" Health perception was measured through the question "How does it characterize its current state of health?" Responses were given on a 4-point Likert scale, ranging from 1 (very bad) to 4 (very good).

### *Socio-demographic characteristics*

Participants were asked about their age, sex, marital status, household members, income and living place. Marital status was dichotomized into living with a partner or living without a partner. The household income was determined based on deciles. Using these data and to create three groups, participants were clustered as followed: 1st to 3rd deciles, 4th to 7th deciles and 8th to 10th deciles. To determine the living place, participants were asked to report if they lived in a large city, in the suburbs or surroundings of a large city, a village or small town, a village or a country house. Participants who indicated that they lived in a large city, in the suburbs or surroundings of a large city were grouped into a new category called "urban area"; participants who reported living in a village or country house were grouped into a new category called "rural area".

### *Data analysis*

The aggregation of the well-being items in the six dimensions started with the standardization of the items, as proposed in the ESS (ESS, 2015; Michaelson et al., 2009). Using the standardized items, a principal component analysis was performed, with Varimax rotation, to group the items with the highest correlation among themselves in different domains. The components' saturation did not allow the creation of components according to the literature (ESS, 2015; Michaelson et al., 2009). Therefore, a reliability analysis was performed on the standardized items of each of the components; alphas were from  $\alpha = 0.7$  to 0.9 (see Table 1).

Posteriorly, these dimensions were aggregated into a total well-being z-score. To facilitate the interpretation of the well-being dimensions and the total score, a transformation metric was performed, which maps the z-scores for each in-

**Table 1.** Items from the well-being ESS module grouped by the dimension they are relate to

Dimensions	ESS survey items	Response range	$\alpha$
Evaluative well-being	How satisfied with life as a whole	0 (extremely dissatisfied) to 10 (extremely satisfied)	0.8
	How happy are you	0 (extremely unhappy) 10 (extremely happy)	
Emotional well-being	Felt sad, how often past week	1 (almost none of the time) to 4 (almost all of the time)*	0.9
	Felt depressed, how often past week	1 (almost none of the time) to 4 (almost all of the time)*	
	Enjoyed life, how often past week	1 (almost none of the time) to 4 (almost all of the time)	
	Were happy, how often past week	1 (almost none of the time) to 4 (almost all of the time)	
	You felt anxious, how often past week	1 (almost none of the time) to 4 (almost all of the time)*	
	You felt calm and peaceful, how often past week	1 (almost none of the time) to 4 (almost all of the time)	
Functioning	Free to decide how to live my life	1 (disagree strongly) to 5 (agree strongly)	0.7
	Little chance to show how capable I am	1 (disagree strongly) to 5 (agree strongly)*	
	Feel accomplishment from what I do	1 (disagree strongly) to 5 (agree strongly)	
	Interested in what you are doing	0 (none of the time) to 10 (all of the time)	
	Absorbed in what you are doing	0 (none of the time) to 10 (all of the time)	
	Enthusiastic about what you are doing	0 (none of the time) to 10 (all of the time)	
	Feel what I do in life is valuable and worthwhile	1 (disagree strongly) to 5 (agree strongly)	
	Have a sense of direction	0 (not at all) to 10 (completely)	
	Always optimistic about my future	0 (none of the time) to 10 (all of the time)	
	There are lots of things I feel I am good at	1 (disagree strongly) to 5 (agree strongly)	
	In general feel very positive about myself	1 (disagree strongly) to 5 (agree strongly)	
	Vitality	Felt everything took effort, how often past week	
Sleep was restless, how often past week		1 (almost none of the time) to 4 (almost of the time)*	
Could not get going, how often past week		1 (almost none of the time) to 4 (almost of the time)*	
Had lot of energy, how often past week		1 (almost none of the time) to 4 (almost of the time)	
Community well-being	Most people can be trusted	0 (can't be too careful) to 10 (most can be trusted)	0.7
	People try to take advantage	0 (most try to take advantage) to 10 (most try to be fair)*	
	Most of the time, people are helpful	0 (mostly don't) to 10 (mostly try to be helpful)	
	Feel people in local area help one another	0 (not at all) to 6 (a great deal)	
	Feel close to the people in local area	1 (disagree strongly) to 5 (agree strongly)	
Supportive well-being	People you can discuss intimate matters	0 (none) to 5 (10 or more)	0.9
	Feel appreciated by those you are close to	0 (not at all) to 10 (completely)	
	Receive help and support	0 (not at all) to 6 (completely)	
	Felt lonely, how often past week	1 (almost none of the time) to 4 (almost all of the time)*	

Note. \* Inverted item.

dicator from 0 to 10, where “0” represents the minimum, and “10” represents the maximum, the “5” corresponds to the mean for the sample (Michaelson et al., 2009). This transformation is described in the following formula:

$$t_i = (Z_i \times 5) / (Z_i \times m_i + C_i) + 5$$

$$M_i = (\min_i + \max_i) / (\min_i - \max_i)$$

$$C = (\min_i \times \max_i \times 2) / (\max_i - \min_i)$$

Descriptive statistics (mean, standard deviation and frequencies) were calculated for all variables. The Student's t-test and Chi-square test were performed to analyse differences between the sexes. Due to the significant differences between men and women in several variables, subsequent analyses were stratified by sex. Associations between the frequency of PA in the previous seven days with health perception and well-being were examined with multivariate linear regression models. Analyses were adjusted for all the socio-demographic variables. Statistical analyses were performed using the IBM SPSS Statistics 22 software, and a

significance level was defined at  $p < 0.05$ .

## Results

The participants' characteristics are presented in Table 2. Most European university students lived without a partner (91.5%), had three to four household members (53.3%) and lived in an urban area (40.7%) or in a town or city (31.6%). On average, men practised PA more often than women did (men:  $4.4 \pm 2.2$ , women:  $4.1 \pm 2.2$ ;  $p < 0.001$ ). In addition, men had a slightly better health perception (men:  $4.2 \pm 0.7$ , women:  $4.1 \pm 0.8$ ,  $p < 0.001$ ) and total well-being score (men:  $5.5 \pm 1.2$ , women:  $5.2 \pm 1.3$ ,  $p < 0.001$ ) than women did.

Table 3 presents the results of multivariate linear regression for the relationship between PA frequency with health perception and well-being. Overall, PA frequency was linearly related to most dimensions of well-being, except for community well-being, to the well-being total score ( $\beta = 0.06$ , 95%CI: 0.04 to 0.09,  $p < 0.001$ ), and health perception ( $\beta = 0.05$ ,

**Table 2.** Participants' socio-demographic characteristics

	Total (n=3143)	Men (n=1456)	Women (n=1687)	p
	n (%) or M±SD	n (%) or M±SD	n (%) or M±SD	
Age	21.3±2.9	21.3±2.9	21.3±2.9	0,908 <sup>a</sup>
Partnership status				<0,001 <sup>b</sup>
Living with a partner	267 (8,5)	81 (5,6)	186 (11,0)	
Living without a partner	2875 (91,5)	1361 (93,5)	1482 (87,8)	
Members of household				<0,001 <sup>b</sup>
1 person	397 (12,3)	218 (15,0)	170 (10,1)	
2 people	531 (16,9)	191 (13,1)	339 (20,1)	
3-4 people	1674 (53,3)	814 (55,9)	860 (51,0)	
≥5 people	551 (17,5)	233 (16,0)	318 (18,8)	
Household income				0,790 <sup>b</sup>
1 <sup>st</sup> to 3 <sup>rd</sup> decile	660 (21,0)	316 (21,7)	344 (20,4)	
4 <sup>th</sup> to 7 <sup>th</sup> decile	861 (27,4)	406 (27,9)	455 (27,0)	
8 <sup>th</sup> to 10 <sup>th</sup> decile	579 (18,4)	266 (18,39)	313 (18,5)	
Living Place				0,014 <sup>b</sup>
Urban area	1279 (40,7)	603 (41,4)	676 (40,1)	
Town or small city	993 (31,6)	485 (33,3)	508 (30,1)	
Rural areas	868 (27,6)	367 (25,2)	501 (29,7)	
PA in the past 7 days (≥20 min/day)	4,2±2,2	4,4±2,2	4,1±2,2	<0,001 <sup>a</sup>
Health perception	4,1±0,8	4,2±0,7	4,1±0,8	<0,001 <sup>a</sup>
Well-being dimensions				
Evaluative well-being	5,8±2,0	5,8±1,9	5,8±2,1	0,828 <sup>a</sup>
Emotional well-being	5,5±1,7	5,7±1,6	5,3±1,7	<0,001 <sup>a</sup>
Functioning	5,2±1,3	5,3±1,2	5,1±1,3	<0,001 <sup>a</sup>
Vitality	5,3±1,5	5,5±1,4	5,2±1,5	<0,001 <sup>a</sup>
Community well-being	5,0±1,2	5,0±1,2	4,9±1,2	0,026 <sup>a</sup>
Supportive relationships	5,2±1,0	5,3±1,0	5,2±1,0	0,126 <sup>a</sup>
Well-being total score	5,3±1,3	5,5±1,2	5,2±1,3	<0,001 <sup>a</sup>

Note. M, mean; SD, standard deviation; PA, physical activity; a Tested by Student t-test; b Tested by Chi-square.

95%CI: 0.03 to 0.06, p<0.001). Regarding men, stronger associations were observed for the vitality (β=0.12, 95%CI: 0.08 to 0.16, p <0.001) and functioning (β=0.07, 95%CI: 0.04 to 0.11, p<0.001) dimensions and for the wellbeing total score (β=0.06, 95%CI: 0.03 to 0.10, p<0.01). For women, the strongest associations were found between PA and vitality (β=0.09, 95%CI:

0.05 to 0.12, p<0.001), evaluative wellbeing (β=0.09, 95%CI: 0.04 to 0.14, p<0.01) and the wellbeing total score (β=0.06, 95%CI: 0.03 to 0.10, p <0.01). For both men and women, health perception was linearly associated with PA frequency (men: β=0.05, 95%CI: 0.03 to 0.07, p<0.001; women: β=0.05, 95%CI: 0.03 to 0.06, p<0.001).

**Table 3.** Linear regression analyses for the relationship between PA with health perception and well-being in European university students

	Physical activity on the last 7 days		
	Total β (95% IC)	Men β (95% IC)	Women β (95% IC)
Health perception	0.05 (0.03, 0.06)***	0.05 (0.03, 0.07)***	0.05 (0.03, 0.06)***
Evaluative wellbeing	0.06 (0.02, 0.10)**	0.04 (-0.01, 0.10)	0.09 (0.04, 0.14)**
Emotional wellbeing	0.05 (0.02, 0.09)**	0.05 (0.01, 0.10)*	0.04 (-0.00, 0.09)
Functioning	0.07 (0.04, 0.09)***	0.07 (0.04, 0.11)***	0.05 (0.02, 0.09)**
Vitality	0.11 (0.08, 0.13)***	0.12 (0.08, 0.16)***	0.09 (0.05, 0.12)***
Community wellbeing	0.00 (-0.02, 0.02)	-0.03 (-0.06, 0.01)	0.03 (-0.01, 0.06)
Supportive relationships	0.03 (0.01, 0.05)**	0.04 (0.01, 0.06)**	0.02 (-0.01, 0.05)
Wellbeing total score	0.06 (0.04, 0.09)***	0.06 (0.03, 0.10)**	0.06 (0.03, 0.10)***

Note. CI, confidence interval; Analyses were adjusted for civil status, members of household, household income, living place; \* p<0,05, \*\* p<0,01, \*\*\* p<0,001.

**Discussion**

This study examined the associations between PA with health perception and the well-being of European university students. Both the total well-being score and health perception were linearly associated with PA frequency. Additionally,

men reported practising PA more often than women and had better health perception and total well-being score.

Among university students, previous findings suggest that men are physically more active than women were (Molina-Garcia et al., 2011). In accordance with that, in this study,

men reported practising PA more often than women did. This finding is not restricted to the university student population as both young and adult men seem to practice more PA than women do.

PA frequency was linearly associated with a health perception and well-being for both sexes. Thus, European university students who practice PA more often seem to have better health perception and well-being. These results are similar to those of previous studies carried out in the adult population and reinforce the idea that practising PA is beneficial to the population's health and well-being (Marques et al., 2016). Furthermore, among university students practising PA more often is associated with benefits in psychological well-being and vitality (Molina-Garcia et al., 2011). This evidence should be viewed as relevant since well-being and health perceptions are important aspects of people's lives (Marques et al., 2016). In addition, well-being is an essential indicator of how people are faring with daily life, and it serves to inform decision-making and policy action in ways that impact people's daily lives (ESS, 2015; Michaelson et al., 2009).

At this stage of life, university students who tend to exhibit low PA and mental health levels (Pengpid et al., 2015; Weitzman, 2004) can be considered an at-risk group. Therefore, it is essential to build PA interventions focused on this population that benefit them physically and psychologically and promote their health and well-being.

The present study has limitations that are important to mention. Data were self-reported rather than objectively measured, which could be subject to bias. The cross-sectional methodology makes it impossible to infer conclusions about causal relations between the studied variables. PA was measured using questionnaires rather than directly (i.e., accelerometer). Despite the limitations, some aspects should be mentioned to highlight the strength of the study. The composition of the sample obtained through the ESS, both in terms of size and variety, is representative of several European countries. Due to the multidimensionality of well-being, the inclusion of various dimensions is recommended and allows the construction of a score that captures more than just life satisfaction (Michaelson et al., 2009).

In conclusion, this study's results suggest the existence of a positive association between PA and the well-being of young European university students. This emphasizes the importance of PA in the university students' lifestyle and the need to develop programmes that reduce physical inactivity.

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# Validity and Reliability of a Smartphone and Digital Inclinometer in Measuring the Lower Extremity Joints Range of Motion

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## Abstract

In clinical settings, available valid and reliable tools are important components in evaluating the lower extremity range of motion. Although the digital inclinometer is highly reliable compared to the universal goniometer, its availability and high cost impede its extensive use. Nowadays, smartphone applications have become widely available to clinicians for assessing the joint range of motion. The present study aims to assess the validity and intra-rater reliability of the smartphone application "Clinometer" for measuring hip, knee, and ankle sagittal ranges of motion, using the digital inclinometer as the reference standard. Active hip, knee flexion and ankle dorsiflexion and plantarflexion range-of-motion measurements were recorded in 102 young, healthy female participants on two separate occasions using Clinometer and a digital inclinometer. Pearson's correlation coefficients ( $r$ ) were used to evaluate the smartphone application's validity against the digital inclinometer. To assess the reliability of the Clinometer app, the intra-class correlation coefficient (ICC), standard error of measurement (SEM), and minimal detectable difference (MDD) were used. Clinometer displayed excellent validity when compared to the digital inclinometer for hip and knee movements ( $r > 0.90$ ), while ankle ROM displayed moderate validity ( $r = 0.52-0.57$ ). Additionally, Clinometer demonstrated excellent reliability ( $ICC > 0.90$ ) for hip and knee sagittal plane motion and moderate reliability for the ankle sagittal plane motion ( $ICC = 0.53-0.67$ ). Clinometer is a portable, low-cost, valid, and reliable tool for assessing active hip and knee range of motions and can be easily incorporated into clinical settings; however, it cannot be used interchangeably for ankle measures.

**Keywords:** *smartphone application; digital inclinometer; ROM; validity and reliability; lower limb joints*



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## Introduction

In a clinical setting, physical therapists encounter many lower limb (LL) pathologies, such as osteoarthritis, muscle strain,

musculoskeletal disorders, and overuse injuries, which can limit the hip, knee, and ankle joints range of motion (ROM) and hinder the daily living activities (Brosseau et al., 1997; Charl-

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ton et al., 2015). A standard goniometer is a physical therapy assessment tool used to measure the range of motion (ROM) limitation, plan the appropriate treatment interventions, and track the treatment effectiveness (Behnoush et al., 2016; Brosseau et al., 1997). Although the universal goniometer (UG) has been extensively used due to its low cost, convenience, and reliability (Norkin & White, 2016), its intra-rater reliability appeared to be higher than its inter-rater reliability. Despite the good general reliability of the UG, this reliability tended to vary from one joint to another and from particular movement to another (Boone et al., 1978; Rothstein et al., 1983; Watkins et al., 1991). In addition, improper goniometer application, such as bony landmark positioning, detection, and maintaining the centre of the goniometer during measurement, can impact the universal goniometer's data validity and reliability (Gajdosik & Bohannon, 1987). Measurement reliability is essential in the clinical setting because patients are often treated and re-evaluated, either by the same or by different physical therapists (Gogia et al., 1987). Currently, a digital inclinometer (DI) has been used because of its higher reliability in comparison with the UG for ROM assessment (Carey et al., 2010; Roach et al., 2013; Santos et al., 2012); however, the only drawback for the DI is its higher cost compared to the UG.

Recently, the American Physical Therapy Association (APTA) has encouraged the integration of technology into the practice of physical therapy, because of its impact on the profession and patients by improving the treatment outcomes and/or reducing the costs of treatment (Swisher & Hiller, 2010). Additionally, the APTA's code of ethics, section 6B, states that "Physical therapists shall take responsibility for their professional development based on critical self-assessment and reflection on changes in physical therapist practice, education, health care delivery, and technology" (Swisher & Hiller, 2010).

Nowadays, smartphone technology provides opportunities for assessing and treating patients in clinical facilities and during the follow-up of the patient progress (Wojciechowski, 2011). To date, many studies have assessed the validity and reliability of the smartphone application against the UG for various joints motion, for instance, the spinal column, knee, and ankle (Alawna et al., 2019; Jones et al., 2014; Mitchell, Gutierrez et al., 2014). However, the validity and reliability of the smartphone application are limited compared to those of the DI in measuring sagittal plane ROM and is still of novel concern due to the importance of these joints in gait. Accordingly, the purposes of the present study were a) To determine the validity of the smartphone "Clinometer" application, using the DI as the reference standard, and b) To explore the intra-rater reliability of Clinometer for quantifying hip, knee, and ankle sagittal ROM.

## Methods

### Participants

A group of 102 young, healthy female participants (mean  $\pm$  SD age: 20.71  $\pm$  0.99 years, height: 158.08  $\pm$  4.95 cm, mass: 57.16  $\pm$  10.95 kg, body mass index (BMI): 22.86  $\pm$  4.05 kg/m<sup>2</sup>) participated in this study. The results of a priori power analysis (G\*Power software) revealed a need for 46 subjects or more to be sufficient to create a statistical power of 0.95, at the pre-set  $\alpha$  level of 0.05 and with a large effect size of 0.5. Participants were included if they had no pain at the time of assessment with a clear history of LL musculoskeletal or neurological injuries within the 12 months prior to participation in the study.

Subjects were screened by completing a questionnaire. If they reported a history of musculoskeletal or neuromuscular disorders, rheumatologic condition, or previous surgeries to the LL, they were excluded from the study. Subjects with an abnormal end-feel other than soft tissue approximation for flexion, presence of pain or lack of full movement of LL joints (hip, knee flexion, ankle dorsiflexion, and plantar flexion) were also excluded from the study. In total, five subjects were excluded from participation in the study on these grounds. Subjects who fulfilled the inclusion criteria provided written informed consent prior to participation. Ethics approval for this study was attained from the Institutional Human Research Ethical Committee (Ethics approval no: P.T.REC/012/002280).

### Instrumentation

The current study used validity and intra-rater reliability design to verify if a smartphone application could be considered a valid and reliable measuring tool for assessing the sagittal plane ROM of hip, knee, and ankle. The validity of the smartphone application was assessed compared to the Baseline DI instrument (Model 12-1057, Fabrication Enterprises, Inc., NY), which is a validated and reliable instrument for assessing the ROM.

The current study utilized a smartphone application known as "Clinometer" (CA) version 4.7. It is accessible on the Google Play store (<https://play.google.com/store/apps>) for free download and as a paid app (US \$2.93) on the Apple iPhone store. A clinometer is a tool for assessing angles of slope (or tilt) using all sides of the device plus the camera. Clinometer uses three various units of measure: degree, per cent, and topo to quantify both inclines and declines. Additionally, it allows for two-way calibration on all sides: For every four sides and the bubble mode, calibration can be done in two procedures, which provides calibration on uneven surfaces. Each path can be calibrated separately to attain optimum precision. The smartphone used in the current study was an iPhone 7 Plus running iOS 4.7.

An expert physical therapist with fifteen years of clinical experience conducted all ROM measurements in the same laboratory on two separate sessions, one week apart. The tested limb was randomly selected, and only active ROM for hip, knee flexion, ankle dorsiflexion, and plantar flexion were measured. The order for ROM assessment (hip and knee flexion; ankle dorsiflexion and plantarflexion) and the measuring tool (DI and CA) were randomly performed (<http://www.randomization.com/>). For each movement direction, three trials were documented then averaged for the analysis purpose.

### Procedure

Hip flexion ROM was assessed while participants assumed supine lying position (Charlton et al., 2015). For each measuring trial of the hip joint, zero points for both DI and CA were set first; then, the measurement of hip flexion was performed. For both DI and CA, the instrument was placed over the thigh's central point (a midpoint between the lateral femoral condyle and greater trochanter measured from a relaxed neutral hip position). The participant was then instructed to flex her hip maximally with a flexed knee position. The hip flexion measurement was recorded when the participant experienced a firm end-feel. The maximum hip flexion is kept for three seconds, during which the measurement was recorded using both CA and DI. For measuring maximum knee flex-

ion range, the participant was positioned in prone lying with an extended knee in neutral rotation then DI was placed just above the lateral malleolus to record the zero point of the knee. This followed by asking the participant to flex her knee maximally and to keep this position for three seconds. The same measurement procedures were repeated using the CA. For measuring the ankle dorsiflexion and plantarflexion ROM, the participant assumed a prone lying position with the knee bent 90° (Rabin et al., 2014). The DI or CA was positioned in the lateral side of the foot (aligned with the fifth metatarsus) while measuring ankle ROM (Gouveia et al., 2014). For all measurements, consistent oral instructions were offered to ensure appropriate performance during the measurement and to confirm reaching the available end-range.

### Statistical analysis

Initially, the normality assumptions of parametric statistical tests were assessed based on the Shapiro-Wilk test, while the variance homogeneity was tested using Levene's test. To assess the validity of the CA compared to the DI, Pearson's correlation coefficients ( $r$ ) were calculated to examine the strength of association between the ROM scores of the CA and DI for the tested joints in sagittal plane movements. The power of the correlation was established according to the following standard:  $r < 0.19$  was considered as a very weak correlation,  $r = 0.2-0.39$  as weak,  $r = 0.40-0.59$  as moderate,  $r = 0.6-0.79$  as strong, and  $r = 0.8-1$  as very strong correlation (Campbell

& Swinscow, 2011). The level of statistical significance was set at  $P < 0.05$ . The statistical analysis was conducted with the Statistical Package for the Social Sciences (SPSS) (version 25, IBM, NY, USA) and Excel (Microsoft Office Excel, 2016). The intra-class correlation coefficient (ICC) was calculated to assess test-retest inter-session reliability of active hip, knee, and ankle ROM.

The ICC reliability values were interpreted as poor reliability ( $< 0.50$ ); moderate (0.50 to 0.75); good (0.75–0.90); and excellent ( $> 0.90$ ) (Portney & Watkins, 2009). The standard error of measurement (SEM) and 95% confidence intervals (CI) were computed to quantify the amount of error linked to the measurement. Calculation of SEM was made using the formula:  $SEM = \sqrt{\Sigma \text{ deviation}^2 / \text{degree of freedom}}$  (Bland & Altman, 1996). Also, the minimal detectable difference (MDD) was analysed to calculate the minimum threshold of measurement to give more confidence that differences between measurements were real and outside the error range. The MDD is calculated from the following formula:  $(SEM * 1.96 * \sqrt{2})$  (Weir, 2005).

### Results

The validity of the CA device against the DI, introduced as means and standard deviations, and ICC are provided in Table 1. The CA exhibited significant very strong correlations ( $r > 0.90$ ) with the DI for hip and knee flexion ROM measurements; however, the two devices displayed significant moderate correlations for ankle joint ROM measurements.

**Table 1.** Validity of the Digital inclinometer and Smartphone application for active hip, knee and ankle ROM. Mean data presented as mean  $\pm$  standard deviation

Variables	Digital Inclinometer	Smartphone application		
	Mean (°)	Mean (°)	ICC (95%CI)	$p$
Hip flexion	112.51 $\pm$ 9.28	112.67 $\pm$ 8.86	0.93 (0.89–0.95)	0.000
knee flexion	130.42 $\pm$ 8.99	129.48 $\pm$ 8.41	0.93 (0.88–0.96)	0.000
Ankle dorsiflexion	17.44 $\pm$ 2.18	17.74 $\pm$ 1.97	0.52 (0.36–0.69)	0.000
Ankle plantar flexion	34.54 $\pm$ 3.78	34.69 $\pm$ 3.54	0.57 (0.29–0.77)	0.000

Note. ICC: Intra-class correlation coefficient; CI: confidence interval;  $p$ : significance set at  $< 0.05$ .

Intra-tester reliability of the CA presented as ICC (95% CI), SEM, and MDD values are provided in Table 2. The ICC values for hip and knee flexion ROM exhibited excellent ( $> 0.90$ ) repeatability apart from ankle dorsiflexion and plantar-

flexion ROM; which reflected values in the moderate range repeatability. Furthermore, the SEM and MDD values can also be seen in Table 2, ranging from 1.45–4.29° and 4.01–11.89°, respectively.

**Table 2.** Intra-tester reliability results of the smartphone application (CA) for active hip, knee and ankle ROM

Variables	Smartphone application		
	ICC (95%CI)	SEM (°)	MDD (°)
Hip flexion	0.92 (0.87–0.96)	4.29	11.89
Knee flexion	0.95 (0.92–0.97)	3.10	8.60
Ankle dorsiflexion	0.67 (0.50–0.77)	1.45	4.01
Ankle plantar flexion	0.53 (0.23–0.70)	3.27	9.07

Note. ICC: Intra-class correlation coefficient; CI: confidence interval; SEM: standard error of measurement; MDD: minimal detectable difference

### Discussion

Although smartphone applications are easily used in clinical practice, it is critical to explore the validity, reliability, and limitations before using them. Having such applications without investigating their validity and measurement variability means that they have no clinical value (Wellmon et al., 2016).

To the best of our knowledge, this is the first study to assess the intra-tester reliability and validity of a smartphone application Clinometer for measuring active hip, knee, and ankle sagittal plane ROM. The CA demonstrated a very strong to a moderate association for LL measurements when compared to the DI. Furthermore, The CA demonstrated excellent reliability

for hip and knee ROM, and moderate reliability for the ankle ROM. Consequently, the present study encourages the use of the smartphone application (i.e., Clinometer) as an alternative to the DI in assessing LL sagittal plane ROM among healthy female participants.

The present study found that the validity and intra-tester reliability of the hip and knee ROM was greater than that of the ankle joint; however, the subsequent SEM and SDD scores were greater in hip flexion ROM, which may have contributed to a relatively greater standard deviation seen in hip flexion ROM, indicating a variation in performance across individuals. This finding is in close agreement with the results of Charlton et al. (2015), who reported excellent association of the smartphone application against the three-dimensional (3D) motion analysis system, with good intra-tester reliability (ICC=0.86). However, no direct comparison can be conducted due to the different populations, the smartphone application used (Hip ROM Tester against the 3D-motion analysis system), and testing the passive flexion ROM of the hip in the previous study.

A lack of similar studies in literature prevents direct comparison with the outcomes of the present study. However, previous studies stated that smartphone applications could be a useful measuring tool to evaluate knee ROM. Many researchers have demonstrated a high agreement of passive (Milanese et al., 2014; Ockendon & Gilbert, 2012; Santos et al., 2012), or active knee flexion ROM (Hambly et al., 2012; Jones et al., 2014) in healthy adults or knee osteoarthritic patients or those after knee arthroplasty (Mehta et al., 2017), and postoperative knee surgery (Pereira et al., 2017) against UG. Interestingly, the current study found excellent intra-tester reliability for the CA in evaluating knee flexion ROM. Such reliability indicated that the knee ROM data were reproducible over the two different testing sessions, with small measurement errors and great confidence in the measured data. This is in close agreement with prior studies that assessed the reproducibility of the iPhone goniometer app for evaluating knee ROM in osteoarthritic knee patients or those after knee arthroplasty (Mehta et al., 2017). They reported excellent reliability of the iPhone goniometer app (ICC=0.97) in evaluating active knee flexion compared to UG.

Moreover, the current study demonstrated a higher ICC value than that reported by Derhon et al. (2017), who showed a good intra-tester correlation (ICC > 0.85) of passive knee flexion ROM in healthy women. The SEM and MDD values in the present study seemed to be extremely similar to those of Mehta et al. (2017), who reported smaller SEM (2.72°) and MDD (6.3°) in assessing knee flexion ROM. However, a direct comparison cannot be made due to the use of a different mobile application, population, and the reliability conducted against UG rather than DI in our study. Consequently, it can be concluded that the CA has greater accuracy in measuring knee ROM when compared to the DI in healthy females.

Unlike the hip and knee, the CA demonstrated moderate correlation and agreement for ankle ROM compared to the DI. Although, the placement on the individual for the two instruments is consistent and the ROM measured by CA was nearly similar to that by DI with similar variability in both measurement tools in our study. Since changes up to 4.01 and 9.07 may be attributable to measurement error in dorsiflexion and plantarflexion ROM, respectively, therapists may be incapable of monitoring and evaluating the therapy on the basis of minor differences. The source of error may be related to

movement measured, the regional anatomy, the complexity of joint motion and the choice of testing position which depends on the purpose of the measurement (Rome & Cowieson, 1996). No study in the existing literature provided the validity of CA with DI in a non-weight bearing ROM position or for measuring plantarflexion ROM; however, previous literature showed excellent validity and reliability for smartphone app weight-bearing dorsiflexion ROM against DI (Vohralik et al., 2015; Williams et al., 2013) or UG (Alawna et al., 2019). This difference may have contributed to the greater stretch attained on soft tissue structures while the participant positioned in a weight-bearing lunge test that could influence the result.

Moreover, the results of our investigation are partially consistent with the outcomes of Vohralik et al. (2015). The SEM value for ankle dorsiflexion ROM of the current study was lower than the values of Vohralik's data (2.68°). However, their intra-tester reliability for the active ankle dorsiflexion ROM was relatively higher (ICC=0.76) than in our study. The difference may be due to different phone applications (iHandy Level) or due to the different measurement techniques (weight-bearing lunge test) used in the previous study. In the present study, the lack of consistency and heightened error in ankle measurements may also be due to poor validity. Consequently, the measure that has poor validity and reliability is not dependable and should not be utilized to obtain clinical decisions (Bhattacharjee, 2012; Norkin & White, 2016).

Regardless of its novel findings, the present study has a number of limitations. Firstly, the sample enrolled in the study was limited to young females. Such a study group could potentially not be able to detect the true variability across the demographic continuum and, consequently, additional research is required on males and also more patients' cohorts. Moreover, the validity and agreement of only sagittal plane ROM were evaluated in this study. Therefore, whether the same results would be achieved when assessing different plane motions is unclear. Future studies should be conducted LL ROM on different planes (e.g., hip frontal plane measurements). Lastly, the study was restricted to non-weight bearing ankle ROM. Therefore, additional studies are required to identify the difference in validity and reliability of smartphone application for ankle ROM in both conditions (weight-bearing versus non-weight-bearing positions).

## Conclusion

The results of the present investigation provided an easy-to-use, fast, portable, valid, and reliable approach for evaluating joint ROM in the sagittal plane. The CA and DI can be used interchangeably with confidence, as "Clinometer" possesses very strong validity. This study showed that the "Clinometer" had excellent repeatability in assessing hip and knee ROM in young, healthy females. Although ankle dorsiflexion and plantarflexion ROM possess values in the moderate range of repeatability, the two instruments cannot be used interchangeably in the clinic for assessing the ankle ROM in a non-weight-bearing position. Future research should be directed to provide the required knowledge about the other plane movements and different measurement positions.

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# Differences in Subject-Specific Competences between Slovenian and Italian Physical Education Teachers

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## Abstract

This cross-sectional study was designed to analyse the differences between the subject-specific competences of Slovenian and Italian physical education (PE) teachers. The participants, 669 Slovenian and 484 Italian PE teachers, evaluated their professional competences with a self-administered questionnaire on a four-level Likert scale. A t-test for independent samples indicates differences in the self-evaluations of the majority of subject-specific competences between both groups. However, in almost all items, Slovenian PE teachers evaluate their competences higher. A multivariate analysis of variance, used to identify the role of some socio-demographic factors (state, gender, years of service), shows that the state has the highest impact on the differences between self-efficiency of both groups ( $p < 0.001$ ,  $\eta^2 = 0.531$ ). The greatest differences (Cohen's  $d > 0.8$ ) are observed on those competences that relate to some of the narrower aspects of PE didactics. The reasons for the perceived differences can be found in some historical and social events, length of education, the different orientation of PE teacher education programmes, and the different responses of both countries to educational policies. The present study outcomes may aid in updating initial PE teacher training and designing a creative system of continuous professional development.

**Keywords:** *physical education, educators, self-efficiency, cross-cultural study*



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## Introduction

In the 1990s, when “competences” became one of the key terms in professional education, many attempts were made to classify and describe this elusive and multi-layered term. The term “competence” involves tacit and explicit knowledge, cognitive and practical skills, as well as motivation, beliefs, value orientations, and emotions (Rychen & Salganik, 2003).

The European network Eurydice (2003) divided teachers' competencies into general (universal or subject-independent) and specific ones closely related to an individual subject area. Physical education (PE) is included in all national school systems as a vital part of children's development; however, national curriculum documents, material conditions for implementing PE, and physical education teacher education (PETE) programmes vary considerably

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between the countries (Hardman, 2008). The competences of PE teachers from different European countries have been studied (Casolo et al., 2019; Gallardo, 2006; Kovač et al., 2008; Romero Granados & Campos Mesa, 2010; Tul et al., 2019; Vitali & Spoltore, 2010). In contrast to the intracultural factors, little attention has been paid to the intercultural factors of PE teachers' self-efficacy. After an extensive literature review, there has only been one cross-cultural study investigating the importance of PE teacher's competences (Nieminen et al., 2008), which showed that Finnish PE students generally value the importance of pedagogical skills and the ability for collaborative relationships as more important than Greek, Japanese and Dutch students do. The authors also reflected that the country's whole culture and educational tradition have a more important influence on the development of the students' perceptions of a PE teacher's competences than the national PETE programmes. Laporte (1997) emphasized the importance of a common (i.e., European) approach to PE, including uniform concepts and PETE programmes. Thus, the question of teachers' competences needs to be set in the wider context of the European Union's work to ensure high quality educational outcomes (European Commission, 2013). Therefore, we conducted a study to evaluate the differences between the self-perceived subject-specific professional competences of Slovenian and Italian PE teachers.

## Methods

### Subjects

The sample included 669 Slovenian and 484 Italian PE teachers. The Slovenian sample consisted of 399 male (59.6%) and 270 female (40.4%); 423 (63.2%) participants were employed at primary schools (6<sup>th</sup> to 9<sup>th</sup> grades), 212 (31.7%) participants at secondary schools (10<sup>th</sup> to 13<sup>th</sup> grades), and the remaining participants were employed elsewhere (1<sup>st</sup> to 5<sup>th</sup> grades, project work). Almost half of the participants had been teaching more than 20 years ( $n=301$ , 45%); the remaining participants are almost equally distributed in the following groups: from 11 to 20 years of working experience ( $n=185$ , 27.7%), and up to 10 years of working experience ( $n=183$ , 27.3%). Most of the participants ( $n=514$ , 76.8%) have finished a four-year university PETE programme.

The sample of Italian PE teachers consisted of 209 male (43.2%) and 275 female (56.8%) from the north-eastern part of Italy; 196 (40.5%) participants were employed at lower secondary schools (6<sup>th</sup> to 8<sup>th</sup> grades), 244 (50.4%) participants at upper secondary schools (9<sup>th</sup> to 13<sup>th</sup> grades), and the remaining participants were employed elsewhere (primary schools, project work). Almost two thirds of participants had been teaching more than 20 years ( $n=338$ , 69.8%); the remaining participants are almost equally distributed in the following groups: from 11 to 20 years of working experience ( $n=68$ , 14%) and up to 10 years of working experience ( $n=78$ , 16.1%). Most of the participants ( $n=402$ , 83.1%) had finished a three-year higher school for PE.

### Procedures

As with previous studies (Gallardo, 2006; Kovač et al., 2008), a self-administered questionnaire was constructed. Before administration, the questionnaire was qualitatively validated for content by a group of 22 PE teachers (10 male

and 12 female) of different ages, amounts of working experience, and working at different teaching levels. The original questionnaire was shortened slightly, as per the recommendations of the validation group. The questionnaire consisted of three parts: demographic (gender, length of work experience, age, teaching level), general competences (36 items) and specific competences (40 items). For the purpose of this paper, only the self-perception of specific competences is used. Teachers reported their current competences on a four-level scale (1-completely incompetent, 2-less competent, 3-competent, and 4-highly competent).

This study was approved in advance by the Ethics Committee of the University of Ljubljana. The questionnaire was sent to all Slovenian primary ( $n=449$ ) and secondary schools ( $n=137$ ) and all Italian lower ( $n=807$ ) and upper ( $n=310$ ) secondary schools in the regions of Friuli-Venezia-Giulia and Veneto by regular mail with an invitation to PE teachers to complete it. Teachers were informed about the objectives of the study and the voluntary and anonymous nature of their participation. A total of 681 Slovenian and 495 Italian questionnaires were returned; 551 questionnaires (80.6%) were fully completed. Twenty questionnaires were missing more than three pieces of data and were thus excluded from the study. Questionnaires with one to three pieces of data missing (Slovenia:  $n=121$ , 17.7%; Italy:  $n=87$ , 17.6%) had the missing data imputed with the use of an E-M algorithm. In the end, 669 Slovenian and 484 Italian questionnaires were included in the analysis. According to data from the Slovenian Ministry of Education, Science and Sport and the Regional School Office of Trieste, Italy, the sample represented approximately 52% of the entire population of PE teachers in Slovenia and 28% in both Italian regions.

### Statistical analyses

The data were analysed with the IBM SPSS Statistics 23.0 software. The basic statistics of variable distribution were calculated. The differences between both groups of teachers were tested with a t-test for independent samples. The statistical significance of the differences between the groups was tested at the  $\alpha = 5\%$  error level. Cohen's  $d$  was used as a standardized measure of the size of differences between the two countries. A multivariate analysis of variance (MANOVA) is used to identify the role of some socio-demographic factors (gender, state, years of service) in explaining the differences between teachers in their teaching competences.

## Results

The entire questionnaire has a high degree of reliability (Cronbach's  $\alpha=.97$ ), while the values of individual segments of the questionnaire vary between .77 and .92.

Both groups have recognized the highest level of competences in the following areas: understanding health aspects of physical activity (PA) and sport; understanding PE curriculum; being able to use different teaching methods and forms of teaching PE; understanding the social importance of sport (Table 1).

Both groups reported low competences in the following areas: understanding financial flow in sport; qualification for working with modern teaching technology (MTT) in sport; and understanding the philosophical aspects of sport (Table 1).

**Table 1.** Descriptive statistics of professional competences items sorted by Cohen's d index size

	t	p	Slovenia		Italy		Cohen's d
			M	SD	M	SD	
Recognizing sport-talented students and their guidance	10.71	<0.001	3.40	0.61	2.98	0.73	0.96*
Qualification for demonstrating skills that are a part of curriculum	9.84	<0.001	3.36	0.60	2.99	0.70	0.89*
Respecting principles of inclusion, individualization and differentiation	3.85	<0.001	3.11	0.62	2.74	0.71	0.84*
Understanding methodical ways in teaching skills that are not a part of the curriculum	9.84	<0.001	3.01	0.64	2.60	0.78	0.84*
Qualification for pedagogical management of class in PE	8.38	<0.001	3.40	0.61	3.09	0.64	0.81*
Understanding physical and motor development of children and youth	8.06	<0.001	3.46	0.57	3.17	0.63	0.81*†
Qualification for working with MTT in sport	9.48	<0.001	2.78	0.70	2.37	0.77	0.78*†
Understanding methodical ways of teaching motor skills from the curriculum	7.29	<0.001	3.51	0.54	3.26	0.65	0.74*†
Qualification for demonstrating skills that are not a part of the curriculum	8.91	<0.001	2.96	0.70	2.56	0.80	0.72*†
Qualification for different ways of assessment and grading knowledge in PE	6.69	<0.001	3.30	0.63	3.04	0.68	0.61*†
Understanding PE curriculum	5.08	<0.001	3.58	0.55	3.38	0.66	0.58*†
Ability to use different teaching methods and forms of teaching PE	5.56	<0.001	3.49	0.55	3.30	0.60	0.58*†
Qualification for encouraging students to be sport active in free time	6.11	<0.001	3.44	0.61	3.21	0.68	0.57*†
Organizational skills and knowledge for the implementation of school and extracurricular programmes	6.61	<0.001	3.37	0.64	3.10	0.75	0.57*†
Understanding general didactics of PE process	5.60	<0.001	3.36	0.60	3.15	0.65	0.54*†
Qualification for evaluation of own pedagogical work in PE	5.54	<0.001	3.25	0.63	3.04	0.63	0.52*†
Qualification for setting goals according to curriculum	4.91	<0.001	3.36	0.63	3.16	0.70	0.45*†
Qualification for encouraging personal progress of a student	4.57	<0.001	3.33	0.61	3.17	0.62	0.45*†
Understanding historical aspects of sport	5.02	<0.001	2.96	0.69	2.75	0.77	0.42*†
Qualification for planning a process according to status analysis and curriculum	4.52	<0.001	3.22	0.63	3.05	0.66	0.42*†
Qualification for formation and conveying of feedback information	4.50	<0.001	3.32	0.60	3.15	0.68	0.42*†
Qualification for efficient conveying of theoretical contents in PE lessons	4.76	<0.001	3.20	0.65	3.00	0.74	0.41*†
Understanding anatomical-functional aspects of sport	3.41	0.001	3.39	0.60	3.27	0.62	0.34*†
Understanding health aspects of PA and sport	2.94	0.003	3.56	0.54	3.46	0.56	0.32*†
Understanding how to use different pedagogic strategies	3.85	<0.001	2.84	0.71	2.67	0.77	0.31*†
Understanding the importance of continuous professional development for PE teacher	3.30	0.001	3.38	0.62	3.25	0.68	0.31*†
Understanding physiological aspects of sport	3.06	0.002	3.35	0.61	3.24	0.63	0.30*†
Qualification for encouraging creativity in finding solutions to motor tasks	3.34	0.001	3.10	0.64	2.96	0.70	0.30*†
Understanding psychological aspects of sport	3.13	0.002	3.25	0.62	3.13	0.69	0.29*†
Understanding theory of practising sport	3.41	0.001	3.18	0.68	3.04	0.75	0.29*†
Qualification for inter-subject connection of PE with other subjects	2.45	0.014	2.96	0.65	2.86	0.72	0.22*†
Understanding social circumstances in PE lessons	2.25	0.024	3.26	0.62	3.18	0.65	0.21*†
Understanding financial flow in sport	2.72	0.006	2.50	0.79	2.37	0.80	0.20*†
Qualification for encouraging student's learning in an instructive and creative way	1.99	0.470	3.18	0.60	3.11	0.66	0.19 †
Understanding biomechanical aspects of sport	2.11	0.030	3.00	0.72	2.90	0.72	0.18*†
Understanding media influence on sport	1.25	0.210	2.88	0.73	2.82	0.77	0.10 †
Understanding cultural aspects of PE	-1.07	0.280	3.17	0.65	3.21	0.68	-0.10 †
Understanding philosophical aspects of sport	-1.56	0.118	2.67	0.81	2.74	0.83	-0.11 †
Understanding social importance of sport	-1.23	0.210	3.40	0.59	3.45	0.61	-0.12 †
Qualification for diagnosing and composing status analysis	-4.80	<0.001	3.07	0.66	3.26	0.63	-0.44*†

Note. M = mean; SD = standard deviation; \* p<0.05 (unadjusted); † p<0.05 (adjusted for gender and years of service).

Statistically significant differences between both groups were shown in 35 out of 40 competences (Table 1, \* symbol); in all (except qualification for diagnosing and composing status analysis), Slovenian PE teachers evaluated their

competences statistically significantly higher than Italian ones did. In most cases, the differences were statistically significant even after adjusted for gender and years of service (Table 1, † symbol). The differences were greater (Cohen's

$d > 0.8$  means large differences; Cohen, 1988) in those competences that related to some of the narrower aspects of PE didactics, such as recognizing sport-talented students and their guidance; respecting principles of inclusion, individualization and differentiation; qualification for demonstrating skills that are a part of the curriculum and that are not

included in it; understanding the methodical ways in teaching skills that are not a part of the curriculum and those in it. There were also differences in perceptions of one's competence in successful pedagogical management during the PE lessons, understanding children and youth's physical and motor development, and working with MTT in PE lessons.

**Table 2.** MANOVA table for the multivariate differences in professional competences between states controlled for gender and years of service

Effect	$\lambda$	F	df (1)	df (2)	p	Eta <sup>2</sup>
State	0.469	15.901	76	1,066	<0.001	0.531
Gender	0.785	3.831	76	1,066	<0.001	0.215
Service (years)	0.717	2.538	152	2,132	<0.001	0.153
State $\times$ gender	0.895	1.642	76	1,066	0.001	0.105
State $\times$ service	0.825	1.417	152	2,132	0.001	0.092
Gender $\times$ service	0.862	1.084	152	2,132	0.236	0.072
State $\times$ gender $\times$ service	0.869	1.024	152	2,132	0.408	0.068

Note.  $\lambda$  = Wilks' lambda; df = degrees of freedom; p = p-value; Eta<sup>2</sup> = partial eta squared (estimate of effect size).

Univariate interactions between professional competences, controlled by state, gender, and years of service were statistically significant for the whole set of variables (Table 2). The biggest differences between the groups are explained by the state ( $p < 0.001$ , Eta<sup>2</sup> = 0.531). Also significant are the two-way interactions of state with gender and state with years of service; however, the interactions between gender and years of service, as well as the three-way interaction between state, gender and years of service, were negligible.

## Discussion

The main findings of the study are i) PE teachers are able to evaluate their own perceived level and, at times, lack of competences critically; ii) the state has the highest impact on differences between both groups of teachers; iii) Slovenian PE teachers perceive their subject-specific competences to be much higher than the Italian ones do; iv) the greatest differences are observed on those competences that relate to some of the narrower aspects of PE didactics.

### *The highest evaluated competences of both groups*

It is known that PE can play a significant role in reducing sedentary behaviour and contributing to public health (McKenzie & Lounsbury, 2013) and that establishing PA as a habitual behaviour in children can result in active adult lifestyles (Pate, 1996). McKenzie and Lounsbury (2013) even emphasize that the survival of PE programmes in schools depends largely on how effective PE teachers are in operating within a public health context. Italian PE teachers have placed the competence understanding health aspects of PA and sport in the first place ( $M = 3.46$ ) and Slovenian in second place ( $M = 3.56$ ). The understanding of the PE curriculum as a basic guideline that supports the teacher's work is highly rated ( $MSLO = 3.59$ ;  $MITA = 3.38$ ) by both groups. Both curricula are quite precise in guiding the teachers to identify PE as a place for learning about and through PA participation, and teachers have been working following them for a long time. Teachers specific didactic knowledge, such as being able to use different teaching methods and forms of teaching, is one of the most valuable competences for PE teachers (Casolo et al., 2019; Romero Granados & Campos Mesa, 2010). Also in this study, teach-

ers' sense of efficacy in student-teacher interaction in practice-learning experience by using different teaching methods and forms of teaching is high ( $MSLO = 3.49$ ,  $MITA = 3.30$ ); this is significant because their self-efficacy is strongly associated with the achievement levels of students (Klassen & Tze, 2014). Both Slovenian and Italian PE teachers perceive themselves to be sufficiently competent in understanding the social importance of sport ( $MSLO = 3.40$ ;  $MITA = 3.45$ ), which has also been confirmed by Vitali and Spoltore (2010), who found that belief in the important social significance of sport is high among Italian PE teachers. Sport can bring people together, especially with great sporting success (Starc, 2010); furthermore, sport is a means of the effective socialization of those children who come from marginalized groups such as immigrants and Roma (Starc & Klinčarov, 2016). Therefore, Slovenian PE teachers pay attention to the wider social context of sport.

### *The lowest evaluated competences of both groups*

Both groups rated their competence for understanding financial flow in sport very low ( $MSLO = 2.50$ ,  $MITA = 2.37$ ). Until recently, PE has been very firmly established in Slovenian educational curricula, and the conditions for work have been continuously improving since 1990; therefore, teachers did not pay attention to the wider financial context and the influence of governing political options, which have been observed in other countries (Kovač, 2011). This is frequently encountered by Italian PE teachers as they have to provide the financial resources to support many out-of-school activities (Tul et al., 2019).

In the digital age, knowledge of MTT applied to subject teaching is fundamental for teachers; even so, the use of MTT is one of the weakest points of European teachers, as it is seldom included in the lessons, presumably due to the insufficient knowledge for its effective use and also negative attitudes toward the new technology (Eurydice, 2019). It has been particularly observed in the present study, as the use of MTT in PE was the lowest marked competence among Italian ( $M = 2.37$ ) and the third-lowest among Slovenian teachers ( $M = 2.78$ ). As the correlation between the ability of teachers to use MTT and their age has been confirmed in other studies (Gianferrari, 2009; Sitar, 2010), the results can be explained by

the age of teachers included in the present study. Despite the low evaluation for understanding the philosophical aspects of sport, there are no statistically significant differences between the groups, presumably due to their lower influence in PE lessons.

#### *The competences in which the groups differ most*

The biggest discrepancy between Slovenian and Italian PE teachers is revealed in some competences related to specific didactic approaches to special groups of students and a narrower sense of teaching PE (Cohen's  $d > 0.8$ ; Table 1). Both groups perceive the average level of their competences; however, in all items, Slovenian PE teachers value their competency higher. Recognizing sport-talented students and their guidance differentiates both groups most (Cohen's  $d = 0.96$ ; Table 1). Sport-talented children require extra attention and an individual approach to PE. Slovenian teachers, especially the older ones, are still very performance-oriented, and most of them consider PE to be the most important environment for recognizing and supporting this group of children (Kovač et al., 2008). Empirical investigations have also shown that the profession of PE teacher is mostly chosen by people who have been successful in sport at a high level (Edmonds & Lee, 2002). Their positive experience in sport influences their understanding of the image of PE teacher and the core mission of PE.

Qualification for working with students with special needs and acceptance of diversity are important characteristics of highly qualified PE teachers (Napper-Owen et al., 2008). Both groups of teachers are critical in assessing how to respect principles of inclusion, individualization and differentiation in PE lessons, particularly when working with students with special needs, behaviourally challenged, health-endangered or children from different cultural environments; however, Italian teachers scored themselves much lower (Cohen's  $d = 0.84$ ). Ethnicity in Italy is highly relevant, as the population in some areas is significantly multicultural, with the number of immigrants from Africa and Asia increasing each year (Tul et al., 2019). At the same time, it seems that low self-efficacy among Slovenian PE teachers about how to work with students with special needs constitutes the most important pedagogical obstacles for their successful inclusion into regular PE classes (Kovač et al., 2008).

There are also differences in the ability to demonstrate various sport skills, which are a part of the PE curriculum (Cohen's  $d = 0.89$ ), and the ability to use appropriate methodical steps in teaching skills that are not a part of the curriculum (Cohen's  $d = 0.84$ ). Tinning (2010) described this pedagogical expression as demonstrate-explain-practice. Demonstration is the most important teaching method in the PE process, especially in all post-socialistic countries; it seems that Slovenian PE teachers still conceptualize skill-based practice in PE dichotomously (Kovač et al., 2008). Therefore, it is understandable that a high value has been given to the ability to demonstrate all sport skills.

The differences in perception are particularly prominent in successful pedagogical management of PE lessons and in understanding the physical and motor development of children and youth (both Cohen's  $d = 0.81$ ). In a time of increased emphasis on academic results and some behavioural problems of students, Slovenian PE teachers seem to be adequately qualified for the successful pedagogical management of PE lessons. Understanding physical and motor development as

part of a broader (social, cognitive, etc.) development is crucial for planning developmentally appropriate pedagogical approaches and developmentally appropriate activity selection. For more than three decades, Slovenian PE teachers have been able to use the data about physical fitness of students from the SLOfit national surveillance system (Kovač et al., 2011). According to didactic theory, planning needs to be done on the grounds of prior analyses in order to take a child's individual potentials and characteristics into account (Casolo et al., 2019); therefore, it is not surprising that the perception of this competence is higher among Slovenian PE teachers than among the Italian ones.

The results of this study show that the state has the highest impact on differences between both groups of teachers. A higher self-efficacy among Slovenian PE teachers can be explained with cultural, historical, social influences on teacher identity formation, as well as the length of education: Slovenian PE teachers are dominated by those who have completed the pre-Bologna four-year study programme, while most of the Italian teachers completed a three-year study programme. The notion of national identification through sport is an important factor, as top Slovenian athletes' exceptional results have the strongest influence on its formation (Starč, 2010). After Slovenian independence in 1991, and the school reform of 1996, which made PE more important in the education system, the position of PE teachers and, above all, the sports infrastructure have also improved (Kovač et al., 2011). The lower estimations of their competences among Italian PE teachers have probably been influenced by historical events, which have defined both the contents and the etymological categorization of PE in Italy as well as a late Italian response to the Bologna reforms (Vitali & Spoltore, 2010). Only in 1998 did Italy fund faculties of sports and motor sciences, which replaced higher schools for PE. However, the stereotypical image of a former PE teacher educated at the old three-year study programme and the classical perception of PE in Italy remain among Italian PE teachers.

We believe that this cross-cultural research could clarify how efficacy beliefs of PE teachers originate under different social and institutional practices. Even more importantly, the findings can help increase the mobility and employability of graduates from different European countries, inform policy decisions about the main purposes of PE, and the importance of the European approach to PE professions. The additional value of this research is the facilitation of a discussion about how different states can systematically encourage teacher self-reflection, review their curricula, (re)design their PETE programmes, and supplement initial teacher training with high-quality further professional development programmes.

When interpreting the results, a certain degree of care is required. First, PE teachers from only one part of Italy are included in the Italian sample. Second, the cross-sectional design of the study gives a rather static view of the dynamic interaction between teachers and society. In the future, longitudinal studies that account for changes over time and provide adequate explanations would be beneficial.

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# Physical Demands in Three Different Basketball Competitions Played By the Same Under-18 Players

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## Abstract

The goal of this study was to compare the physical demands of the same team in three different basketball competitions (EBA league (EBA), U18 regional league (U18L), and a U18 international tournament (U18T)) during the same season. Data from eleven U18 players (age:  $16.92 \pm 0.67$  years) were collected using inertial movement units. As external load variables, Player Load (PL), accelerations (ACC), decelerations (DEC), changes of direction (COD), and jumps (JUMP) were expressed in their total (t) and high intensity (h) values. The analysis of variances (ANOVA) and effect size (ES, Cohen's d) with their respective 90% confidence intervals were applied to identify differences between the competitions. U18T showed the highest values in PL, tACC, tDEC, hDEC, tCOD, tJUMP, and hJUMP (small to moderate ES). However, the hACC and hCOD values were greater in EBA (small ES) than in U18L and U18T. In conclusion, all three competitions presented different external load demands for the same group of players. This data could help basketball coaches to optimize the training process based on the competition in which their team plays. Furthermore, data could also indicate the most suitable competition for players' development.

**Keywords:** *basketball, inertial movement analysis, team sport, monitoring, training load*



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PHYSICAL DEMANDS OF THREE DIFFERENT COMPETITIONS

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## Introduction

Basketball is a team-based and opposition sport in which high-intensity actions, such as accelerations, changes of direction, and jumps, are combined with moments of pause during games (Stojanovic et al., 2018). It is important to quantify the physical demands experienced by players during different basketball competitions to improve the process of players' sports growth from lower categories until professionalism (Petway et al., 2020).

Most clubs have programmes to select and develop the tal-

ent of young basketball players. Through training, their final purpose is to prepare potential players for the training and competition demands of professional basketball (Sotiriadou & Shilbury, 2013). Various strategies have been proven to be successful during the development of sports talent, such as the theories of 10 years and deliberate practice (Ericsson et al., 1993; Newell & Rosenbloom, 1981). However, there are critical moments in the maturation process at which development can be greatly stimulated if the players engage with situations

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that require them to perform at their optimal level (Berry et al., 2008). These situations can range from applying the appropriate training stimulus at a certain age to being able to face the best possible rival to demanding the greatest technical, tactical, and physical requirements.

At present, the competition model that exists in Spain allows players who are still in training age categories (Junior/U18) to participate in professional or semi-professional competitions at the national level. This is a structured development model forming part of a long-term strategic one (Calleja-González et al., 2016). However, there are challenges in the physical demands that players must overcome to compete at the professional level. As the league level increases, players are required to perform a greater number of high-intensity and moderate-intensity actions than in the minor leagues (Scanlan et al., 2011), which suggests planning long-term training. Furthermore, players need to be prepared for a greater demand for high-intensity moments and shorter breaks between situations (Feriolli et al., 2020). In addition to higher physical demands, professional competitions also show increased technical and tactical demands and a higher percentage of effectiveness to which junior players must adapt (Conte et al., 2017).

Differences between U18, professional, and senior players have been described (Trapero et al., 2020). However, all studies compared different samples (one of the senior players and the other of under-18 players). Therefore, there is a comparative bias due to a multitude of factors, such as the game model, the rival, the player's physical characteristics, and the refereeing of the match (Fox et al., 2019). To the authors' knowledge, there is no study comparing the same players competing at different levels. This study would provide valuable information to assess

the differences that may exist in basketball physical demands more accurately.

This work aimed to describe and compare the physical demands of the same team of basketball players during three different competitions completed in the same season. The results could help to optimize the training process of players simultaneously participating in different competitions. Moreover, this investigation could also aid in understanding the optimal competition for the players to receive adequate physical stimulation during training.

## Methods

### Design

Data were collected in three different competitions: EBA League (EBA, 4<sup>th</sup> senior category of Spanish basketball); Regional League of under 18 players (U18L); international tournament of the under-18 category (U18T). Two leagues (EBA and U18L) were analysed from September to March, corresponding to the first part of the competitive period. The U18T took place between the 3<sup>rd</sup> and 6<sup>th</sup> of January; four games were played in three days between junior international players. All games of the three competitions were monitored through inertial movement units (IMU). In total, 32 matches were recorded (15 from EBA, 13 from U18L, and four from U18T).

### Participants

Eleven players in the U18 age-level (age:  $16.92 \pm 0.67$  years) participated in the study. Before the study, the anthropometric data for each player were collected following the standards of technical measurement recommended by the International Working Group of Kinanthropometry, ISAK (Table 1).

**Table 1.** Anthropometric values of the players participating in the study. Total values are presented as mean  $\pm$  standard deviation (SD)

Participants	Height (cm)	Weight (kg)	BMI (kg/m <sup>2</sup> )	Sum 8 Skinfolds (mm)	Body fat (%)	Muscle mass (%)
Player 1	195.5	98.4	25.7	66.5	8.1	50.6
Player 2	185.0	76.1	22.2	57.6	6.6	49.5
Player 3	186.6	69.1	19.8	45.6	7.5	49.1
Player 4	209.0	97.6	22.3	103.1	13.3	41.6
Player 5	200.1	84.0	21.0	52.0	5.8	48.7
Player 6	203.0	75.1	18.2	36.0	3.2	51.0
Player 7	200.1	74.7	18.6	46.5	4.9	48.4
Player 8	202.5	91.7	22.4	65.0	7.8	49.0
Player 9	201.7	84.6	20.9	71.0	9.3	46.0
Player 10	211.6	100.7	22.5	65.0	6.1	49.2
Player 11	202.0	76.2	18.6	46.2	4.7	42.6
Team average	200.9 $\pm$ 7.2	84.4 $\pm$ 11.1	21.1 $\pm$ 2.2	58.5 $\pm$ 18.1	6.9 $\pm$ 2.6	48.3 $\pm$ 2.4

The anthropometric material used was previously approved and calibrated: wall height rod (precision, 1 mm); Tanita scale (precision, 100 g); Rossraf metric metal tape, which was narrow and inextensible (precision, 1 mm); Holtain small bone diameter pachymeter (precision, 1 mm); Cescorf2 calliper (precision, 0.5 mm); complementary material (demographic pencil to mark the subject); and anthropometric bench of 40  $\times$  50  $\times$  30 cm. Carter (1982) and Lee's (Lee et al., 2000) formulas were used to calculate fat and muscle percentages. All players belonged to the same team of a Euroleague (European first division) and ACB (Spanish first division) academy in Spain and simultaneously participated in the three competitions. Players

completed a total of five to six weekly basketball training sessions, along with two to three strength-training sessions and two games each week. Each player was informed about the study requirements by the club, and each one gave his written and verbal consent. Moreover, all the ethical procedures used in this study were in accordance with the Declaration of Helsinki, Fortaleza update (Harris & Atkinson, 2015) and were approved by the Basque Country University Ethics Committee.

### External load variables

As in previous studies in basketball (Svilar et al., 2018; Salazar et al., 2020), a combination of variables was used to

estimate the frequency of total and high-intensity actions. This strategy provided more detailed information on the physical demands of the players (Fox et al., 2020). Inertial variables included PlayerLoadTM (PL); changes of direction ((COD), directions from -135° to -45° to the left, and from 45° to 135° to the right); decelerations ((DEC), direction from -135° to 135°); accelerations ((ACC), directions from -45° to 45°); and jumps (JUMP). The PL was recorded by the accelerometer of the inertial devices whose sampling frequency is 100-Hz, and it is represented by the square root of the changes in acceleration in the three spatial axes (Nicoletta et al., 2018). The variables COD, DEC, ACC, and JUMP are shown in their total (t) and high intensity (h) values. We established the threshold at  $\pm 3 \text{ m}\cdot\text{s}^{-2}$  for COD, DEC, and ACC, and at  $> 40 \text{ cm}$  for JUMP. All data were relativized to the total minutes in the field of each player (min-1), excluding rest times and time-outs. This allowed a comparison regardless of the difference in minutes played by each player during the matches analysed.

#### Procedures

All official matches were based on FIBA basketball rules, with four quarters of 10 minutes of live time, two minutes of rest between quarters, and 15 between halves. We used inertial devices to monitor external load during matches (Catapult T6, Catapult Innovations, Melbourne, Australia). This technology had been previously validated in small field indoor sports (Luteberget et al., 2018). Devices were placed in the players' scapular area through a custom vest and were turned on 30 minutes before the matches started. Players were familiar with the devices as they used them in their day-to-day training at the club. After their recording,

all data was exported through specific software (OpenField version 2.3.1, Catapult Innovations, Melbourne, Australia). Afterwards, the data was processed in an Excel workbook for Mac (Microsoft Excel version 16, Microsoft Corporation, Redmond, WA, USA).

#### Statistical analysis

All descriptive data are shown as mean  $\pm$  standard deviation (SD). The normality of the data distribution and the sphericity was confirmed using the Shapiro-Wilk test and the test of Levene for the equality of variances, respectively. The differences between groups were examined using a statistical analysis of variances (ANOVA) and taking the "competition" dimension (EBA, U18L and U18T) as a factor. Statistical significance was established at  $p < 0.05$ . Subsequently, effect sizes (ES) were calculated using Cohen's  $d$  with their respective confidence intervals of 90%. The ES were interpreted based on the following thresholds:  $< 0.2$ , trivial; 0.2-0.6, small; 0.6-1.2, moderate; 1.2-2.0, long; and  $> 2.0$ , exceptionally long (Batterham and Hopkins, 2006). All of the analyses were performed by using the Microsoft Excel software for Mac (Microsoft Excel version 16, Microsoft Corporation, Redmond, WA, USA) and the free statistical package JASP version 0.9.2 (University of Amsterdam, <https://jasp-stats.org/>).

#### Results

Table 2 shows the absolute values of all external load variables analysed for each competition (EBA, U18L, and U18T). The highest values for PL, tCOD, tACC, DEC component, and JUMP variables were found in U18T competition. EBA competition showed the greatest demands in hCOD and hACC.

**Table 2.** Mean  $\pm$  standard deviation (SD) for each of the external load variables in the three competitions analysed: EBA League (EBA), U18 autonomic league (U18L) and international tournament (U18T)

	EBA	U18L	U18T	<i>p</i>
PL (ua·min <sup>-1</sup> )	11.02 $\pm$ 0.93	11.08 $\pm$ 1.09	11.39 $\pm$ 1.37	<i>p</i> = 0.17
tCOD (ua·min <sup>-1</sup> )	9.98 $\pm$ 2.64	9.50 $\pm$ 2.47	10.34 $\pm$ 2.44	<i>p</i> = 0.11
hCOD (ua·min <sup>-1</sup> )	0.65 $\pm$ 0.31	0.57 $\pm$ 0.28	0.59 $\pm$ 0.35	<i>p</i> = 0.05
tDEC (ua·min <sup>-1</sup> )	2.18 $\pm$ 0.48	2.21 $\pm$ 0.55	4.07 $\pm$ 3.54 <sup>b c</sup>	<i>p</i> < 0.01
hDEC (ua·min <sup>-1</sup> )	0.18 $\pm$ 0.08	0.18 $\pm$ 0.09	0.28 $\pm$ 0.24 <sup>b c</sup>	<i>p</i> < 0.01
tACC (ua·min <sup>-1</sup> )	2.25 $\pm$ 0.61	2.11 $\pm$ 0.62	2.26 $\pm$ 0.71	<i>p</i> = 0.13
hACC (ua·min <sup>-1</sup> )	0.38 $\pm$ 0.16 <sup>a</sup>	0.32 $\pm$ 0.16	0.33 $\pm$ 0.20	<i>p</i> = 0.02
tJUMP (ua·min <sup>-1</sup> )	1.55 $\pm$ 0.42	1.56 $\pm$ 0.42	1.67 $\pm$ 0.52	<i>p</i> = 0.35
hJUMP (ua·min <sup>-1</sup> )	0.19 $\pm$ 0.11	0.20 $\pm$ 0.12	0.22 $\pm$ 0.13	<i>p</i> = 0.30

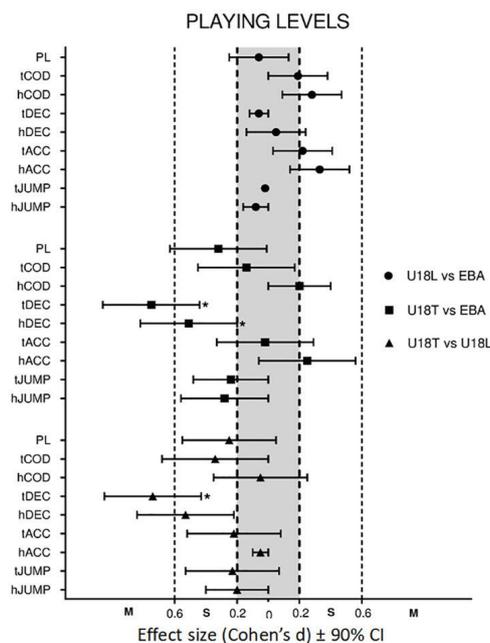
Note. a, significant differences EBA vs U18L; b, significant differences EBA vs U18T; c, significant differences U18L vs U18T. PL is Player Load per player; tCODmin is the total number of movements to the right / left; hCOD is the number of movements to the right / left above the high-intensity threshold ( $> 3 \text{ m}\cdot\text{s}^{-2}$ ); tDEC is the total number of decelerations; hDEC is the number of high-intensity decelerations ( $< -3 \text{ m}\cdot\text{s}^{-2}$ ); tACC is the total number of accelerations; hACC is the number of high-intensity accelerations ( $> 3 \text{ m}\cdot\text{s}^{-2}$ ); tJUMP is the total number of jumps; and hJUMP is the number of jumps above 40 cm. Bold letters highlight the higher value of the three competition levels.

Figure 1 presents ES for all variables, comparing all competitions. Results of the EBA and U18L comparison showed small ES on hCOD ( $p > 0.05$ ), tACC ( $p > 0.05$ ), and hACC ( $p = 0.02$ ) (EBA  $>$  U18L).

The comparison between U18T and EBA is displayed in the medial part of Figure 1. In this case, small ES are shown in the PL ( $p > 0.05$ ), hDEC ( $p > 0.05$ ), and JUMP ( $p > 0.05$ ) variables (U18T  $>$  EBA). tDEC was the only variable that

showed a significant moderate effect in favour of U18T ( $p < 0.01$ ); in contrast, a small non-significant difference was found in hACC between the competition in favour of EBA.

In the lowest part of Figure 1, results from a comparison between the two U18 competitions are shown. All variables expressed higher demands in U18T with a significant moderate ES in the tDEC variable. In addition, PL, tCOD, and hDEC presented small ES compared to U18L.



**Figure 1.** Effect size (Cohen's d) with 90% confidence interval on the differences between the three competitions (EBA, U18L and U18T). PL is Player Load per player; tCODmin is the total number of movements to the right / left; hCOD is the number of movements to the right / left above the high-intensity threshold ( $> 3 \text{ m} \times \text{s}^{-2}$ ); tDEC is the total number of decelerations; hDEC is the number of high-intensity decelerations ( $< -3 \text{ m} \times \text{s}^{-2}$ ); tACC is the total number of accelerations; hACC is the number of high-intensity accelerations ( $> 3 \text{ m} \times \text{s}^{-2}$ ); tJUMP is the total number of jumps; hJUMP is the number of jumps above 40 cm.

**Discussion**

This study aimed to describe and compare the physical demands of the same U18 players in three different basketball competitions during the same season. This is the first investigation that examines the differences in the external load demands of different competitions in the same basketball players using micro-technology to the authors' knowledge. The main conclusion of the study is that competition with teams of international level (U18T) causes higher physical requirements, expressed in most variables (except for hCOD and hACC), than regional competitions (U18L and EBA senior level).

The higher level U18 players who belonged to academies of professional teams showed higher anthropometric values and a better physical condition (Vernillo et al., 2012). These differences were revealed during matches with teams of international calibre, requiring a higher physical level. Additionally, players with better physical condition showed their capacities during the match, increasing the distance travelled at high speed (Abdelkrim et al., 2010).

Differences between international players and national players have been studied. The former expressed a higher percentage of maximum heart rate and a higher blood lactate concentration during international competitions (Rodriguez-Alonso et al., 2003). However, the players included in the study corresponded to two different groups. Nevertheless, the anthropometric characteristics of participants in the present study are similar to U18 European players (Jelicic et al., 2002; Vázquez-Guerrero et al., 2019). Consequently, it could be assumed that the competitive level of the analysed team corresponds to the physical demands of the international tournament (U18T), with the U18L level being below its potential capacities.

The Spanish EBA league is the fourth senior age category within Spanish basketball. Despite this, many junior players compete in this league as a preliminary step in their sports

development, seeking later professionalism in basketball. Nevertheless, only the hCOD and hACC variables presented higher values in this category compared to U18L and U18T. Both variables were expressed in their magnitude of high intensity. This result can be explained by the fact that players can compete in senior years in the EBA league, with stronger and faster players playing basketball of greater intensity than the U18 categories.

In the U18L, a greater number of tCOD and tACC movements were found. These results are in line with a recent study showing that U18 players express a higher frequency in the total number of ACC and DEC compared to elite players (Trapero et al., 2020). However, it is important to highlight that the mentioned study used two different groups of players that competed at two different levels, which can hamper possible comparisons.

Regarding high-intensity actions and their intermittence, the values of high-intensity actions tend to increase as the level of the league increases (Ferioli et al., 2020; Scanlan et al., 2011). One explanation for this phenomenon is that, at a higher level, players show a more efficient technical-tactical behaviour compared to young or sub-elite players. High-level players cover less distance at medium speeds and show a lower frequency in the number of actions (Zhang et al., 2017; Petway et al., 2020). Furthermore, this technical-tactical efficiency during basketball matches is a qualitative indicator of performance (Sampaio et al., 2015). However, the EBA league is a non-professional league; therefore, the anthropometric profile and the player's level of physical condition may not demand a high external load in most variables compared to international U18T.

Another point to highlight is the different competitive format of the analysed competitions. While the EBA and U18L matches were played during the in-season period (i.e., one game per week), the four U18T games were congested in a

three-day period. Instead, the highly competitive density, the external load values of most variables were higher in U18T than in EBA and U18L. These results show that accumulated fatigue in tournament-based competition could not be a major limitation for physical performance during official basketball tournaments (Moreira et al., 2012). Indeed, the context (opponents, supporters, motivation, etc.) might favour the levelling of physical demands during competition.

In the end, we must acknowledge certain limitations of this study. First, there are no internal load variables included. Nevertheless, although we did not analyse how the external load influences the players' internal responses, the external load only showed small variations in the three different competitions. Second, this study shows only average values of the selected variables, which may underestimate the most demanding scenarios during the competition. Finally, future research should incorporate the analysis of the emotional and psychological component and how this influences the physical demands when facing matches in one competition or another.

In conclusion, the present study describes how different competition levels suppose different external load stimuli in the same players with the same game model. Therefore, basketball coaches and strength and conditioning specialists must consider the level of competition at which players are incorporated. This must be based on the physical characteristics and the need to develop their path to professionalism. This information must be used to compete at the optimal level, where players can express the greatest possible physical demands and promote their development.

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# Effects of Mentha Piperita Essential Oil Uptake or Inhalation on Heart Rate Variability and Cardiopulmonary Regulation during Exercise

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## Abstract

This study compares the effects of the uptake or inhalation of 50uL *Mentha piperita* (MP) essential oil for 10 days on heart rate variability (HRV) and cardiopulmonary regulation during various exercise intensities. Forty-eight healthy male subjects were randomly assigned to MP uptake (MPU; n=16), MP inhalation (MPI; n=16), and control group (C; n=16). All participants were measured resting HRV, respiratory, cardiovascular, and metabolic parameters during aerobic, anaerobic, and graded exercise tests (GXT) before and after treatment. There were significant increases in the low-frequency area (LFa;  $1.8 \pm 0.1$  vs  $2.2 \pm 0.2$  ms<sup>2</sup>), the ratio of low frequency to respiration frequency area (LFa/RFa;  $0.9 \pm 0.1$  vs  $1.3 \pm 0.1$ ) at resting and carbon dioxide production ( $V_{CO_2}$ ;  $41.2 \pm 4.0$  vs  $49.2 \pm 6.8$  mL/min<sup>-1</sup>/kg<sup>-1</sup>), ventilation per minute ( $V_E$ ;  $80.2 \pm 4.3$  vs  $97.5 \pm 5.5$  L/min<sup>-1</sup>), and respiratory rate (RR;  $38.2 \pm 1.9$  to  $44.3 \pm 2.1$  breath/min<sup>-1</sup>) in an anaerobic test following MPU intervention. In GXT, maximal carbon dioxide production ( $V_{CO_{2max}}$ ;  $51.9 \pm 3.5$  to  $59.1 \pm 6.4$  mL/min<sup>-1</sup>/kg<sup>-1</sup>), maximal ventilation per minute ( $V_{E_{max}}$ ;  $126.4 \pm 6.5$  to  $138.4 \pm 5.4$  L/min<sup>-1</sup>) and maximal respiratory rate ( $RR_{max}$ ;  $52.7 \pm 3.6$  to  $60.1 \pm 2.3$  breath/min<sup>-1</sup>) significantly increased in MPU. The correlations of  $\Delta LFa$  with  $\Delta V_{CO_{2max}}$ ,  $\Delta V_{E_{max}}$  and  $\Delta RR_{max}$  in the MPU group were significant. Continuous uptake or inhalation of 50uL MP oil for 10 days does not improve aerobic capacity and maximal exercise performance, but 10 days' uptake of MP essential oil increased sympathetic activity at rest and may relate to respiratory regulation under high-intensity exercise.

**Keywords:** essential oil, ergogenic aids, aromatherapy, ventilation threshold, carbon dioxide



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MENTHA PIPERITA ESSENTIAL OIL ON EXERCISE PERFORMANCE

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## Introduction

Many scientific researchers are still attempting to increase athletic performance with supplemented natural plant prod-

ucts. Various essential oils extracted from aromatic plants have biological effects on human health (Elshafie & Camele, 2017). *Mentha piperita* (MP) is a common species in the La-

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miaceae family (Watanabe et al., 2015) and has been shown to have the effects of anti-spasticity, anti-pain, anti-inflammation, anti-oxidation, and analgesic effects (Gobel et al., 1994; McKay & Blumberg, 2006; Scherer et al., 2013). MP essential oil has also been proven to be related to the treatment of headaches (Mauskop, 2001), irritable bowel disease (Alammar et al., 2019), and other diseases (McKay & Blumberg, 2006). MP essential oil contains more than 40 kinds of chemicals, primarily menthol at about 29% and menthone at about 20-30% (Rohloff, 1999), and has confirmed the safety of eating mint (Ahijevych & Garrett, 2004).

Whether through direct nasal inhalation or mixing with the aqueous solution for oral uptake, is MP essential oil effective in enhancing athletic performance? Previous studies found inhalation of MP essential oil was effective in improving physical fitness (including running speed, handgrip strength, and the number of push-ups) (Raudenbush et al., 2001), increasing oxygen saturation ( $SpO_2$ ), heart rate (HR), blood pressure (BP), and mean arterial pressure (MAP) during a 15-minute treadmill stress test (Raudenbush et al., 2002), typing performance test (Barker et al., 2003) and aerobic performance and reaction time (Asghar, 2011).

Furthermore, Jaradat et al. (2016) demonstrated that inhaling MP essential oil through the nose improved expiratory volume in the first second ( $FEV_1$ ), forced vital capacity (FVC), running speed (Jaradat et al., 2016). However, Pournemati et al. (2009) found that there was no effect on maximal heart rate ( $HR_{max}$ ), running time, maximal oxygen uptake ( $VO_{2max}$ ), oxygen consumption ( $VO_2$ ), ventilation per minute ( $V_E$ ), and respiratory exchange rate (RER) in female soccer athletes following MP inhalation (Pournemati et al., 2009). To date, the impact and mechanism of inhaling MP essential oil on athletic performance remain controversial.

There are many ways to orally uptake MP essential oil, including direct oral consumption, drink in aqueous solutions of essential oil, or infusion of mint leaves. Sönmez et al. (2010) confirmed that oral intake supplemented with a water solution soaked in mint leaves offered no improvement in running speed on a 400-metre test, but lactic acid in the blood and muscular pain level significantly decreased as compared to other treatments. Meamarbashi and Rajabi (2013) found that subjects who drank 50uL MP aqueous solution for 10 consecutive days enhanced FVC, peak expiratory flow (PEF), peak inspiratory flow (PIF), and time to exhaustion, maximal workload, and  $VO_{2max}$ . Moreover, direct oral administration of 50uL MP oil increased grip strength, standing vertical jump, standing long jump,  $FVC_1$ , PIF, PEF, and reaction times (Meamarbashi, 2014). Similarly, the benefits of uptake MP essential oil are still questioned. Shepherd and Peart (2017) indicated that uptake of MP essential oil for 10 consecutive days does not improve aerobic capacity.

Many essential oils affect the autonomic nervous system by stimulation (increased sympathetic activity) or soothing (increased parasympathetic activity). Previous studies have also confirmed essential oils with a stimulating effect, such as grapefruit (Horii et al., 2015), peppermint, estragon, and fennel (Haze et al., 2002), while other essential oils such as lavender, geranium, chamomile, sandalwood, bergamot, rosemary, and similar, have soothing effects (Chang & Shen, 2011; McCaffrey et al., 2009; Seo, 2009). The fragrance is an essential oil that stimulated the olfactory system and thus affected brain waves (Lorig & Schwartz 1988) or stimulated or

inhibited brain functions (Manley 1993). Komori et al. (1995) pointed out that fragrance affects the endocrine function by changing the concentration of cortisol and dopamine in urine and the activity of the natural killer cell, which also affects the immune function. In an animal experiment, menthol vapour was proved to reduce surfactant and further change lung surface tension and lung function (Zänker et al., 1980). Previous studies have also shown that fragrance had effects on the autonomic nervous system by observed on HR, skin conductance, respiration (Brauchli et al. 1995), and blood pressure (Heuberger et al. 2001).

Based on the above studies, the results of enhanced athletic performance by continuous uptake or inhalation of MP essential oil are still conflicting. The present study designs a randomized controlled trial (RCT) to validate the effects of 50 uL MP essential oil uptake or inhalation on autonomic, respiratory, cardiovascular, and metabolic systems and to identify possible mechanisms by which MP essential oil affects athletic performance. The purposes of this study are as follows: first, confirm the effects of 50uL intake or inhalation of MP essential oil on athletic performance for 10 consecutive days, including in aerobic, anaerobic, and maximum exercise performance. Second, confirm whether 50uL intake or inhalation of MP essential oil for 10 days will affect the autonomic nervous system and thus change respiratory, cardiovascular, and metabolic regulation during exercise.

## Methods

Healthy male participants aged 20 to 30 years old were recruited from two universities. The conditions of the participants were: not smoking, no cardiovascular diseases, no lung-related diseases, no neurological diseases or metabolic diseases, no daily use of any dietary supplement, and no use of mint-related products during the experiment. This research was approved by the Institutional Review Board of Catholic St. Mary's Hospital (No. IRB104001). All participants agreed and signed informed consent before the study. The 48 participants were randomly assigned to MP Uptake Group (MPU, n=16), MP Inhalation Group (MPI, n=16), and Control Group (C, n=16) by a random table. Two subjects of the MPI group unable to complete the study due to personal reasons. The experimental achievement rate was thus 95.83%.

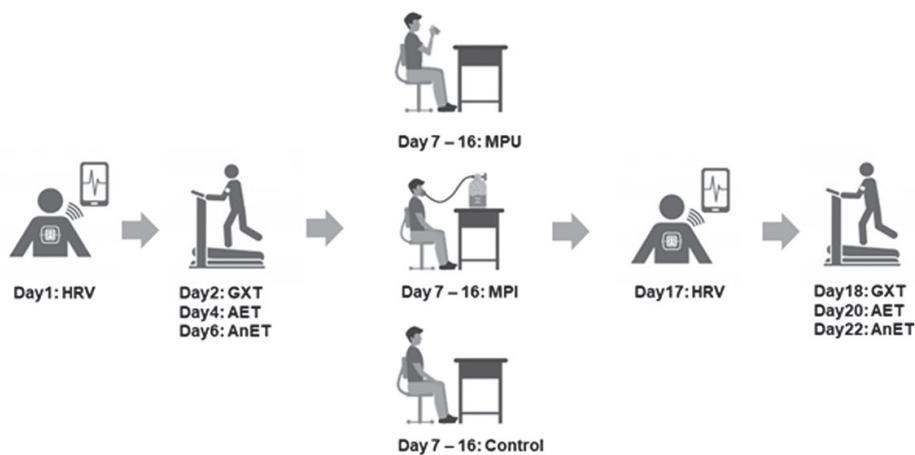
Before the formal MP uptake or inhalation experiments were performed, the subjects underwent two sensitization tests. Each participant underwent an anthropological parameter measurement and a skin sensitization test that involved the application of 1% of MP on the inner skin of the upper arm in order to monitor whether the participant exhibited any allergic responses (redness, swelling, heat, or pain). If an allergic response was observed within 24 hours, the subject was subsequently excluded. After that, the participants performed an MP spray inhalation sensitization test. Each inhaled 1% of MP (50  $\mu$ L) for 10 min and was then monitored for 24 hours. As in the previous test, if an allergic response was observed or if any participant experienced discomfort, they were excluded. All participants had passed the sensitization tests before participating in the follow-up intervention experiment.

This study designed a single-blind RCT due to the strong taste of MP essential oil that may result in different results between inhalation and uptake intervention. The single-blind design was expected to minimize the recognition of the researchers by the groups of subjects. After signing the informed

consent, the heart rate variability (HRV) test was measured on Day 1. All subjects were given a treadmill graded exercise test (GXT) on Day 2 to measure the maximal exercise performance. On Day 4 (an interval of 48 hours), subjects were given a six-minute 80% ventilatory threshold (VT) exercise test for aerobic endurance. On Day 6 (an interval of 48 hours), subjects were given a six-minute  $\Delta 50$  (50% of VT and maximal workload) exercise test for anaerobic endurance.

The MPU group drank peppermint oil-water (500mL aqueous solution containing 50uL of MP essential oil) for 10 consecutive days under the laboratory researchers' monitoring; the daily intake was recorded, and the study monitored whether there were any adverse reactions. For the MPI group, 50uL of peppermint oil spray was inhaled for 10 consecutive days in the laboratory. The spray was manufactured using an ultrasonic spray-manufacturing machine (KUN-808, K. SONIC, Taiwan),

and the nose of the subject was connected with a catheter and a mask. The laboratory researchers confirmed that the subject completely inhaled 50 uL MP essential oil spray to ensure the dosage of MP essential oil was consistent between MPU and MPI groups. Subjects' daily intake and any adverse reactions were recorded by laboratory researchers. The Control Group was asked to maintain a daily lifestyle and prohibited using MP essential oil products during the experiment. MP essential oil was selected from the products manufactured by the U.S. dealer FBI, and the products had qualified production and safety certificates (Name: *Mentha piperita*; No: 31060). The amount of MP essential oil in this study was determined according to previous studies and confirmed the dosage was lower than the daily uptake recommended by the U.S. FDA (Meamarbashi, 2014; Meamarbashi & Rajabi, 2013; Nair, 2001). The experimental design of this study is shown in Figure 1.



**Figure 1.** Experimental design of this study. HRV: heart rate variability; GXT: Graded exercise test; AET: Aerobic endurance test; AnET: Anaerobic endurance test; MPU: *Mentha piperita* essential oil uptake group; MPI: *Mentha piperita* essential oil inhalation group; Control: control group.

The GXT was performed by modified Bruce protocol. In short, the subjects were rested in a sitting position for two minutes before the test. When the  $VO_2$  and HR were stable, the subjects started to walk on the treadmill at a speed of 2.7  $km \cdot h^{-1}$  with a slope of 0% for three minutes. The speed and slope were adjusted every three minutes until the subjects were exhausted (ACSM, 2013).

The exhaustion criteria of the subjects were as follows: 1) the rating of perceived exertion (RPE) is greater than 19; 2) RER is greater than 1.1; 3) the estimated  $HR_{max}$  is more than 90%. When more than two criteria were simultaneously reached, the researchers determined that was the test termination standard. Various respiratory, cardiovascular, and metabolic parameters, such as tidal volume ( $V_t$ ), RR,  $V_E$ , HR, BP,  $VO_2$ ,  $VCO_2$ ,  $V_E/VO_2$ , and  $V_E/VCO_2$ , were collected during GXT using an energy metabolic measurement system (Cortex Metamax 3B, Germany).

The study determined that VT was according to the criterion that  $V_E/VO_2$  increased gradually accompanied by exercise intensity, but  $V_E/VCO_2$  did not increase simultaneously.  $V_E$  gradually deviated from the linear curve (Beaver et al., 1986). The determination of VT was according to the time chart drawn by the computer, and then VT was independently confirmed by two experienced exercise physiologists. If the determination results were inconsistent, then exercise physiologists were re-evaluated to reach a consensus. For the aerobic endurance test, subjects performed under 80% VT an intensity

treadmill exercise for six minutes. For the aerobic endurance test, subjects performed a treadmill exercise for six minutes under  $\Delta 50$  intensity (50% of VT and maximum workload).

HRV was measured with a personal heart rate monitor (Medeia, Bulgaria). All subjects were placed on the ECG patch and rested for ten minutes in a sitting position. Then researchers started to collect HRV data for five minutes. HRV parameters included respiration-frequency area (RFa, index of parasympathetic activity), low-frequency area (LFa, index of sympathetic activity), and LFa/RFa ratio (index of sympathovagal balance).

All respiratory, cardiovascular, and metabolic data collected during the various exercise tests were calculated at an average of 10 seconds. If there was an obvious deviation in the data, such as extreme values due to the subject sneezing or coughing, then that data was deleted. If the average exceeded four standard deviations, then the values were listed as extreme values and deleted. The data were analysed using SPSS for Windows 23.0. One-way ANOVA was used to analyse the anthropometric parameters (age, height, weight, body mass index, systolic blood pressure (SBP), diastolic blood pressure (DBP), and HR) of all groups. The differences of HRV (RFa, Lfa, and LFa/RFa), respiratory ( $V_E$ ,  $V_t$ , and RR), cardiorespiratory (HR, SBP, and DBP), and metabolic ( $VO_2$ ,  $VCO_2$ ,  $V_E/VO_2$ , and  $V_E/VCO_2$ ) parameters in various exercise tests before and after interventions for different groups (timing \* groups) were analysed using repeated-measure ANOVA. The Scheffé meth-

od was used to determine the main effect was significant. Pearson correlation was used to analyse the relationship between HRV and respiratory parameters. The significant level of statistical testing was determined to be  $\alpha = .05$ .

**Results**

A total of 46 subjects in this study completed all the experiments. The anthropometric data of the three groups of subjects show no significant difference (Table 1).

**Table 1.** Basic anthropometrical parameters of the three groups

Parameters	MPU (n=16)	MPI (n=14)	C (n=16)	F-value	P-value
Age (yr)	23.2±2.1	21.8±2.0	22.8±1.8	1.94	0.16
Height (cm)	172.8±6.3	175.1±8.4	173.0±5.4	1.94	0.16
Weight (kg)	67.6±10.6	67.2±9.2	66.7±8.0	0.05	0.96
BMI (kg/m <sup>2</sup> )	23.3±4.0	22.0±2.9	22.3±2.4	0.73	0.49
SBP (mm Hg)	120.3±14.8	118.0±6.2	117.9±13.2	0.20	0.82
DBP (mm Hg)	73.8±11.0	72.5±10.7	70.5±9.8	0.38	0.68
HR (beat/m-1)	66.0±10.2	63.6±13.4	70.3±14.8	0.97	0.39

Note. Data present as mean ± SD. \*p<.05 calculated by one-way ANOVA to compare three groups. MPU: *Mentha piperita* essential oil uptake group; PI: *Mentha piperita* essential oil inhalation group; C: control group. BMI: body mass index. SBP: systolic blood pressure. DBP: diastolic blood pressure. HR: heart rate.

Under HRV measurement, there were no significant differences between the three groups before treatments. After MPU intervention, LFa (1.8±0.1 vs 2.2±0.2 ms<sup>2</sup>, p = .03) and

LFa/RFa ratio (0.9±0.1 vs 1.3±0.1, p = .01) increased compared to pre-intervention. There was no significant difference in the MPI and C groups (Table 2).

**Table 2.** HRV parameter changes after three interventions

Parameters	MPU (n=16)		MPI (n=14)		C (n=16)	
	Pre	Post	Pre	Post	Pre	Post
RFa, ms <sup>2</sup>	2.3±0.2	2.0±0.3	2.2±0.2	2.0±0.2	2.2±0.2	1.8±0.3
LFa, ms <sup>2</sup>	1.8±0.1	2.2±0.2*	1.9±0.2	2.0±0.2	1.8±0.1	1.7±0.1
LFa/RFa	0.9±0.1	1.3±0.1*	0.9±0.1	1.1±0.1	0.9±0.1	1.1±0.1

Note. Data present as mean ± SD. \*p<.05 calculated by repeated-measure ANOVA to compare three groups and pre and post interventions. MPU: *Mentha piperita* essential oil uptake group; MPI: *Mentha piperita* essential oil inhalation group; C: control group. Pre: pre-intervention; Post: post-intervention. RFa: respiration frequency area (parasympathetic activity). LFa: low-frequency area (sympathetic activity). LFa/RFa: sympathovagal balance.

For the aerobic exercise test, there was no significant difference in VO<sub>2</sub>, VCO<sub>2</sub>, V<sub>E</sub>, V<sub>t</sub>, and RR of the three groups before or after the interventions. For the anaerobic exercise test, there was no significant difference in VO<sub>2</sub>, VCO<sub>2</sub>, V<sub>E</sub>, V<sub>t</sub>, and

RR in the MPI and C groups. However, VCO<sub>2</sub> (41.2±4.0 vs 49.2±6.8 mL/min<sup>-1</sup>/kg<sup>-1</sup>, p = .03), V<sub>E</sub> (80.2±4.3 vs 97.5±5.5 L/min<sup>-1</sup>, p = .01), and RR (38.2±1.9 vs 44.3±2.1 breath/min<sup>-1</sup>, p = .04) increased significantly after MPU intervention (Table 3).

**Table 3.** Respiratory and metabolic parameters at aerobic and anaerobic endurance tests after three interventions

Parameters	MPU (n=16)		MPI (n=14)		C (n=16)	
	Pre	Post	Pre	Post	Pre	Post
Aerobic endurance test						
VO <sub>2</sub> (mL/min <sup>-1</sup> /kg <sup>-1</sup> )	26.2±9.0	25.0±6.8	28.1±5.1	29.7±2.8	28.7±5.3	29.7±3.7
VCO <sub>2</sub> (mL/min <sup>-1</sup> /kg <sup>-1</sup> )	24.1±7.7	23.5±5.0	26.6±5.6	25.0±5.2	24.0±3.1	25.9±2.9
V <sub>E</sub> (L/min <sup>-1</sup> )	60.2±9.3	59.2±7.5	60.2±9.1	63.8±8.0	60.6±7.8	59.8±6.5
V <sub>t</sub> (L/min <sup>-1</sup> )	1.8±0.6	1.7±0.4	1.9±0.5	1.9±0.3	1.8±0.4	1.8±0.5
RR (breath/min <sup>-1</sup> )	33.4±3.4	34.1±2.9	31.7±3.0	33.6±2.5	33.7±1.8	33.2±2.7
Anaerobic endurance test						
VO <sub>2</sub> (mL/min <sup>-1</sup> /kg <sup>-1</sup> )	37.0±3.1	35.3±4.4	32.4±7.0	33.3±4.4	35.2±6.4	34.9±3.2
VCO <sub>2</sub> (mL/min <sup>-1</sup> /kg <sup>-1</sup> )	41.2±4.0	49.2±6.8*	40.4±3.9	41.8±2.7	42.3±4.4	40.4±4.2
V <sub>E</sub> (L/min <sup>-1</sup> )	80.2±4.3	97.5±5.5*	83.5±5.8	82.3±4.6	84.4±10.1	81.5±9.7
V <sub>t</sub> (L/min <sup>-1</sup> )	2.1±1.0	2.2±1.3	2.0±0.9	2.1±1.5	2.1±1.3	2.0±0.9
RR (breath/min <sup>-1</sup> )	38.2±1.9	44.3±2.1*	41.8±2.6	39.2±3.2	40.2±1.8	40.8±2.0

Note. Data present as mean ± SD. \*p<.05 calculated by repeated-measure ANOVA to compare three groups and pre and post interventions. MPU: *Mentha piperita* essential oil uptake group; MPI: *Mentha piperita* essential oil inhalation group; C: control group. Pre: pre-intervention; Post: post-intervention. VO<sub>2</sub>: oxygen consumption; VCO<sub>2</sub>: carbon dioxide production; V<sub>E</sub>: ventilation per minute; V<sub>t</sub>: tidal volume; RR: respiratory rate; HR: heart rate.

Before the intervention, the respiratory, cardiovascular, and metabolic parameters had no significant differences at VT or

maximal exercise during GXT among the three groups. After 10 days of intervention, while there was no significant differ-

ence in exercise time,  $VO_2$ ,  $VCO_2$ ,  $V_E$ ,  $V_t$ ,  $RR$ ,  $V_E/VO_2$ , and  $V_E/VCO_2$  following MPI and C interventions. However,  $VCO_{2max}$  ( $51.9 \pm 3.5$  vs  $59.1 \pm 6.4$  mL/min<sup>-1</sup>/kg<sup>-1</sup>,  $p = .01$ ),  $V_{Emax}$  ( $126.4 \pm 6.5$

vs  $138.4 \pm 5.4$  L/min<sup>-1</sup>,  $p = .01$ ),  $RR_{max}$  ( $52.7 \pm 3.6$  vs  $60.1 \pm 2.3$  breath/min<sup>-1</sup>,  $p = .01$ ), and  $V_E/VCO_2$  ( $33.1 \pm 4.0$  vs  $38.9 \pm 4.9$ ,  $p = .01$ ) significantly increased in the MPU group (Table 4).

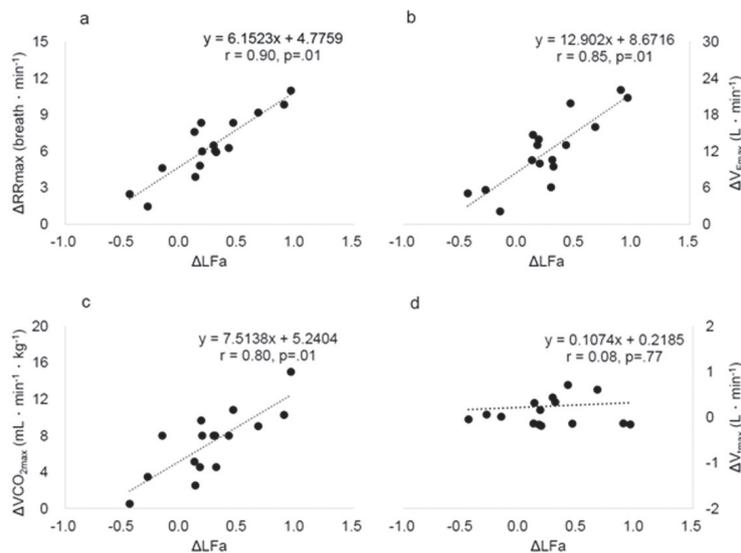
**Table 4.** Respiratory, cardiovascular and metabolic parameters during GXT after three interventions

Groups Parameters	MPU (n=16)		MPI (n=14)		C (n=16)	
	Pre	Post	Pre	Post	Pre	Post
Ventilatory threshold						
Exercise time (s)	581.0±90.8	557.0±100.4	612.3±114.3	583.1±103.2	565.1±84.8	559.5±110.0
$VO_2$ (mL/min <sup>-1</sup> /kg <sup>-1</sup> )	37.5±5.7	37.3±7.6	35.4±5.9	33.4±9.7	36.8±5.6	37.9±6.5
$VCO_2$ (mL/min <sup>-1</sup> /kg <sup>-1</sup> )	36.8±6.7	37.6±7.2	34.0±6.2	32.2±8.9	36.4±5.5	37.2±6.4
$V_E$ (L/min <sup>-1</sup> )	70.2±13.5	69.7±14.5	67.2±9.9	68.3±7.8	65.5±10.8	63.9±15.1
$V_t$ (L/min <sup>-1</sup> )	1.9±0.4	1.9±0.4	1.7±0.4	1.7±0.3	1.8±0.4	2.0±0.6
RR (breath/min <sup>-1</sup> )	37.4±8.1	36.6±8.3	41.4±7.7	42.9±10.0	36.9±8.3	33.3±7.5
$V_E/VO_2$	25.6±3.4	25.1±4.1	27.3±2.7	27.4±4.2	26.9±5.9	24.7±2.4
$V_E/VCO_2$	27.7±2.9	29.8±3.1	30.4±4.0	30.7±3.8	30.4±6.2	29.9±2.7
Maximal exercise						
Exercise time (s)	847.4±127.3	861.1±89.0	907.7±156.4	891.3±156.5	828.0±74.0	821.7±81.8
HR (breath/min <sup>-1</sup> )	187.3±6.0	189.8±5.6	189.9±9.8	188.8±8.9	191.4±11.7	193.6±10.1
$VO_2$ (mL/min <sup>-1</sup> /kg <sup>-1</sup> )	52.0±5.9	55.5±6.0	50.8±6.8	52.5±8.6	51.0±7.1	53.9±7.6
$VCO_2$ (mL/min <sup>-1</sup> /kg <sup>-1</sup> )	51.9±3.5	59.1±6.4*	53.4±7.5	52.3±4.7	50.8±3.0	48.9±4.1
$V_E$ (L/min <sup>-1</sup> )	126.4±6.5	138.4±5.4*	121.8±7.3	126.6±4.4	123.1±5.1	120.8±7.9
$V_t$ (L/min <sup>-1</sup> )	2.4±0.4	2.3±0.4	2.2±0.5	2.3±0.6	2.4±0.4	2.4±0.4
RR (breath/min <sup>-1</sup> )	52.7±3.6	60.1±2.3*	55.4±4.0	55.0±2.1	51.3±6.4	50.3±5.1
$V_E/VO_2$	31.6±4.9	34.2±5.1	34.8±5.2	36.6±5.1	33.1±4.6	34.8±3.7
$V_E/VCO_2$	33.1±4.0	38.9±4.9*	33.7±7.0	36.3±5.1	33.1±4.3	32.2±2.6

Note. Data present as mean ± SD. \* $p < .05$  calculated by repeated-measure ANOVA to compare three groups and pre and post interventions. MPU: *Mentha piperita* essential oil uptake group; MPI: *Mentha piperita* essential oil inhalation group; C: control group. Pre: pre-intervention; Post: post-intervention. HR: heart rate;  $VO_2$ : oxygen consumption;  $VCO_2$ : carbon dioxide production;  $V_E$ : ventilation in minutes;  $V_t$ : tidal volume; RR: respiratory rate.

Correlation analysis was used to explore the HRV and respiratory parameters following MPU. After analysis the difference values of LFa at baseline and RR,  $V_E$ ,  $VCO_2$ , and  $V_t$  at maximal

exercise before and after MPU intervention, the correlations of  $\Delta LFa$  with  $\Delta RR_{max}$  ( $r = .90$ ,  $p = .01$ ),  $\Delta V_{Emax}$  ( $r = .85$ ,  $p = .01$ ), and  $\Delta VCO_{2max}$  ( $r = .80$ ,  $p = .01$ ) were significant (Figure 2).



**Figure 1.** Correlation analysis of pulmonary parameters and HRV by Pearson correlation in MPU. MPU: *Mentha piperita* essential oil uptake group.  $\Delta RR_{max}$ : difference values of respiratory rate between baseline and maximal exercise.  $\Delta V_{Emax}$ : difference values of ventilation in minute between baseline and maximal exercise.  $\Delta VCO_{2max}$ : difference values of carbon dioxide production between baseline and maximal exercise.  $\Delta V_{tmax}$ : difference values of tidal volume between baseline and maximal exercise.  $\Delta LFa$ : difference values of low-frequency area (sympathetic activity) between pre-intervention and post-intervention.

## Discussion

To the authors' best knowledge, this is the first study to design RCT experiments with a relatively large sample size to investigate the effects of MP essential oil uptake or inhalation for 10 consecutive days on HRV on resting and respiratory, cardiovascular, and metabolic regulation during various exercise intensities. The main findings of this study are 1) both the uptake and the inhalation of 50uL of MP essential oil for 10 consecutive days does not increase aerobic capacity and maximal exercise performance; 2) the 10 days' uptake of MP essential oil enhances the sympathetic activity and sympathovagal balance at rest, but the inhalation of MP essential oil does not have this effect; 3) the 10 days' uptake of MP essential oil increases RR,  $V_E$ , and  $VCO_2$  at higher intensity exercise (higher than VT), which may relate to sympathetic activation.

The results of this study are similar to those of Shepherd and Peart, who found that the uptake of 50uL of MP essential oil for 10 consecutive days did not improve maximal performance or aerobic capacity (Shepherd & Peart, 2017). However, Meamarbashi and Rajabi (2013) recruited 12 healthy male subjects for the uptake of 50 uL of MP essential oil for 10 consecutive days and found that the maximum oxygen uptake increased by 10.5%, exhaustion exercise time increased by 24.9%, and work volume increased by 51.5%. Although the results of Meamarbashi and Rajabi (2013) determined that the uptake of MP essential oil was helpful for aerobic capacity and athletic performance, the experimental design lacked a control group, and thus the supplementary benefit may be the learning effect of the subjects after two exercise tests. To be cautious, this study designed an aerobic endurance test (80% VT) and showed no significant change in  $VO_2$ ,  $VCO_2$ ,  $V_E$ , Vt, and RR regardless of uptake or inhalation of MP essential oil. Therefore, we confirm that uptake or inhalation of 50uL MP essential oil for 10 days has no significant effect on aerobic capacity.

Previous studies have confirmed that the uptake of MP essential oil has beneficial effects on lung functions. Meamarbashi and Rajabi (2013) noted that lung function (such as FVC, PEF, and PIF) increased significantly after 10 days of supplementation of MP essential oil. Meamarbashi (2014) used the supplementation of MP essential oil once and found that FVC<sub>1</sub>, PEF, and PIF all increased significantly after 5 minutes of supplementation. A more recent study by Shepherd and Peart (2017) pointed out that there was no obvious change in FVC, FVC<sub>1</sub>, and FVC<sub>1</sub>/FVC under the supplementation of peppermint oil for 10 consecutive days, and there was only an increase in chest circumference at the maximum exhale. Although the lung function was not measured in this study,  $V_E$  and RR during anaerobic exercise and GXT significantly increased following MPU intervention. This might be the benefit of improved lung part of function after MP essential oil uptake, but even if respiratory-regulated functions were improved in a short period, there were not sufficient to enhance aerobic, anaerobic, and maximal exercise performance.

The notable findings of this study are that LFa, LFa/RFa,  $V_E$ , RR, and  $VCO_2$  increased after 10 days' MP essential oil uptake. The changes of  $V_E$ , RR, and  $VCO_2$  are related to sympathetic activity activation. Sönmez (2010) randomly divided subjects into a mint group, placebo group, and control group. The mint group was supplemented with a water solution soaked in mint leaves and did not affect 400-metre running speed. However, the concentration of lactic acid in the blood was significantly lower than that of the placebo group and the control group.

Sönmez (2010) indicated that mint oil may be related to accelerating lactic acid clearance, but the detailed mechanism for affecting lactic acid clearance was not explained. Another research result of Meamarbashi and Rajabi (2013) showed that  $VCO_2$ ,  $V_E$ , RR, and Vt increased significantly, while  $P_{ET}CO_2$  decreased significantly. The increase in  $VCO_2$  was similar to our study, in which the increase in  $V_E$ , RR, and  $VCO_2$  occurred under anaerobic and GXT. This may mean that respiratory regulation changed by MP essential oil uptake may participate in the removal of anaerobic metabolites.

Nair (2001) indicated that isolated clinical cases of peppermint oil and/or its constituents had been reported; peppermint oil (8%) was not a sensitizer when tested using a maximization protocol; however, the concentration of Pulegone in peppermint oil should not exceed 1% to ensure safety. Meamarbashi and Rajabi (2013) recruited 30 healthy male college students as subjects and randomly assigned them to the experimental and control groups. The experimental group was orally supplemented with 50ul of peppermint essential oil (100%). Meamarbashi (2014) designed 12 healthy male students who supplemented 500mL aqueous solution with 0.05mL peppermint essential oil (0.01%) for ten consecutive days. Shepherd and Peart (2017) recruited seven healthy subjects who supplemented with 0.05ml in 500ml mint water solution (0.01%) for 10 days. A relatively recent study indicated that 19 subjects were treated with repeated menthol mouth swilling (0.1% concentration). Repeated menthol mouth swilling does not improve strength or power performance. (Best et al., 2020). In this study, the MPU group was supplemented with 50 uL MP essential oil dissolved in a 500mL aqueous solution (0.01%). In the MPI group, 50uL MP essential oil was dissolved in 50ml of aqueous solution (0.1%) and inhaled odour, which was produced by an ultrasonic spray manufacturing machine. Therefore, the concentration of MP essential oil in this study was between 0.01-0.1%, which is similar to the MP essential oil dosage of previous studies (Best et al., 2020; Meamarbashi, 2014; Meamarbashi & Rajabi, 2013; Shepherd & Peart, 2017).

This study confirmed that 50ul MP essential oil intake for 10 consecutive days increased sympathetic nerve activity in resting and which relative to increase RR,  $V_E$ , and  $VCO_2$  during high-intensity exercise. It is speculated that the possible mechanisms are that the menthol of MP essential oil stimulated sympathetic nerve activity to 1) increase bronchiectasis (Zänker et al. 1980), thus affecting FVC, PEF, and PIF (Jaradat et al. 2016; Meamarbashi 2014; Meamarbashi and Rajabi 2013); 2) promote stimulation of adrenaline secretion (Haze et al. 2002), increase liver glucose release, and changed the energy metabolism. Acidic substances after carbohydrate metabolism would stimulate respiratory regulation and assist in the elimination of lactic acid (Sönmez et al., 2010). Although this study did not analyse blood biochemical parameters from the subjects, it observed that timings of VT at GXT were earlier following uptake or inhalation of MP essential oil. These all indirectly indicate the possible effects of MP essential oil on respiratory and metabolic regulation during exercise.

Although all variables that may affect the experimental results were controlled as much as possible, there were still many research restrictions beyond our control. 1) Because MP essential oil has a strong fragrance, it is difficult to design a placebo with a similar fragrance but a different composition; thus, it is difficult to adopt the double-blind experimental design. 2) This study only discussed the effects of the uptake or

inhalation of MP essential oil on HRV, respiratory cardiovascular, and metabolic regulation at various exercise intensities but did not analysis the blood biochemical values of subjects. In this study, we attempted to find possible mechanisms by using non-invasive methods. Therefore, many complex phenomena cannot be further explained. We suggested that future studies explore the possible effects of MP essential oil on integrative physiological regulation by blood biochemical values.

To our knowledge, this is the first study that compared the effect of uptake or inhalation of MP essential oil on HRV at resting, respiratory, cardiovascular, and metabolic regulation at various exercise intensities. This study confirmed that the uptake or inhalation of 50uL of MP essential oil for 10 consecutive days does not improve the aerobic capacity and maximal exercise performance of healthy young males. However, the uptake of MP essential oil for 10 consecutive days does enhance sympathetic activity and the sympathovagal balance. The activation of sympathetic nerve activity is related to the enhanced respiratory regulation during high-intensity exercise.

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Revised May 2021

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- Maximum number of references is 30;
- Maximum combined total of 6 Tables/Figures.

Review papers should provide concise in-depth reviews of both established and new areas, based on a critical examination of the literature, analyzing the various approaches to a specific topic in all aspects of sports science and medicine, such as all clinical aspects of exercise, health, and sport; exercise physiology and biophysical investigation of sports performance; sport biomechanics; sports nutrition; rehabilitation, physiotherapy; sports psychology; sport pedagogy, sport history, sport philosophy, sport sociology, sport management; and all aspects of scientific support of the sports coaches from the natural, social and humanistic side.

Open Submissions

Indexed

Peer Reviewed

Review papers should be:

- Up to 6000 words (excluding title, abstract, tables/figures, figure legends, Acknowledgements, Conflict of Interest, and References);
- A structured abstract of less than 250 words;
- Maximum number of references is 100.

Editorials are written or commissioned by the editors, but suggestions for possible topics and authors are welcome. It could be peer reviewed by two reviewers who may be external or by the Editorial Board.

Open Submissions

Indexed

Peer Reviewed

Editorials should be:

- Up to 1000 words (excluding title, abstract, tables/figures, figure legends, Acknowledgements, Conflict of Interest, and References);
- A structured abstract of less than 250 words;
- Maximum number of references is 10.

Short reports of experimental work, new methods, or a preliminary report can be accepted as two page papers. Your manuscript should include the following sections: Introduction, Methods, Results, and Discussion.

Open Submissions

Indexed

Peer Reviewed

Short reports should be:

- Up to 1500 words (excluding title, abstract, tables/figures, figure legends, Acknowledgements, Conflict of Interest, and References);
- A structured abstract of less than 250 words;
- Maximum number of references is 15.

Peer review - fair review provides authors who feel their paper has been unfairly rejected (at any journal) the opportunity to share reviewer comments, explain their concerns, and have their paper reviewed for possible publication in MJSSM.

Open Submissions

Indexed

Peer Reviewed

Peer review - fair review should be:

- Up to 1500 words (excluding title, abstract, tables/figures, figure legends, Acknowledgements, Conflict of Interest, and References);
- A structured abstract of less than 250 words;
- Maximum number of references is 15.

Invited papers and award papers include invited papers from authors with outstanding scientific credentials. Nomination of invited authors is at the discretion of the MJSSM editorial board. MJSSM also publishes award papers selected by the scientific committee of the MSA annual conference.

Open Submissions

Indexed

Peer Reviewed

Invited papers and award papers should be:

- Up to 3000 words (excluding title, abstract, tables/figures, figure legends, Acknowledgements, Conflict of Interest, and References);
- A structured abstract of less than 250 words;
- Maximum number of references is 30;
- Maximum combined total of 6 Tables/Figures.

Meeting Abstracts contain conference abstracts of the sports science papers presented at the MSA annual conference and MSA-sponsored meetings. This publication offers a first look into the current research in the field of Sports Science.

Open Submissions

Indexed

Peer Reviewed

Meeting Abstracts should be:

- Restricted to 250 words (including title, authors and institutions) and must include the following separate sections: [1] purpose; [2] methods; [3] results; [4] conclusion;
- Without references;
- Without Tables/Figures.

### 1.3. Submission

MJSSM only accepts electronic submission to the e-mail of the Journal Office: **office@mjssm.me**.

Submitted material includes:

- A manuscript prepared according to the Guidelines for the Authors;
- A signed form that states the study was not previously published, nor has been submitted simultaneously for consideration of publication elsewhere, that states that all of the authors are in agreement with submission of the manuscript to MJSSM, and that, for studies that use animal or human individuals, authors must include information regarding their institution's ethics committee, and which identifies the official approval number;
- A signed form that there is no conflict of interest.

Name the files according to the family name of the first author. Authors submitting revised versions of the manuscript can use the identification number of their manuscript as provided by the Journal Office. *See example:*

- ✓ FAMILY NAME-manuscript.doc – (main manuscript file)
- ✓ FAMILY NAME-statement.PDF – (authorship statement)
- ✓ FAMILY NAME-declaration.PDF – (declaration of potential conflict of interest)
- ✓ FAMILY NAME-fig1.tiff – (Figure 1)

### 1.4. Peer Review Process

An original manuscript submitted for publication will be submitted to the review process as long as it fits the following criteria:

- The study was not previously published, nor has been submitted simultaneously for consideration of publication elsewhere;
- All persons listed as authors approved its submission to MJSSM;
- Any person cited as a source of personal communication has approved the quote;
- The opinions expressed by the authors are their exclusive responsibility;
- The author signs a formal statement that the submitted manuscript complies with the directions and guidelines of MJSSM.

The editors-in-chief, executive editor and associate editors will make a preliminary analysis regarding the appropriateness, quality, originality and written style/grammar of the submitted manuscript. The editors reserve the right to request additional information, corrections, and guideline compliance before they submit the manuscript to the ad-hoc review process.

MJSSM uses ad-hoc reviewers, who volunteer to analyze the merit of the study. Typically, one or two expert reviewers are consulted in a double-blind process. Authors are notified by e-mail when their submission has been accepted (or rejected). Minor changes in the text may be made at the discretion of the editors-in-chief, executive editor and/or associate editors. Changes can include spelling and grammar in the chosen language, written style, journal citations, and reference guidelines. The author is notified of changes via email. The final version is available to the author for his or her approval before it is published.

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## 1.6. After Acceptance

After the manuscript has been accepted, authors will receive a PDF version of the manuscripts for authorization, as it should look in printed version of MJSSM. Authors should carefully check for omissions. Reporting errors after this point will not be possible and the Editorial Board will not be eligible for them.

Should there be any errors, authors should report them to the Office e-mail address [office@mjssm.me](mailto:office@mjssm.me). If there are not any errors authors should also write a short e-mail stating that they agree with the received version.

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MJSSM is hosting the Code of Conduct Ethics Committee of Publications of the **COPE** (the Committee on Publication Ethics), which provides a forum for publishers and Editors of scientific journals to discuss issues relating to the integrity of the work submitted to or

published in their journals.

## 2. MANUSCRIPT STRUCTURE

### 2.1. Title Page

The first page of the manuscripts should be the title page, containing: title, type of publication, running head, authors, affiliations, corresponding author, and manuscript information. *See example:*

Transfer of Learning on a Spatial Memory Task between the Blind and Sighted People Spatial Memory among Blind and Sighted

Original Scientific Paper

Transfer of learning on a spatial memory task

Selcuk Akpinar<sup>1</sup>, Stevo Popović<sup>1,2</sup>, Sadettin Kirazci<sup>1</sup>

<sup>1</sup>Middle East Technical University, Physical Education and Sports Department, Ankara, Turkey

<sup>2</sup>University of Montenegro, Faculty for Sport and Physical Education, Niksic, Montenegro

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*S. Popovic*

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*Faculty for Sport and Physical Education*

*Narodne omladine bb, 84000 Niksic, Montenegro*

*E-mail: stevop@ac.me*

Word count: 2,980

Abstract word count: 236

Number of Tables: 3

Number of Figures: 3

#### 2.1.1. Title

Title should be short and informative and the recommended length is no more than 20 words. The title should be in Title Case, written in uppercase and lowercase letters (initial uppercase for all words except articles, conjunctions, short prepositions no longer than four letters etc.) so that first letters of the words in the title are capitalized. Exceptions are words like: “and”, “or”, “between” etc. The word following a colon (:) or a hyphen (-) in the title is always capitalized.

#### 2.1.2. Type of publication

Authors should suggest the type of their submission.

#### 2.1.3. Running head

Short running title should not exceed 50 characters including spaces.

#### 2.1.4. Authors

The form of an author's name is first name, middle initial(s), and last name. In one line list all authors with full names separated by a comma (and space). Avoid any abbreviations of academic or professional titles. If authors belong to different institutions, following a family name of the author there should be a number in superscript designating affiliation.

#### 2.1.5. Affiliations

Affiliation consists of the name of an institution, department, city, country/territory(in this order) to which the author(s) belong and to which the presented / submitted work should be attributed. List all affiliations (each in a separate line) in the order corresponding to the list of

authors. Affiliations must be written in English, so carefully check the official English translation of the names of institutions and departments.

Only if there is more than one affiliation, should a number be given to each affiliation in order of appearance. This number should be written in superscript at the beginning of the line, separated from corresponding affiliation with a space. This number should also be put after corresponding name of the author, in superscript with no space in between.

If an author belongs to more than one institution, all corresponding superscript digits, separated with a comma with no space in between, should be present behind the family name of this author.

In case all authors belong to the same institution affiliation numbering is not needed.

Whenever possible expand your authors' affiliations with departments, or some other, specific and lower levels of organization.

#### **2.1.6. Corresponding author**

Corresponding author's name with full postal address in English and e-mail address should appear, after the affiliations. It is preferred that submitted address is institutional and not private. Corresponding author's name should include only initials of the first and middle names separated by a full stop (and a space) and the last name. Postal address should be written in the following line in sentence case. Parts of the address should be separated by a comma instead of a line break. E-mail (if possible) should be placed in the line following the postal address. Author should clearly state whether or not the e-mail should be published.

#### **2.1.7. Manuscript information**

All authors are required to provide word count (excluding title page, abstract, tables/figures, figure legends, Acknowledgements, Conflict of Interest, and References), the Abstract word count, the number of Tables, and the number of Figures.

## **2.2. Abstract**

The second page of the manuscripts should be the abstract and key words. It should be placed on second page of the manuscripts after the standard title written in upper and lower case letters, bold.

Since abstract is independent part of your paper, all abbreviations used in the abstract should also be explained in it. If an abbreviation is used, the term should always be first written in full with the abbreviation in parentheses immediately after it. Abstract should not have any special headings (e.g., Aim, Results...).

Authors should provide up to six key words that capture the main topics of the article. Terms from the Medical Subject Headings (MeSH) list of Index Medicus are recommended to be used.

Key words should be placed on the second page of the manuscript right below the abstract, written in italic. Separate each key word by a comma (and a space). Do not put a full stop after the last key word. *See example:*

### **Abstract**

Results of the analysis of...

*Key words: spatial memory, blind, transfer of learning, feedback*

## **2.3. Main Chapters**

Starting from the third page of the manuscripts, it should be the main chapters. Depending on the type of publication main manuscript chapters may vary. The general outline is: Introduction, Methods, Results, Discussion, Acknowledgements (optional), Conflict of Interest (optional), and Title and Abstract in Montenegrin (only for the authors from former Yugoslavia, excluding Macedonians and Slovenes). However, this scheme may not be suitable for reviews or publications from some areas and authors should then adjust their chapters accordingly but use the general outline as much as possible.

### **2.3.1. Headings**

Main chapter headings: written in bold and in Title Case. *See example:*

✓ **Methods**

Sub-headings: written in italic and in normal sentence case. Do not put a full stop or any other sign at the end of the title. Do not create more than one level of sub-heading. *See example:*

- ✓ *Table position of the research football team*

### 2.3.2 Ethics

When reporting experiments on human subjects, there must be a declaration of Ethics compliance. Inclusion of a statement such as follow in Methods section will be understood by the Editor as authors' affirmation of compliance: "This study was approved in advance by [name of committee and/or its institutional sponsor]. Each participant voluntarily provided written informed consent before participating." Authors that fail to submit an Ethics statement will be asked to resubmit the manuscripts, which may delay publication.

### 2.3.3 Statistics reporting

MJSSM encourages authors to report precise p-values. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Use normal text (i.e., non-capitalized, non-italic) for statistical term "p".

### 2.3.4. 'Acknowledgements' and 'Conflict of Interest' (optional)

All contributors who do not meet the criteria for authorship should be listed in the 'Acknowledgements' section. If applicable, in 'Conflict of Interest' section, authors must clearly disclose any grants, financial or material supports, or any sort of technical assistances from an institution, organization, group or an individual that might be perceived as leading to a conflict of interest.

## 2.4. References

References should be placed on a new page after the standard title written in upper and lower case letters, bold.

All information needed for each type of must be present as specified in guidelines. Authors are solely responsible for accuracy of each reference. Use authoritative source for information such as Web of Science, Medline, or PubMed to check the validity of citations.

### 2.4.1. References style

MJSSM adheres to the American Psychological Association 7th Edition reference style. Check the Publication Manual of the American Psychological Association (2019), Seventh Edition that is the official source for APA Style, to ensure the manuscripts conform to this reference style. Authors using EndNote® to organize the references must convert the citations and bibliography to plain text before submission.

### 2.4.2. Examples for Reference citations

One work by one author

- ✓ In one study (Reilly, 1997), soccer players...
- ✓ In the study by Reilly (1997), soccer players...
- ✓ In 1997, Reilly's study of soccer players...

Works by two authors

- ✓ Duffield and Marino (2007) studied...
- ✓ In one study (Duffield & Marino, 2007), soccer players...
- ✓ In 2007, Duffield and Marino's study of soccer players...

Works by three or more authors: cite only the name of the first author followed by et al. and the year

- ✓ Bangsbo et al. (2008) stated that...
- ✓ In one study (Bangsbo et al., 2008), soccer players...

Works by organization as an author: cite the source, just as you would an individual person

- ✓ According to the American Psychological Association (2000)...
- ✓ In the APA Manual (American Psychological Association, 2003), it is explained...

Two or more works in the same parenthetical citation: citation of two or more works in the same parentheses should be listed in the order they appear in the reference list (i.e., alphabetically); separated by a semi-colon

- ✓ Several studies (Bangsbo et al., 2008; Duffield & Marino, 2007; Reilly, 1997) suggest that...

### 2.4.3. Examples for Reference list

#### Works by one author

Borg, G. (1998). *Borg's perceived exertion and pain scales*: Human Kinetics.

#### Works by two authors

Duffield, R., & Marino, F. E. (2007). *Effects of pre-cooling procedures on intermittent-sprint exercise performance in warm conditions*. *European Journal of Applied Physiology*, 100(6), 727–735. <https://doi.org/10.1007/s00421-007-0468-x>

#### Works by three to twenty authors

Nepocatych, S., Balilionis, G., & O'Neal, E. K. (2017). Analysis of dietary intake and body composition of female athletes over a competitive season. *Montenegrin Journal of Sports Science and Medicine*, 6(2), 57–65. <https://doi.org/10.26773/mjssm.2017.09.008>

#### Works by more than twenty authors

Krustrup, P., Mohr, M., Amstrup, T., Rysgaard, T., Johansen, J., Steensberg, A.,... Bangsbo, J. (2003). The yo-yo intermittent recovery test: physiological response, reliability, and validity. *Medicine & Science in Sports & Exercise*, 35(4), 697–705. <https://doi.org/10.1249/01.mss.0000058441.94520.32>

#### Works by group of authors

NCD-RisC. (2017). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet*, 390(10113), 2627–2642. [https://doi.org/10.1016/s0140-6736\(17\)32129-3](https://doi.org/10.1016/s0140-6736(17)32129-3)

#### Works by unknown authors

*Merriam-Webster's collegiate dictionary* (11th ed.). (2003). Merriam-Webster.

#### Journal article (print)

Scruton, R. (1996). The eclipse of listening. *The New Criterion*, 15(3), 5–13.

#### Journal article (electronic)

Aarnivala, H., Pokka, T., Soinen, R., Mottonen, M., Harila-Saari, A., & Niinimäki, R. (2020). Trends in age- and sex-adjusted body mass index and the prevalence of malnutrition in children with cancer over 42 months after diagnosis: a single-center cohort study. *European Journal of Pediatrics*, 179(1), 91–98. <https://doi.org/10.1007/s00431-019-03482-w>

#### Thesis and dissertation

Pyun, D. Y. (2006). *The proposed model of attitude toward advertising through sport*. [Unpublished Doctoral Dissertation]. The Florida State University.

#### Book

Borg, G. (1998). *Borg's perceived exertion and pain scales*: Human Kinetics.

#### Chapter of a book

Armstrong, D. (2019). Malory and character. In M. G. Leitch & C. J. Rushton (Eds.), *A new companion to Malory* (pp. 144–163). D. S. Brewer.

#### Reference to a Facebook profile

Little River Canyon National Preserve (n.d.). *Home* [Facebook page]. Facebook. Retrieved January 12, 2020 from <https://www.facebook.com/lirinps/>

## 2.5. Tables

All tables should be included in the main manuscript file, each on a separate page right after the Reference section.

Tables should be presented as standard MS Word tables.

Number (Arabic) tables consecutively in the order of their first citation in the text.

Tables and table headings should be completely intelligible without reference to the text. Give each column a short or abbreviated

heading. Authors should place explanatory matter in footnotes, not in the heading. All abbreviations appearing in a table and not considered standard must be explained in a footnote of that table. Avoid any shading or coloring in your tables and be sure that each table is cited in the text.

If you use data from another published or unpublished source, it is the authors' responsibility to obtain permission and acknowledge them fully.

### 2.5.1. Table heading

Table heading should be written above the table, in Title Case, and without a full stop at the end of the heading. Do not use suffix letters (e.g., Table 1a, 1b, 1c); instead, combine the related tables. *See example:*

- ✓ **Table 1.** Repeated Sprint Time Following Ingestion of Carbohydrate-Electrolyte Beverage

### 2.5.2. Table sub-heading

All text appearing in tables should be written beginning only with first letter of the first word in all capitals, i.e., all words for variable names, column headings etc. in tables should start with the first letter in all capitals. Avoid any formatting (e.g., bold, italic, underline) in tables.

### 2.5.3. Table footnotes

Table footnotes should be written below the table.

General notes explain, qualify or provide information about the table as a whole. Put explanations of abbreviations, symbols, etc. here. General notes are designated by the word Note (italicized) followed by a period.

- ✓ *Note.* CI: confidence interval; Con: control group; CE: carbohydrate-electrolyte group.

Specific notes explain, qualify or provide information about a particular column, row, or individual entry. To indicate specific notes, use superscript lowercase letters (e.g. <sup>a,b,c</sup>), and order the superscripts from left to right, top to bottom. Each table's first footnote must be the superscript <sup>a</sup>.

- ✓ <sup>a</sup>One participant was diagnosed with heat illness and n = 19.<sup>b</sup>n = 20.

Probability notes provide the reader with the results of the tests for statistical significance. Probability notes must be indicated with consecutive use of the following symbols: \* † ‡ § ¶ || etc.

- ✓ \*P<0.05, †p<0.01.

### 2.5.4. Table citation

In the text, tables should be cited as full words. *See example:*

- ✓ Table 1 (first letter in all capitals and no full stop)
- ✓ ...as shown in Tables 1 and 3. (citing more tables at once)
- ✓ ...result has shown (Tables 1-3) that... (citing more tables at once)
- ✓ ...in our results (Tables 1, 2 and 5)... (citing more tables at once)

## 2.6. Figures

On the last separate page of the main manuscript file, authors should place the legends of all the figures submitted separately.

All graphic materials should be of sufficient quality for print with a minimum resolution of 600 dpi. MJSSM prefers TIFF, EPS and PNG formats.

If a figure has been published previously, acknowledge the original source and submit a written permission from the copyright holder to reproduce the material. Permission is required irrespective of authorship or publisher except for documents in the public domain. If photographs of people are used, either the subjects must not be identifiable or their pictures must be accompanied by written permission to use the photograph whenever possible permission for publication should be obtained.

Figures and figure legends should be completely intelligible without reference to the text.

The price of printing in color is 50 EUR per page as printed in an issue of MJSSM.

### 2.6.1. Figure legends

Figures should not contain footnotes. All information, including explanations of abbreviations must be present in figure legends. Figure legends should be written below the figure, in sentence case. *See example:*

- ✓ **Figure 1.** Changes in accuracy of instep football kick measured before and after fatigued. SR – resting state, SF – state of fatigue, \*p>0.01, †p>0.05.

### 2.6.2. Figure citation

All graphic materials should be referred to as Figures in the text. Figures are cited in the text as full words. *See example:*

- ✓ Figure 1
- × figure 1
- × Figure 1.
- ✓ ...exhibit greater variance than the year before (Figure 2). Therefore...
- ✓ ...as shown in Figures 1 and 3. (citing more figures at once)
- ✓ ...result has shown (Figures 1-3) that... (citing more figures at once)
- ✓ ...in our results (Figures 1, 2 and 5)... (citing more figures at once)

### 2.6.3. Sub-figures

If there is a figure divided in several sub-figures, each sub-figure should be marked with a small letter, starting with a, b, c etc. The letter should be marked for each subfigure in a logical and consistent way. *See example:*

- ✓ Figure 1a
- ✓ ...in Figures 1a and b we can...
- ✓ ...data represent (Figures 1a-d)...

## 2.7. Scientific Terminology

All units of measures should conform to the International System of Units (SI).

Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples.

Decimal places in English language are separated with a full stop and not with a comma. Thousands are separated with a comma.

Percentage	Degrees	All other units of measure	Ratios	Decimal numbers
✓ 10%	✓ 10°	✓ 10 kg	✓ 12:2	✓ 0.056
× 10 %	× 10 °	× 10kg	× 12 : 2	× .056

Signs should be placed immediately preceding the relevant number.

✓ 45±3.4	✓ p<0.01	✓ males >30 years of age
× 45 ± 3.4	× p < 0.01	× males > 30 years of age

## 2.8. Latin Names

Latin names of species, families etc. should be written in italics (even in titles). If you mention Latin names in your abstract they should be written in non-italic since the rest of the text in abstract is in italic. The first time the name of a species appears in the text both genus and species must be present; later on in the text it is possible to use genus abbreviations. *See example:*

- ✓ First time appearing: *musculus biceps brachii*
- ✓ Abbreviated: *m. biceps brachii*



ISSN 1451-7485

Sport Mont (SM) is a print (ISSN 1451-7485) and electronic scientific journal (eISSN 2337-0351) aims to present easy access to the scientific knowledge for sport-conscious individuals using contemporary methods. The purpose is to minimize the problems like the delays in publishing process of the articles or to acquire previous issues by drawing advantage from electronic medium. Hence, it provides:

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- Community-based dialogue on articles;
- Worldwide media coverage.

SM is published three times a year, in February, June and October of each year. SM publishes original scientific papers, review papers, editorials, short reports, peer review - fair review, as well as invited papers and award papers in the fields of Sports Science and Medicine, as well as it can function as an open discussion forum on significant issues of current interest.

SM covers all aspects of sports science and medicine; all clinical aspects of exercise, health, and sport; exercise physiology and biophysical investigation of sports performance; sport biomechanics; sports nutrition; rehabilitation, physiotherapy; sports psychology; sport pedagogy, sport history, sport philosophy, sport sociology, sport management; and all aspects of scientific support of the sports coaches from the natural, social and humanistic side.

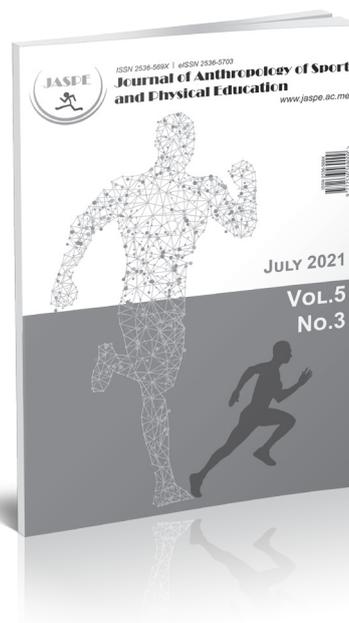
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**Publication date:** Autumn issue – October 2021  
Winter issue – February 2022  
Summer issue – June 2022



## **Journal of Anthropology of Sport and Physical Education**



ISSN 2536-569X

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- Fast publication time;
- Peer review by expert, practicing researchers;
- Post-publication tools to indicate quality and impact;
- Community-based dialogue on articles;
- Worldwide media coverage.

JASPE is published four times a year, in January, April, July and October of each year. JASPE publishes original scientific papers, review papers, editorials, short reports, peer review - fair review, as well as invited papers and award papers in the fields of Anthropology of Sport and Physical Education, as well as it can function as an open discussion forum on significant issues of current interest.

JASPE covers all aspects of anthropology of sport and physical education from five major fields of anthropology: cultural, global, biological, linguistic and medical.

Prospective authors should submit manuscripts for consideration in Microsoft Word-compatible format. For more complete descriptions and submission instructions, please access the Guidelines for Authors pages at the JASPE website: <http://www.jaspe.ac.me/?sekcija=page&p=51>. Contributors are urged to read JASPE's guidelines for the authors carefully before submitting manuscripts. Manuscripts submissions should be sent in electronic format to [jaspe@ucg.ac.me](mailto:jaspe@ucg.ac.me) or contact JASPE's Editor:

**Bojan MASANOVIC**, *Editor-in Chief* – [bojanma@ucg.ac.me](mailto:bojanma@ucg.ac.me)

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## MONTENEGRIN SPORTS ACADEMY

Founded in 2003 in Podgorica (Montenegro), the Montenegrin Sports Academy (MSA) is a sports scientific society dedicated to the collection, generation and dissemination of scientific knowledge at the Montenegrin level and beyond.

The Montenegrin Sports Academy (MSA) is the leading association of sports scientists at the Montenegrin level, which maintains extensive co-operation with the corresponding associations from abroad. The purpose of the MSA is the promotion of science and research, with special attention to sports science across Montenegro and beyond. Its topics include motivation, attitudes, values and responses, adaptation, performance and health aspects of people engaged in physical activity and the relation of physical activity and lifestyle to health, prevention and aging. These topics are investigated on an interdisciplinary basis and they bring together scientists from all areas of sports science, such as adapted physical activity, biochemistry, biomechanics, chronic disease and exercise, coaching and performance, doping, education, engineering

and technology, environmental physiology, ethics, exercise and health, exercise, lifestyle and fitness, gender in sports, growth and development, human performance and aging, management and sports law, molecular biology and genetics, motor control and learning, muscle mechanics and neuromuscular control, muscle metabolism and hemodynamics, nutrition and exercise, overtraining, physiology, physiotherapy, rehabilitation, sports history, sports medicine, sports pedagogy, sports philosophy, sports psychology, sports sociology, training and testing.

The MSA is a non-profit organization. It supports Montenegrin institutions, such as the Ministry of Education and Sports, the Ministry of Science and the Montenegrin Olympic Committee, by offering scientific advice and assistance for carrying out coordinated national and European research projects defined by these bodies. In addition, the MSA serves as the most important Montenegrin and regional network of sports scientists from all relevant subdisciplines.

The main scientific event organized by the Montenegrin Sports Academy (MSA) is the annual conference held in the first week of April.

Annual conferences have been organized since the inauguration of the MSA in 2003. Today the MSA conference ranks among the leading sports scientific congresses in the Western Balkans. The conference comprises a range of invited lecturers, oral and poster presentations from multi- and mono-disciplinary areas, as well as various types of workshops. The MSA conference is attended by national, regional and international sports scientists with academic careers. The MSA conference now welcomes up to 200 participants from all over the world.

It is our great pleasure to announce the upcoming 19th Annual Scientific Conference of Montenegrin Sports Academy "Sport, Physical Activity and Health: Contemporary Perspectives" to be held in Dubrovnik, Croatia, from 7 to 10 April, 2022. It is planned to be once again organized by the Montenegrin Sports Academy, in cooperation with the Faculty of Sport and Physical Education, University of Montenegro and other international partner institutions (specified in the partner section).

The conference is focused on very current topics from all areas of sports science and sports medicine including physiology and sports medicine, social sciences and humanities, biomechanics and neuromuscular (see Abstract Submission page for more information).

We do believe that the topics offered to our conference participants will serve as a useful forum for the presentation of the latest research, as well as both for the theoretical and applied insight into the field of sports science and sports medicine disciplines.





**Faculty for sport  
and physical education  
NIKŠIĆ**



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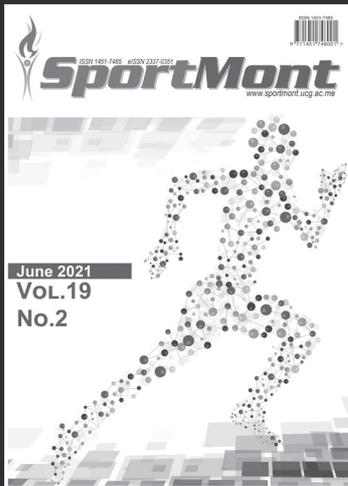
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## Sports Science and Medicine Journals from Montenegrin Sports Academy

We have expanded the quality of our journals considerably over the past years and can now claim to be the market leader in terms of breadth of coverage.

As we continue to increase the quality of our publications across the field, we hope that you will continue to regard MSA journals as authoritative and stimulating sources for your research. We would be delighted to receive your comments and suggestions, mostly due to the reason your proposals are always welcome.

## Look Inside!



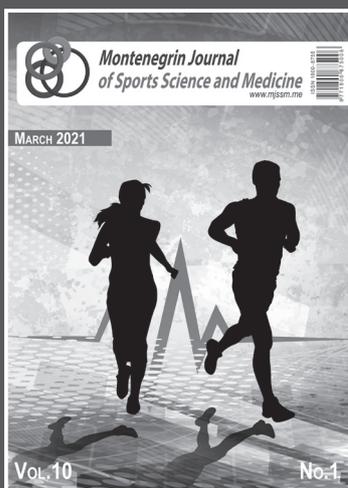
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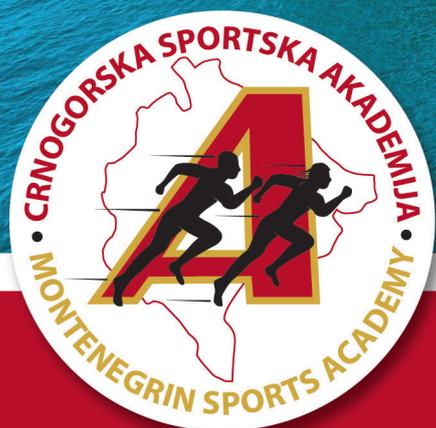
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MSA Conference 2022

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7<sup>th</sup> - 10<sup>th</sup> April 2022,  
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