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Effect of glucose and sodium chloride mouth rinses on neuromuscular fatigue: a preliminary study

Teng Keen Khong[1,2], Victor S Selvanayagam[1], and Ashril Yusof[1]

[1]Centre of Sport and Exercise Sciences, University of Malaya, Kuala Lumpur, Malaysia

[2]Department of Sports Sciences, Faculty of Applied Sciences, Tunku Abdul Rahman University College, Kuala Lumpur, Malaysia

Corresponding Author: Ashril Yusof.

e-mail: ashril@um.edu.my

Running title: Effect of CHO and NaCl Mouth Rinse on Neuromuscular Fatigue

Abstract

Carbohydrate (CHO) mouth rinse has been shown to improve endurance performance and maintain the central drive of contracting muscles. Salt (NaCl) mouth rinse solution, often used in dentistry to desensitise the oral cavity to pain, could also activate cortical areas of the brain. Hence, the objective of this preliminary study was to investigate whether CHO (glucose) and NaCl mouth rinses could attenuate the reduction in maximum voluntary contraction (MVC) and sustained MVC (sMVC) following an endurance exercise (30-minute cycling at 70% VO_2max). Ten subjects (male, age: 22 ± 1 years, weight: 65.3 ± 12.4 kg, height: 164.5 ± 7.5 cm, VO_2max: 48.3 ± 6.1 $mL \cdot kg^{-1} \cdot min^{-1}$) completed three trials of 30-minute cycling exercise. In a randomised cross-over study, in each trial, the participants rinsed using either water, 6% glucose, or 6% NaCl solution for 5 seconds immediately prior to and every 10 minutes during the cycling exercise. The MVC and sMVC were measured pre and post cycling. Analysis of variance showed significant interaction and time effects for MVC, while for sMVC there was a significant interaction with time and group effects. Both MVC and sMVC were higher post cycling in the glucose and NaCl groups compared to the

water group, which suggests that activation of glucose and NaCl oral receptors could better preserve post-exercise force production. This is the first study to show that NaCl mouth rinse can produce a comparable effect to glucose. Hence, mouth rinses may be able to activate other distinct pathways that could attenuate fatigue.

Keywords: Carbohydrate, Sodium Chloride, Central Fatigue, Mouth Rinse, Maximal Voluntary Contraction

Introduction

The oral cavity is responsive to tastants such as sweetness and saltiness that indicate the presence of sugar (i.e., glucose) and salt (NaCl), respectively. The taste receptors activate the nervous system at the peripheral level which, in turn, transmit signals to the respective areas of the brain cortex to produce an appropriate response (gustatory excitation) (Roper, 2013). In recent years, studies have shown that carbohydrate (CHO) mouth rinse can improve endurance performance, as shown by improved time trials (Carter, Jeukendrup, & Jones, 2004; Chambers, Bridge, & Jones, 2009; Lane, Bird, Burke, & Hawley, 2012; Pottier, Bouckaert, Gilis, Roels, & Derave, 2010; Rollo, Williams, Gant, & Nute, 2008) and time-to-exhaustion (Fares & Kayser, 2011). These findings indicate that the oral CHO receptors activate the insular and motor cortices, which subsequently excite the neuromuscular pathways (Chambers *et al.*, 2009) and hence improve performance. It has been reported that both insular and motor cortical activities spike sharply during a sustained contraction, whereas a small increase in the magnitude of both occurs during an intermittent contraction (Liu *et al.*, 2003). Hence, it is possible that oral receptors other than the CHO receptor, such as salty, sour and bitter tastants, could also stimulate areas of the brain and thereby activate neural drive (Leow *et al.,* 2007).

Carter *et al.* (2004) were the first to show that rinsing the mouth with a maltodextrin (6.4%) solution results in a higher power output during a 1-h cycling time trial. Since then, many studies on the effect of CHO mouth rinse have shown similar outcomes for prolonged exercise performance (Chambers *et al.*, 2009; Kasper *et al.*, 2016; Pottier *et al.*, 2010). However, a few studies have reported a lack of improvement using CHO mouth rinse (Beelen *et al.*, 2009; Whitham & McKinney, 2007). The satiety level seems to affect the outcomes of mouth rinse, where better performance has been observed in unfed (Fares & Kayser, 2011) as compared to fed (Beelen *et al.*, 2009) states. When unfed, low muscle and liver glycogen seem to aggravate the positive effects of CHO mouth rinse (Beelen *et al.*, 2009). Other factors that may also influence the outcome are the concentration of CHO solution and the rinse duration. A review by de Ataide e Silva *et al.* (2013) suggests that a 6–10% range of CHO concentration and a 5–10-s rinse time are adequate to produce beneficial effects. In addition to improving prolonged cycling/running performance, maltodextrin mouth rinse has also been found to better preserve maximum voluntary contraction (MVC), as reported by Jeffers *et al.* (2015). However, in their study, the preservation of muscular force could not be attributed to changes in the central factor because voluntary activation (VA) does not differ between the maltodextrin and placebo mouth rinse groups.

The potential to activate the insular and motor cortices is not confined to CHO tastants alone, as NaCl is also able to produce fMRI responses in the right insular cortex (Prinster *et al.*, 2017), which is linked to other parts of the brain that are involved in cardiovascular regulation during exercise (Waldrop, Eldridge, Iwamoto, & Mitchell, 1996). In addition, electromyography (EMG) recordings have shown that a stronger contraction and higher activation rate in the muscles involved in swallowing occur when NaCl is prescribed (Ding, Logemann, Larson, & Rademarker, 2003). These findings suggest that the stimulation of the oral NaCl receptors could increase muscle activity, and therefore could potentially increase force production.

As CHO mouth rinse has no influence on metabolic processes (Carter *et al.*, 2004), it is believed that changes in central drive or motivation level contribute to the reported improvements in performance. Therefore, it is pertinent to investigate the effect of mouth rinse on exercise that involves high central drive, such as MVC. In addition, it seems likely that other tastants such as NaCl could potentially produce changes in the brain and thereby preserve MVC. Hence, the aim of the current study was to investigate the effects of glucose and NaCl mouth rinse solutions on MVC after a 30-minute cycling activity at 70% of VO_2max. In this study it was hypothesised that both glucose and NaCl would improve MVC post cycling as compared to water.

Methods

Participants

Ten males (n = 10, age: 22 ± 1 years, weight: 65.3 ± 12.4 kg, height: 164.5 ± 7.5 cm, VO_2max: 48.3 ± 6.1 $mL \cdot kg^{-1} \cdot min^{-1}$) provided their informed consent to participate in the experiment for this study. They were non-smokers, free from any metabolic diseases and injuries, and exercised for at least 30 minutes 3 days per week.

Study design

In a randomised cross-over study with a 7-day washout period, the participants underwent three trials with water, 6% glucose, and 6% NaCl mouth rinse solutions. They refrained from intense exercise, caffeine, and alcohol for 24 hours, and fasted overnight prior to the trials. All trials were performed during a similar time of day (around 08.00) in a controlled laboratory environment. Prior to the experimental trials, participants underwent a VO_2max test to determine their maximum aerobic capacity and cycling intensity for the trial, which was followed by a familiarisation session that simulated the actual trial. On the trial day, after warming up, participants took part in MVC and sustained MVC (sMVC) tests. They then cycled for 30 minutes at an intensity corresponding to 70% VO_2max. Thirty minutes of cycling at 70% VO_2max was selected for the following reasons: first, the duration of 30 minutes was chosen because it is the minimum amount of time for an endurance exercise in which a CHO mouth rinse has been observed to improve exercise performance, as reviewed by Jeukendrup *et al*. (2013). Second, the exercise intensity of 70% VO_2max was chosen because it is sufficient to induce a lower force output post exercise (Khong *et al.*, 2018). A 1-minute gaseous exchange was collected every 5 minutes during the cycle using the COSMED Quark CPET metabolic cart (Rome, Italy) and presented in Figure 3, and the cycle ergometer's (Monark 828E, Sweden) load was adjusted against a set RPM (60) to ensure that exercise intensity (70% VO_2max) was maintained throughout each trial; when the VO_2 value is >73% of VO_2max the load was decreased and when the VO_2 value was 67% < of VO_2max the load was increased. The designated mouth rinse was given immediately before the cycling exercise, and at every 10 minutes (10th, 20th, and 30th minute) during the trial. The solution was rinsed for 5 seconds and then spat out. The 5-second rinse time was chosen to minimise obstructions to breathing and to concentration on maintaining the required cycling revolutions per minute (rpm). Moreover, the review by de Ataide e Silva *et al.* (2013) indicated that a 5-second rinse time would be sufficient for the purposes of the current study. MVC and sMVC data was collected again immediately upon the cessation of cycling. The participants were encouraged to perform to their maximal capability and to try to beat their previous performance, regardless of the order in which the solutions were administered.

MVC and sMVC recordings

The participants were seated on a dynamometer (HUMAC NORM, MA, USA) with their chest, pelvis and thigh secured with straps to avoid extraneous body movements during MVC. For the MVC recordings, participants performed three sets of maximal isometric knee extension contractions at 60º, followed by a 2-minute rest. The highest torque produced from the three sets was used as the MVC. For the sMVC recordings, a sustained maximal isometric knee extension at 60º was performed for 90 seconds. During the recording of both the MVC and sMVC, participants were verbally encouraged to produce their maximal efforts.

Statistical analysis

All statistical analyses were performed using SPSS 23.0 (IBM Corp., Armonk, NY, USA). Data normality was examined and confirmed for $p > 0.05$ using the Shapiro–Wilks test. A mixed model repeated measure two-way analysis of variance (ANOVA) was used to determine the effect of the mouth rinse on sMVC and MVC. A Tukey post-hoc analysis was used to determine pairwise difference. Cohen's d analysis was used to measure the effect sizes of the post-test results between groups. Values are presented as mean ± SD and statistical significance is set at $p < 0.05$.

Results

MVC

For MVC, two-way ANOVA showed a significant interaction effect ($F_{2, 27} = 7.412$, p = 0.0027) and time effect ($F_{2, 27} = 104.2$, p < 0.0001). Water produced the greatest post-cycling MVC loss when compared to glucose (p = 0.0004; 95% CI for differences = -75.8 to 23.2) and NaCl (p

= 0.0045; 95% CI for differences = -78.9 to 20.1), while the reductions produced by NaCl and glucose were similar (p = 0.6420; 95% CI for differences = -46.4 to 52.6). The results are illustrated in Figure 1. Cohen's d analysis showed medium effect sizes for post-test results between water and salt (d = 0.67), water and sugar (d = 0.69), and salt and sugar (d = 0.72).

sMVC

For sMVC, two-way ANOVA showed a significant interaction effect ($F_{15, 180} = 1.832$, p = 0.0332) with time ($F_{3, 36} = 5.835$, p = 0.0023), as well as a group effect ($F_{3, 119} = 77.56$, p < 0.0001). Water produced lower post-cycling sMVC peak force when compared to salt (p = 0.04; 95% CI for differences = -57.26 to -1.591) and glucose (p = 0.004; 95% CI for differences = -43.31 to -9.321). The sMVC force results were similar at the 30th, 60th, and 90th second. The results are illustrated in Figure 2.

Discussion

This study is the first to report the effects of 6% glucose and 6% NaCl mouth rinses on force production following a 30 minutes endurance exercise at 70% VO_2max. Both mouth rinses can generate greater MVC and sMVC post endurance exercise as compared to water. These findings suggest that the biochemical properties of glucose and NaCl may be able to activate the nervous system and parts of the brain to sustain the neural drive of force production.

Firstly, the findings show that the glucose mouth rinse preserved MVC post the endurance cycling exercise. This is similar to previous studies which showed that MVC is preserved when 6.4% maltodextrin mouth rinse is administered during a cycling exercise at 75% power output (Jeffers *et al.*, 2015), and when 8.0% maltodextrin mouth rinse is prescribed during a fatiguing resistance exercise (Jensen, Stellingwerff, & Klimstra, 2015). In contrast, a few studies that used similar concentrations of maltodextrin did not observe this phenomenon following a 120 minutes constant load cycling at 55% W_{max} followed by a time trial (Luden *et al.*, 2016), 4-km time trial (Pires *et al.*, 2018), and 50% MVC contraction until exhaustion (Black, Schubert, Szczyglowski, & Wren, 2018). These differences in the findings could be due to variations in the study variables such as the intensity of the fatiguing exercise and the inclusion feeding prior to exercise. It seems that a relatively low intensity exercise such as that as employed by Luden *et al.* (2016) is not sufficient to activate central responses compared to higher intensity exercises (Jeffers *et al.*, 2015). Another important factor is feeding prior to the fatiguing exercise, where fasting for 2 h prior to exercise showed a lack of difference in MVC between mouth rinse and placebo groups (Black *et al.*, 2018; Luden *et al.*, 2016; Pires *et al.*, 2018), in contrast to the unfed condition (Jeffers *et al.*, 2015) or an overnight fast (Jensen *et al.*, 2015). Secondly, the current study also found that NaCl mouth rinse preserved MVC post exercise at a similar level to the 6% glucose mouth rinse. To our knowledge, this observation has never been reported. It appears that NaCl may also influence the neural drive in producing maximal force. In other words, taste sensation and/or taste receptors in the mouth could induce these reactions in favour of improving muscular performance.

The preservation of MVC by glucose and NaCl mouth rinses may be associated with neural and neuromuscular processes involving the oral cavity (glucose and NaCl receptors), brain, and working muscles. Although the exact mechanism is still unclear, it has been proposed that activation of the anterior cingulate cortex and ventral striatum via the gustatory cortex and orbital frontal cortex following oral CHO stimulation influences motor behaviour (Jeukendrup *et al.*, 2013). In the case of NaCl, activation of 'salty' taste receptors in the oral cavity might have an influence on the insular cortex and/or opioid receptor activities (Gosnell & Majchrzak, 1990;

Pastuskovas, Cassell, Johnson, & Thunhorst, 2003) which, in turn, could also activate the parasympathetic nervous system that regulates cardiac autonomic activity (Williamson, McColl, & Mathews, 2004) via intracortical communication between the insular cortex and motor cortex, which could improve cycling performance (Williamson *et al.*, 1997). Furthermore, the activity of μ-opioidergic receptors has been shown to play a role in the development of fatigue and is exercise intensity dependent (Hiura *et al.*, 2017; Sidhu *et al.*, 2014). These proposed mechanisms are speculative because the link between the activation of NaCl oral receptors and the brain is yet to be empirically established. Therefore, future work should focus on establishing the role of the insular cortex and modulating the responsiveness of μ-opioid receptors with NaCl mouth rinse during a fatiguing exercise.

In other non-CHO mouth rinse studies, it has been shown that the cool sensation from menthol mouth rinse improves endurance performance when exercising in the heat (Flood, Waldron, & Jeffries, 2017; Jeffries, Goldsmith, & Waldron, 2018; Stevens *et al.*, 2016), although its effect on strength/MVC has not been investigated. While the exact mechanisms involved are still unclear, it has been hypothesised that menthol is able to reduce the perception of thermal sensation, thus attenuating inhibitory psychophysiological perception responses (Stevens *et al.*, 2017). These findings imply that the oral cavity can interact with other liquid contents and not just CHO.

The current study also found that glucose and NaCl mouth rinses preserved sMVC post exercise. At all the time points during sMVC, the force generated by both the glucose and NaCl mouth rinses was higher than that generated by water. It should be noted that the reduction in force with time during the sMVC test in all groups followed the same decreasing pattern, where a large difference was observed in WAT, which suggests that glucose and NaCl mouth rinses are able to prevent loss of maximum force generating capacity particularly during the early part of the 90 seconds of sustained contraction. Generally during a sMVC, a high neural drive from the central nervous system must be continuously produced to sustain a maximal force output (Bigland-Ritchie *et al.*, 1978). Hence, the comparatively greater MVC and sMVC reductions observed in WAT indicates that the drive was preserved by glucose and NaCl mouth rinses which may be attributed to central factors because these mouth rinses do not elicit any metabolic responses. In support of this assumption, at least for glucose mouth rinse, increases in corticospinal responsiveness (motor-evoked potential (MEP) amplitude) (Gant *et al.*, 2010) and MEP latency (Jeffers *et al.*, 2015) have previously been reported. However, changes in the VA have not been observed alongside changes in force output (Black *et al.*, 2018; Jeffers *et al.*, 2015). Also, although not consumed in the form of a mouth rinse, CHO has been reported to result in a higher central activation ratio, which is a central activity measure employed during sustained contraction (Khong, *et al.*, 2018; Nybo, 2003). Other neurophysiological variables such as cortical MEP, short interval intracortical inhibition, and intracortical facilitation (see Table 1 in Khong *et al.*, (2017)), as well as biochemical variables (brain serotonin, BCAA) (Blomstrand, 2006) could provide information on the central mechanisms involved in fatigue. In addition, measurements such as the central activation ratio recorded during a sustained contraction as reported in Khong *et al*. (2018) and Nybo (2003) could also provide time course information on neural output.

When considering the findings of the current study there are two main limitations to bear in mind. Firstly, although the participants were not aware of the study objectives, the mouth rinses were not taste-matched or fully blinded to them, so they were able to distinguish the taste despite not knowing the actual content of the mouth rinses. Therefore, to minimise any anticipation of treatment effects, the participants were encouraged to beat their previous MVC and sMVC measurements, regardless of the order in which the solutions were administered. Secondly, water mouth rinse was used as the control. However, this could potentially produce a placebo effect, hence the use of a no-rinse or taste-matched controls could minimise this risk (Gam *et al.,* 2015).

In practical terms, the findings of this study could be applied as a strategy by athletes to prevent neuromuscular fatigue. As a precautionary note, the use of CHO mouth rinse may cause tooth cavities as it has been noted that sugar residues remain present for longer than 1 hour (Luke *et al.*, 1999) and may exacerbate mouth infections (National Institute of Diabetes and Digestive and Kidney Diseases). Hence a NaCl mouth rinse would be more practical as its usage could avoid such issues. In addition, the use of a NaCl rinse may help to relieve ulcer and/or gum infections (Huynh *et al.*, 2016).

To summarise, while several studies have investigated the effects of CHO ingestion on the activation of the regions of the brain that contribute to the delay of fatigue/improvement in performance, it seems that no studies have been conducted on potential effects of NaCl. The current study sought to address this gap by conducting a randomised cross-over trial the findings of which showed that glucose and NaCl mouth rinses have similar effects in preserving MVC and sMVC post endurance exercise. Hence, it seems that activation of glucose and NaCl oral receptors could lead to central activation in maintaining the neural drive to contract muscles.

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Declaration of Interest

The authors have no conflict of interest to declare.

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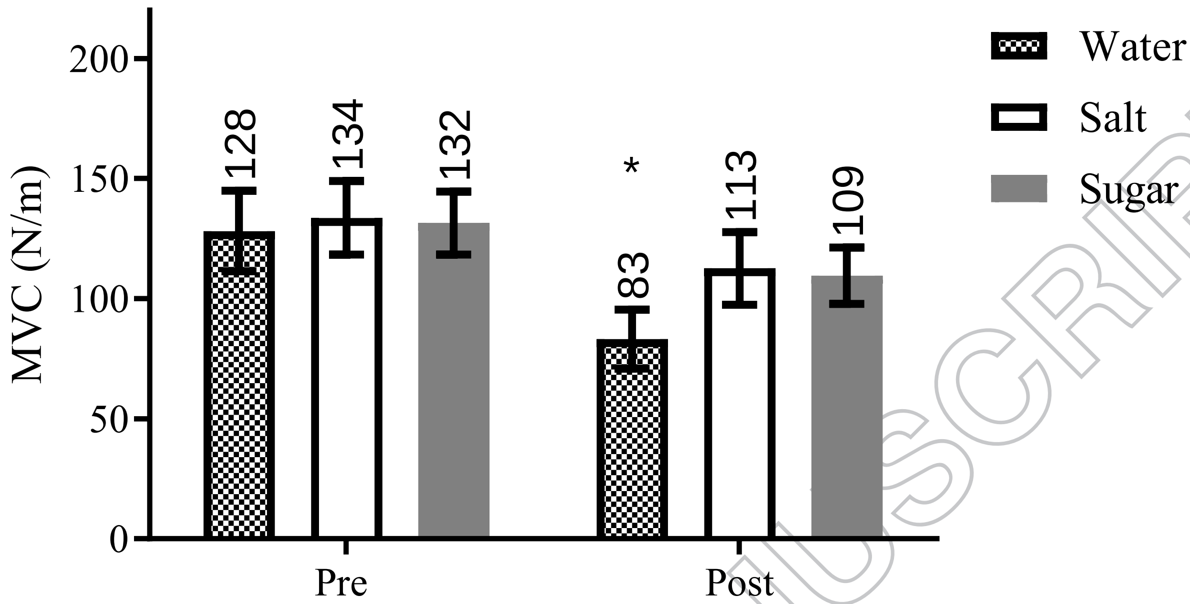

Figure 1: MVC measured at pre-cycling running and immediately after 30-minute cycling at 70% VO2max. *Water showed greater drop in MVC compared to Salt and Sugar.

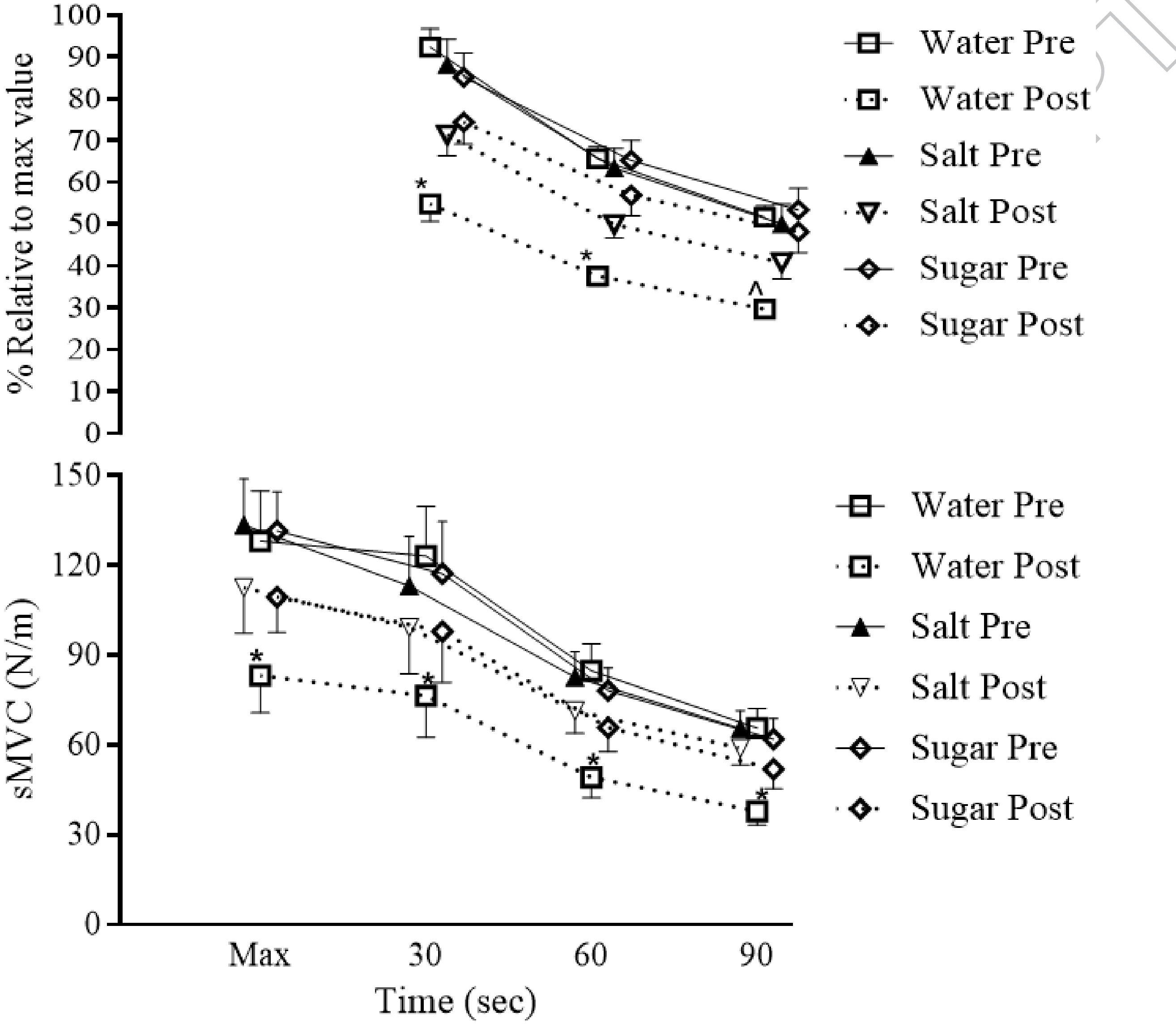

Figure 2: sMVC measured at pre-cycling running and immediately after 30-minute cycling at 70% VO2max, presented in mean ± standard error. *greater drop throughout the sMVC compared to Salt post and Sugar post.

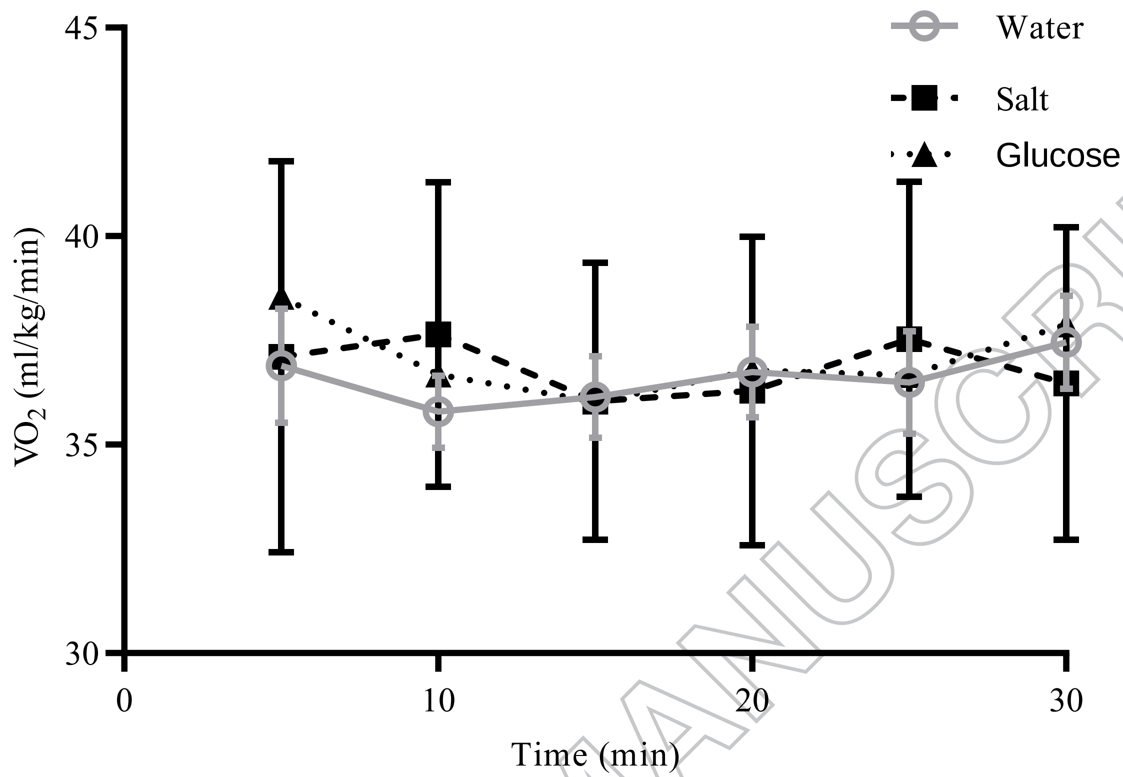

Figure 3: VO_2 values throughout the 30-minute cycling sessions in all trials. No significant differences between groups and no significant changes within each session.