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Sports massage with ozonised oil or non-ozonised oil: Comparative effects on recovery parameters after maximal effort in cyclists

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ABSTRACT

Objectives: To study the effects of passive rest (PR) and sports massage with (SMOZO) and without (SM) ozonised oil on sports performance psycho-physiological indices in competitive amateur cyclists after 3 pre-fatiguing Wingate cycle and post-recovery ramp tests.
Design: An intra-subjects experimental design with repeated measures.
Setting: Department of Human Anatomy and Physiology, University of Padua.
Participants: Fifteen male competitive cyclists (age: 27 ± 3.5 years, body weight: 77.6 ± 8.3 kg, height: 178 ± 7.7 cm) were studied.
Main outcome measures: Subjects' power output (P), heart rate (HR), Visual Analogue Scale (VAS) score and blood lactate (BL) clearance in response to PR, SMOZO and SM recoveries were compared.
Results: There were no significant differences in cyclists' heart rate patterns in the three experimental conditions ($p > 0.05$). After SMOZO recovery, athletes showed a higher P_{max} ($p < 0.05$) and a lower perceived fatigue VAS score ($p < 0.033$) in the ramp test. Blood lactate decreased more at T2 (mid-time point of treatment) and T3 (final time point of treatment) than T1 (beginning of treatment) compared to SM and PR conditions.
Conclusions: These findings suggest that use of ozonised oil during sports massage increases blood lactate removal, improves performance and reduces the perception of fatigue in cyclists from 3 Wingate tests.

1. Introduction

A widely accepted view is that fatigue does not arise from a single process but many distinct mechanisms, both central and peripheral. Moreover, it is known that the involvement of each mechanism can vary from one type of exercise to another. There are several controversies in the literature about which kind of recovery is best used to attenuate fatigue development. The reduction of lactate production or the increase of its removal rate has been a goal pursued by many investigators and is linked to the lactate-fatigue hypothesis (Fitts & Holloszy, 1976). Several researchers have used high-intensity cycling exercises such as the Wingate test in order to induce lactate-fatigue characteristics in studied subjects (Martin, Zoeller, Robertson, & Lephart, 1998; Robertson, Watt, & Galloway, 2004). This is because the Wingate test has been shown to rely on anaerobic glycogenolysis as the primary energy pathway, producing blood lactate concentrations ranging from 6 to 15 times above resting levels (Baror, 1987). Monedero & Donne (2000) showed that active recovery was more efficient in blood lactate removal than massage alone and that the combined recovery (massage plus active recovery) was the most efficient intervention for maintaining maximal performance time during a simulated 5 km maximal effort cycling tests. Moreover, it is known that sports massage is effective on the reduction of harmful effects of eccentric exercise (Rodenburg, Steenbeek, Schiereck, & Bar, 1994), delayed-onset muscle soreness (DOMS) (Hilbert, Sforzo, & Swensen, 2003), swelling (Zainuddin, Newton, Sacco, & Nosaka, 2005)

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and the perception of fatigue (Mori, Ohsawa, Tanaka, Taniwaki, Leisman, & Nishijo, 2004). Sports massage can be applied before exercise to improve subsequent exercise performance (Arroyo-Morales et al., 2011) and as a post-exercise treatment to support the recovery of muscle performance (Brooks, Woodruff, Wright, & Donatelli, 2005) and to lessen post-exercise fatigue (Ogai, Yamane, Matsumoto, & Kosaka, 2008). Indeed, Arroyo-Morales et al. (2011) showed a significant decrease in the tension subscale of the Profile of Mood States (POMS) in a massage group compared to a placebo group indicating a role for sports massage in athletes prone to excessive pre-event tension. Moreover, Brooks et al. (2005) found that manual massage to the forearm and hand after maximal exercise was associated with greater effects than non-massage on post-exercise power grip performances in healthy adults (Brooks et al., 2005). Furthermore, Ogai et al. (2008) showed that sports massage was effective for decreasing perceived lower limb fatigue and muscle stiffness, and for recovery of total power during bicycle ergometer pedalling. Techniques like effleurage (stroking) and petrissage (kneading) are commonly used in sports massage to induce post-exercise intramuscular oedema resolution, by facilitating fluid movement and increasing circulation (Callaghan, 1993). During sports massage, several athletes use creams and oils enriched with Bioperoxoil®, a compound of 30% ozonised sunflower seed oil with 0.5% alpha-lipoic acid (Rodrigues, Cardoso, Caputo, Carvalho, Fiorini, & Schneedorf, 2004), to improve recovery and to reduce post-exercise fatigue. The ozonised oils have been widely studied in their chemical structure (Travagli, Zanardi, Valacchi, & Bocci, 2010), in their pharmacology and toxicology and in their dermatologic compatibility (Travagli, Zanardi, Bernini, et al., 2010; Travagli, Zanardi, Valacchi, et al. 2010) giving guarantees on the perfect tolerability in dermatologic applications. The levels of toxicity of ozonised oils are perfectly comparable with those of natural oils from which they originate. Ozonised oils are stable enough to be included in cosmetic preparations for topical applications (Kim et al., 2009). Some of the biological effects of ozone have been attributed to rapid reactions of the gas with unsaturated fatty acids present in vegetable oils and cellular compartments, releasing endogenous lipid peroxides and hydroperoxides able to penetrate cellular membranes (Rodrigues et al., 2004). In particular, topical ozone therapy has been reported to activate local microcirculation, increase oxygen tension, improve cellular oxygen uptake, stimulate oxidative defensive enzymatic systems and improve granulation and tissue growth (Rodrigues et al., 2004; Valacchi et al., 2011). For these reasons the use of ozonised oils in sports massage may improve sports performance and post-exercise recovery. In recent years massage with ozonised oil of muscles engaged in strenuous activity during various sporting events has become increasingly popular. However, there are no data about the effects of sports massage with ozonised oil on the performance of exercised athletes. Therefore, the purpose of this study was to examine the effects of passive rest and sports massage with and without ozonised oil on the performance, heart rate, subjective feelings of fatigue and blood lactate clearance in competitive amateur cyclists after Wingate cycle tests.

2. Methods

2.1. Experimental design

This study was an intra-subjects experimental design with repeated measures under three experimental conditions. All subjects performed: (i) a warm up period, (ii) a maximal pre-fatiguing exercise phase, (iii) a recovery period (RP) and (iv) a ramp test (Fig. 1). During the recovery period (iii) cyclists were subjected to one of three experimental conditions: 15 min passive recovery [PR]; 15 min recovery by sports massage with oil enriched with Bioperoxoil (30% ozonised sunflower seed oil with 0.5% of alpha-lipoic acid) [SMOZO] or 15 min recovery by sports massage [SM] with oil but without Bioperoxoil (placebo treatment). The setting of the conditions was determined by random assignment. Fourteen days before the test session, familiarization was completed to ensure that all subjects knew the protocol and could complete the amount of work required. In particular, during the familiarization session all subjects performed a warm up period, three successive 30-S Wingate Tests, a recovery period by sports massage and a ramp test by cyclo ergometer. Dietary intake (food and fluid) was recorded for two days before the familiarization visit, and subjects then replicated this exactly in the two days before each testing session. During these days cyclists were instructed to refrain from heavy physical activity. In this way in agreement with Robertson et al. (2004) we should have ensured that pre-exercise muscle glycogen content and acid-base balance were similar before each testing session (Robertson et al., 2004). Hence, any results should be treatment-related rather than linked to extraneous elements. Each testing session was separated from the following one by 2 days.

Moreover, subjects were instructed not to take any medication (anabolic steroids, other hormones, metabolic modulators, diuretics, non-steroidal anti-inflammatory drugs NSAIDs, etc) or to undergo any medical treatment (blood transfusion, blood donation)

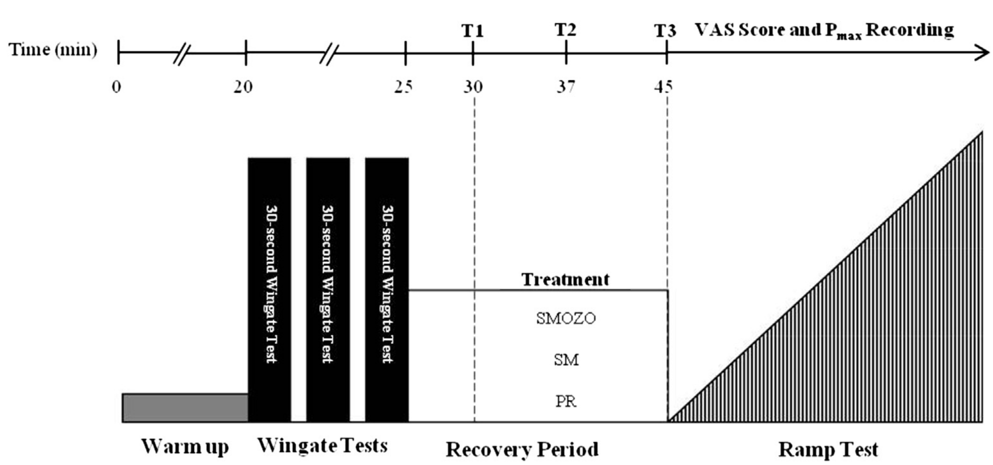

Fig. 1. Experimental design. Legends: SMOZO: SPORTS massage with ozonised oil; SM: Sports massage; PR: Passive recovery; T1: Blood lactate sampling at beginning of treatment (baseline); T2: Blood lactate sampling at mid-time point of treatment; T3: Blood lactate sampling at final time point of treatment.

during the experimental period and not to consume food, caffeine, or alcohol during the 3 h before the test. Each cyclist was questioned about his compliance with the instructions on arrival for each test session. Based on published findings (Dotan, Ohana, Bediz, & Falk, 2003; Leicht & Perret, 2008) a sample size evaluation was performed using previous results as a gold standard – the significance level was set at 0.05, standard deviation was set at 1.25 and power was set at 0.80. Regarding the main dependant variable; the difference that we expected among groups (PR-SM-SMOZO) was set at 1.40 mmol/l.

2.2. Subjects

Fifteen healthy men (age: 27 ± 3.5 years, body weight: 77.6 ± 8.3 kg, height: 178 ± 7.7 cm, training history: 8 ± 4 years, travelling distance/week: 380 ± 50 km) from two teams of competitive amateur cyclists volunteered to take part in this study. Inclusion/exclusion criteria for subjects were: to be competitive amateur cyclists; no history of orthopaedic, cardiovascular or metabolic disorders that may affect the subjects' ability to perform a high-intensity test; no contra – indications to sports massage.

2.3. Procedures

Every testing session included: a warm up period, a maximal pre-fatiguing exercise phase, a recovery period and a ramp test (Fig. 1). The warm up protocol consisted of 20 min standardized light activity including 15 min of cycling at 60 rpm and 80 Watt followed by 5 min at 100 Watt. The maximal pre-fatiguing exercise phase included three successive 30-s Wingate Tests on Cardioline Xr200n (Cardioline – Medical Devices SpA – Milano, Italy) cyclo – ergometer with electromagnetic brake. Each 30-s Wingate Test was separated by a 2-min recovery period from the following one (Martin et al., 1998). In agreement with Baror (1987) we asked the subjects to perform 30 s of "all out" (supramaximal) cycling at a very high braking force that was indexed to body weight (0.75 kg × body weight). In this way the Wingate test has been shown to produce a greater amount of blood lactate (Baror, 1987). After the third 30-s Wingate Tests subjects started the recovery period. During the first five minutes participants remained seated on the cycle ergometer without pedalling in order to allow blood lactate levels to reach peak post-exercise levels (Dotan et al., 2003; Leicht & Perret, 2008), and at the end of this 5-min period one of the three recovery treatments was immediately initiated. In passive recovery subjects rested for ~8 min in supine posture and for ~8 min in prone position; during the sports massage, with or without ozonised oil, athletes were rubbed down for ~8 min in supine and prone, respectively. We used a ~4-min sports massage routine for each leg in prone and supine, respectively. During the first two minutes of massage we used effleurage technique, defined as any stroke that glides over the skin without attempting to move the deep muscle masses (Martin et al., 1998); as opposed to petrissage technique used during the last two minutes, consisting of kneading manipulations that compress and roll the skin and muscle under the hands/fingers (Martin et al., 1998). The same professional masseur with 10 years of experience administered all massages using oil (40 ml was used per massage) with or without Bioperoxoil (Bioperoxoil® by Ozonoil SA, Barcelona, ES). The professional masseur and participants were blinded in terms of experimental protocols: SMOZO or SM, respectively. Plasma lactate was measured with SensLab Lactate Scout–Test strips (Bautzner Staβe, Leipzig, Germany) using capillary blood collected from the index finger. Blood samples were obtained at the ~5th (T1), ~13th (T2) and ~20th minute (T3) of the recovery period. We selected the 5th minute as lactate baseline as it is the hypothesized time point in which blood lactate levels should have reached peak post-exercise levels (Dotan et al., 2003; Leicht & Perret, 2008), and the 13th and 20th minute of total recovery period as the middle and final time points of treatment phase, respectively. Following recovery, athletes performed a ramp test using the Cardioline Xr200n cyclo ergometer with electromagnetic brake (Cardioline – Medical Devices SpA – Milano, Italy). Following 3 min of baseline cycling at 60 W, work-rate was increased by 1 W every 2 s (i.e. 30 W/min) until the participant was unable to continue despite vigorous encouragement (Poole, Wilkerson, & Jones, 2008). The participants cycled at a self-selected pedal rate, which remained constant throughout the test (70–90 rev/min). The heart rate was monitored during several experimental phases by a Polar heart beat monitor (Polar S810; Polar, Kemple, Finland). We recorded the maximal power reached (P_{max}) and immediately after the incremental test we recorded the athletes' perceived fatigue level. To analyse the level of perceived fatigue we used the Visual Analogue Scale (VAS), which is 100 mm long vertically. The bottom of the scale (at 0 mm) is indicated as 'no fatigue' and the top of the scale (at 100 mm) is indicated as extreme fatigue (Lee, Hicks, & Nino-Murcia, 1991). To evaluate the normality we performed the *Shapiro–Wilk's W* test. All variables (Blood lactate 0.86; Heart rate 0.98; P_{max} 0.83; VAS 0.91) were normally distributed.

2.4. Reliability and reproducibility

The same investigator took all the measurements. Before experimentation, all subjects were invited twice to the physiological laboratory in order to standardize all data collection procedures.

2.4.1. Wingate test

In agreement with Weinstein, Bediz, Dotan, & Falk (1998) we showed that the Wingate test had good intraclass reliability coefficients for peak blood lactate concentration, peak heart rate and mean power of 0.94, 0.91, and 0.94 respectively.

2.4.2. Ramp test

The ramp test was performed two times with ICC values of 0.89 for P_{max} and, in agreement with Lee et al. (1991), 0.88 for the VAS test.

2.5. Statistical analysis

One-way ANOVA was used to compare group variables at baseline, VAS and P_{max} followed by Tukey post hoc testing when indicated. A multivariate ANOVA for repeated measurement (treatment × time) was used for blood lactate. Statistical significance was set at $p < 0.05$ for all tests. All data were analysed by using Prism5 GraphPad software (Abacus Concepts GraphPad Software, San Diego, CA). Statistica software vers. 8.0 (Tulsa, USA) were used to obtain ICC values and for a sample size evaluation. All values are expressed as means ± SD.

3. Results

Athletes showed similar blood lactate levels ($p > 0.05$) at time T1 after high-intensity cycling exercise [PR: 10.92 ± 1.28 mmol/l, SM: 10.74 ± 1.25 mmol/l; SMOZO: 10.79 ± 1.22 mmol/l, $p > 0.05$]. This demonstrated that subjects underwent the three kinds of recovery treatments starting from the same blood lactate levels. We observed similar blood lactate clearance patterns (Fig. 2) in the cyclists in response to the three different kinds of recovery. Indeed, we showed that blood lactate levels significantly decreased at T2 vs. T1 and at T3 vs. T2 in all experimental conditions (PR, SMOZO and

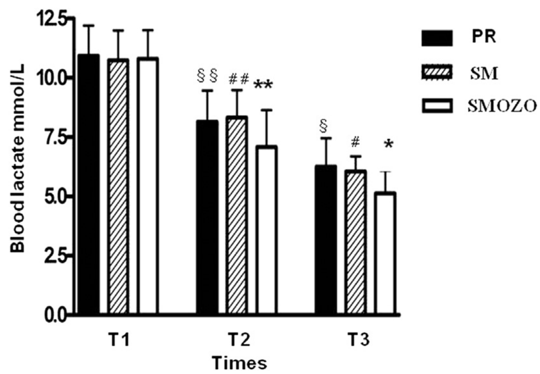

Fig. 2. Mean blood lactate concentration at T1, T2 and T3 times in response to passive, SMOZO and SM recoveries. $^{*,\#,\S}P < 0.05$ T3 vs. T1; $^{**,\#\#,\S\S}P < 0.05$ T2 vs. T1.

SM) ($p < 0.002$). However, we observed a higher blood lactate clearance in response to SMOZO treatment (Table 1). Indeed, SMOZO group showed a lower blood lactate than SM and PR at T2 vs. T1 [SMOZO (34.3%) vs. SM (22.5%), $\Delta = 11.8\%$; SMOZO (34.3%) vs. PR (25.4%), $\Delta = 8.9\%$] and T3 vs. T2 [SMOZO (27.6%) vs. SM (27.2%), $\Delta = 0.4\%$; SMOZO (27.6%) vs. PR (23.2%), $\Delta = 4.4\%$] time points. Moreover, we did not show any significant differences in cyclists' heart rate patterns before, during and after the three kinds of experimental conditions ($p > 0.05$) (Table 2). Instead, during ramp incremental test, after SMOZO treatment athletes showed a higher P_{max} compared to when they were treated by PR and SM (P_{max}–SMOZO: 370 ± 60 W vs. P_{max}–PR: 344 ± 56 W, $p < 0.05$; P_{max}–SMOZO: 370 ± 60 W vs. P_{max}–SM: 340 ± 55 W, $p < 0.05$). The recovery with massage significantly reduced athletes' perceived fatigue in comparison with passive recovery. Indeed, VAS scores were significantly lower ($p < 0.033$) in SMOZO than SM and PR and in SM compared to PR (Fig. 3).

4. Discussion

The major finding of this study is that sports massage with ozonised oil promoted a higher blood lactate clearance and better ramp test performance than passive recovery or simple sports massage in male competitive amateur cyclists following high-intensity anaerobic activity. On the other hand we found that blood lactate clearance in cyclists in response to SM and PR showed no difference. These findings support the results of Hemmings, Smith, Graydon, and Dyson (2000) which showed that massage did not improve blood lactate clearance compared to passive recovery in repeated sports performance. In agreement with Robertson et al. (2004) the lack of an observed effect on lactate clearance in the SM group compared to the PR group implied that there was no change in muscle blood flow and/or lactate efflux during the massage intervention and that lactate removal from the circulation was unaffected by massage (Robertson et al., 2004). Therefore, the higher blood lactate clearance in response to SMOZO treatment may be solely attributable to the biological effects of ozonised oil. Furthermore it is known that heart rate is an important physiological factor involved in lactate clearance (Perret, Labruyère, Mueller, & Strupler, 2012) and in our study we found that cyclists' heart rate patterns did not show any significant differences during the three types of treatment. For this reason we can exclude the possibility that heart rate patterns explained the differences in post-exercise blood lactate clearance.

The influence of massage with ozonised oil appeared early on in the process - from T1 to T2 the SMOZO group showed a higher percentage decrease of blood lactate (34.3%) than T2 vs. T3 (27.6%). Overall these findings suggest that sports massage with the Bioperoxoil enriched oil may increase lactate removal rate from blood tissue in competitive amateur cyclists. Topical ozone therapy-related biological effects (Rodrigues et al., 2004) could explain the improvement of blood lactate clearance influencing post-exercise performance recovery. Indeed, several authors suggested that lactate elimination from the exercising muscle is dependent on the capillary supply (Tesch & Wright, 1983), metabolic rate and systemic blood flow (Brooks, 1986). Ozone, once introduced into the body, acts by influencing the phospholipid component of the erythrocyte membrane, increasing elasticity and correspondingly the ability of blood to deliver oxygen to tissues (Bocci, Travagli, & Zanardi, 2009; Kim et al., 2009; Travagli, Zanardi, Silvietti, & Bocci, 2007). Also Coppola et al. (1995) demonstrated that oxygen-ozone treatment reduces both plasma and whole blood viscosity (Coppola et al., 1995).

An increase in antioxidant defence efficiency and lactate clearance (Rodrigues et al., 2004; Valacchi et al., 2011) could be one reason that the SMOZO group showed a lower fatigue and a higher power output than PR and SM groups in response to the ramp test. Indeed, several reports indicate that blood lactate and reactive oxygen species (ROS) contribute to induce muscular fatigue (Fitts & Holloszy, 1976; Powers & Jackson, 2008; Robertson et al., 1986). In particular metabolic acidosis has been shown to be a major factor in muscular fatigue during short-term, high-intensity exercise (Robertson et al., 1986). The resultant decrease in muscle force production consequent to lactate accumulation, although not the only factor affecting the fatigue and performance, could represent the rate limiting for peak anaerobic exercise performance (Marcora & Staiano, 2010). Furthermore, low blood pH has been shown to excite pain receptors, contributing to an amplified perception of physical exertion and decreased muscular performance (Robertson et al., 1986). Therefore, accelerating blood lactate clearance immediately post-exercise may be beneficial for a subsequent bout of high-intensity exercise, particularly during athletic competitions that require multiple performances in a single day such as boxing (Hemmings et al., 2000). In this way, the use of ozonised oil in sports massage could increase the known massage-related psychophysical effects on fatigue perception. Indeed, in agreement with Ogai et al. (2008) the SM group showed a lower perception of fatigue ($p < 0.05$) than the PR group (Ogai et al., 2008). This is probably because massage is known to reduce the concentration of cortisol, indicating alleviation of general stress and increased dopamine and serotonin values, signifying changes

Table 1
Blood lactate clearance during recovery period.

Kind of treatment	Lactate (mmol/L)			Percentage decrease		
	T1	T2	T3	T1 vs. T2	T1 vs. T3	T2 vs. T3
PR	10.92 ± 1.28	8.14 ± 1.33	6.2 ± 1.21	25.4%	42.7%	23.2%
SMOZO	10.79 ± 1.22	7.08 ± 1.55	5.1 ± 0.97	34.3%	52.5%	27.6%
SM	10.74 ± 1.25	8.32 ± 1.16	6.0 ± 0.64	22.5%	43.6%	27.2%

Legends: T1: beginning of treatment (baseline), T2: mid-time point of treatment, T3: final time point of treatment.

Table 2
Mean (SD) heart rate response (beats/min) at the end of each of the following stages: Warm up, 30-s Wingate tests (W1, W2, W3), 2′ recovery, recovery period and ramp test.

Treatment	Rest	Warm up	Maximal pre-fatiguing exercise phase					Recovery	Ramp test
			W1	2′Recovery	W2	2′Recovery	W3		
PR	62 ± 6	90 ± 11	188 ± 12	123 ± 15	184 ± 11	129 ± 11	191 ± 9	84 ± 10	195 ± 13
SMOZO	63 ± 8	92 ± 6	190 ± 11	126 ± 16	187 ± 12	125 ± 10	195 ± 15	81 ± 9	193 ± 11
SM	60 ± 10	95 ± 10	192 ± 13	128 ± 12	189 ± 15	130 ± 9	193 ± 8	87 ± 12	196 ± 15

PR: Passive Recovery; SMOZO: Sports Massage with ozonised oil; SM: Sport Massage.

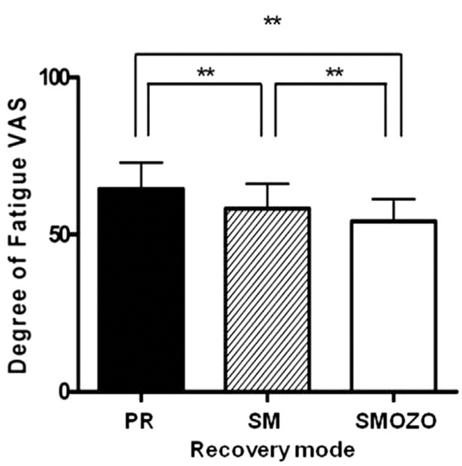

Fig. 3. Mean of Visual Analogue Scale (VAS) scores after ramp test in cyclists from passive, SMOZO and SM recoveries. *$P < 0.05$ SMOZO vs. SM; **$P < 0.05$ SMOZO vs. PR; ***$P < 0.05$ SM vs. PR.

of subjective experience of exercise stress (Field, Hernandez-Reif, Diego, Schanberg, & Kuhn, 2005).

Several studies in the literature that investigated the effects of sports massage did not indicate length, intensity, type of treatment and dietary intake during the days preceding laboratory tests. In our study we standardized the procedure to analyse the effects of sports massage with ozonised oil on performance, subjective feelings of fatigue and blood lactate clearance in competitive amateur cyclists subjected to Wingate cycle tests. However, in the present study we did not include for comparison a recovery by stretching methodology. This is a limitation of our study because stretching is one of the main recovery techniques and many people stretch before or after engaging in athletic activity. Usually though the purpose of stretching is to reduce risk of injury, decrease soreness after exercise, or improve athletic performance. A necessary area for future study includes biochemical analyses to investigate the metabolic pathways through which the ozonised oil exerts its positive effects on lactate clearance reported in the present study.

5. Conclusion

Topical application of Ozone during sports massage appears to be a promising way to reduce recovery time and attenuate some components of muscle fatigue. In particular it could be useful to introduce sports massage with ozonised oil in physical activities requiring repeated performance, such as boxing, in which massage is commonly used as recovery methodology. The use of ozonised oil in sports massage may lead to greater lactate removal and improved sports performance.

Conflict of interest

None declared.

Ethical approval

The study was conducted in accordance with the principles of the Helsinki declaration and was approved by the Departmental Ethical Committee (University of Padova).

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