Final Report

UEFA ACADEMY
Research Grant Programme
10th Cycle

2019 - 2020

The UEFA 120 min Study

By
Ioannis G. Fatouros, Ph.D.,
Professor

University of Thessaly
Department of Physical
Education & Sport
Sciences, Karies, Trikala
42100

PERFORMANCE AND PHYSIOLOGICAL ANALYSIS OF OVERTIME AND IMPLICATIONS FOR RECOVERY AND TRAINING

THE UEFA 120 MIN STUDY

Ioannis G. Fatouros, Ph.D., Professor

Department of Physical Education & Sport Sciences, University of Thessaly, Karies, Trikala 42100, Greece

Magni Mohr, Professor

Department of Sports Science and Clinical Biomechanics, SDU Sport and Health Sciences Cluster (SHSC), University of Southern Denmark, Odense, Denmark

Georgios Ermidis, Ph.D.

University of Naples “Parthenope”, Naples, Italy

Table of Contents

1. OFFICIAL COVER LETTER

UNIVERSITY OF THESSALY
DEPARTMENT OF PHYSICAL EDUCATION & SPORT SCIENCES
Trikala, Karies 42100
Greece

Ioannis G. Fatouros, *Ph.D.*
Professor
Tel.: +30 24310 47047, email: ifatouros@pe.uth.gr, fatouros@otenet.gr

April 25th, 2020

Official Letter to UEFA

It is with pleasure that I write this letter to describe the progress made with the study entitled "Performance and Physiological Analysis of Overtime: implications for recovery and training" sponsored by UEFA Academy. As the scientific coordinator of our project's consortium (University of Thessaly, Southern Denmark University, University of Naples "Parthenope"), I have the pleasure to send you our final report. All aspects of this funded project have been completed (experimental phase, 90% of the measurements, statistical analysis, results). We need to complete three more analyses (plasma glycerol, muscle lactate and histochemical glycogen depletion per fiber time). These analyses, although they started early, they have not been completed due to the lockdown of our University facilities. We believe that these measurements will be completed in late May 2020. However, as you will notice in our final report, we do have a clear picture of all dimensions of overtime in football. We hope to start submitting the first scientific manuscripts from this work in June 2020. We are looking forward to presenting this work to your esteem committee as soon as possible.

Sincerely,

Ioannis G. Fatouros, Ph.D.

Professor

2. EXECUTIVE SUMMARY

During the last EURO 2016 and World Cup 2018, 31% of games exposed players to extra-time periods during the knockout stage (5 games out of 16). In fact, during the last World Cup, one of the finalists played three 120-minute matches, probably creating accumulated fatigue to the players. The 120-minute elite football match has been insufficiently studied and its importance for winning big tournaments is underrated. In recent decades, changes in tournament structure with more knockout games, the intensity of play and changes in defensive and offensive strategies have made the 120-minute match much more frequent and impacted many of the key factors for success. The 120-minute match of today is very physically demanding and in the last 15-minute period highly dependent on physical power. It is also very tactically, technically and mentally demanding for fitness coaches and players to prepare for this challenge when going into major tournaments.

The overtime in football is understudied compared to other areas of football science. The mechanisms of match-related fatigue involved in soccer match with extra time play are likely to be multifaceted in origin. For this reason, there is enormous interest to search how a 120-min football match impacts the physiological, metabolic and musculoskeletal machinery of the human body and to explore which mechanisms are involve in the development of fatigue after 120 minutes. Moreover, increasing interest exists for the practitioners to be informed of how to prepare for knockout matches that may result in overtime (i.e. training protocols, nutritional support, and recovery techniques). Taking into consideration the rise in the number of 120-min matches, the increase in the intensity of match-play and fitness requirements and the progressive rise of congested schedules of official football matches of modern football, it would be valuable to investigate the impact of a 120-min match on the physiological and metabolic responses of players as well as the required recovery kinetics of performance.

The present investigation attempted to determine: (i) the impact of overtime on the physiological, metabolic, physical and mental load imposed on the players compared to a 90-min match; (ii) the impact of two consecutive 120-min matches performed 72 hours apart on the physiological, metabolic, physical and mental load imposed on the players and what are the recovery kinetics of performance; and (iii) the effect of carbohydrate supplementation on performance recovery kinetics when players participate in two consecutive 120-min matches performed 72 hours apart.

The results of this study clearly suggest that (i) overtime represent a substantial accumulation of physical, metabolic, mental and technical overload when compared to a 90-min match; (ii) playing two consecutive 120-min matches performed 72 hours apart results in prolonged performance deterioration that may affect field performance during the second match; and (iii) carbohydrate supplementation may not only restore glycogen contents in time but also enhances performance during the second match when players participate in two consecutive 120-min matches performed 72 hours apart.

3. BACKGROUND - INTRODUCTION

Football is an intermittent, self-paced sport characterized by abrupt high-intensity actions interspersed with periods of low- to moderate-intensity efforts.[3] Typically, a football match includes two 45-min halves interspersed by a 15-min half time (HT) rest interval. During a match, players run 9-12 km of which 2-4 km are covered by high intensity running and sprinting.[7,33] These numbers represent ~1350 runs, including 220 high-intensity runs, with the activity pattern changing every 4-6 seconds.[4,33] Players typically reach an average heart rate that approximates 85% of its maximal value[21] and an average aerobic loading that corresponds to 75% of the maximal oxygen uptake (VO_{2max}).[4,33] In addition, blood lactate concentration may reach 2-14 mM while muscle lactate may exceed 15-20 mM/kg d.w. indicating a high anaerobic energy turnover rate.[24,36] Immediately after a match, 50% of the muscle fibers are almost empty or empty of glycogen.[24] Collectively, these data indicate that football match-play is a high-intensity activity that engages both oxidative ('aerobic") and non-oxidative metabolism for energy acquisition.[24] Moreover, the high number of explosive muscle actions during a match, i.e. accelerations, decelerations, changes of direction, jumps, impacts, shots and tackles,[45] incorporate a powerful eccentric component associated with exercise-induced muscle damage (EIMD) which has been associated with deterioration of performance.[11,21] Although there is limited data, fatigue development during a match may also negatively affect technical performance, e.g. passing precision,[39] that could consequently affect team success.[38]

Fatigue may be defined as inability to sustain performance expressed as an increase in the perceived effort to produce a desired amount of force or power and/or a decline in the capacity to produce maximal force or power.[12] Football players experience fatigue during the last 15-min period of a match as well as temporarily following high-intensity activity during a match.[24,33,37] Players demonstrate a dramatic decline in high-intensity running towards the end of a game[33] causing a marked impairment in repeated sprint ability (RSA),[24,32] intense intermittent exercise,[24] countermovement jump[29,30] and strength performance[23,27] that may persist for as long as 24-72 hours after a match.[9,11,21,36] Fatigue is mediated by neuromuscular mechanisms which may vary according to the exercise or contraction mode and the characteristics of exercise (intensity, duration).[28] These neuromuscular mechanisms may be categorized as either central (due to changes in the synaptic concentration of neurotransmitters within the central nervous system) or peripheral (refers to the motor units and includes processes associated with mechanical (e.g. EIMD) and cellular (e.g. glycogen depletion) changes in the muscular system).[12] Match-related fatigue is attributed to a combination of central and peripheral factors.[37] Central fatigue may be incriminated for the deterioration seen in strength and sprint performance especially during or shortly after a match whereas peripheral fatigues may contribute to more prolonged decline of performance.[37] EIMD may be one of the most important contributors to peripheral fatigue developed in response to a single of successive football matches.[21,29,37] Other peripheral factors associated with match-related fatigue are various intramuscular metabolic disturbances (i.e. ion homeostasis, acidosis, interstitial K^+) which can reduce sarcolemmal excitability.[20] It has been shown that when football matches are played within a 3-day frame, performance is reduced during the second match and more pronounced EIMD is developed.[29] The recovery kinetics of performance following football match-play are probably related to muscle glycogen repletion[2,24,31] as well as the resolution of the inflammatory response associated with EIMD and the skeletal muscle healing process.[43]

During the knockout phase of various football tournaments (e.g. the FIFA World Cup, the UEFA EURO tournament, the UEFA Champions League etc.), when matches are tied, an extra 30-min period of match-play (consisting of 15-min halves separated by a 2-min break), called overtime, is applied. Overtime starts 5 min after the end of regulation (90 min) with teams swap ends of the pitch. Overtime was first introduced back in 1897

by the English Football Association and has been included in the FIFA regulations in its current form since 2004. Overtime has become a deciding factor of the final outcome in recent tournaments. Since the 1986 FIFA World Cup tournament, overtime has been played in 33% of knockout matches that required the extra 30 minutes of play. In the FIFA World cups during the period 2002-2010, 25 to 38% of the matches used an overtime whereas during the last decade this number increased even at 50% (World Cup of 2014). In the 2016 Union of European Football Associations (UEFA) championships, a single National team (Portugal) completed ~60 minutes more match-time than their counterparts (France) in the final match of that tournament. It appears that elite players may cover an additional distance of ~3 km, 12 more sprints, 207 more accelerations and ~150 m of high-speed running during overtime .[35,40,47] During the 2014 FIFA World Cup, 99 outfield players from seven matches that required ET covered an extra 2,962-m distance and performed 9 more sprints during overtimes compared to 90-min matches.[35]

Although overtime is a central feature of football match-play during the last three decades, there is very limited data about its physiological, metabolic, and recovery profile. Considering that the fatigue associated with a 90-min match play has been documented to impair performance and increase injury risk,[9,-11,16,21,29,36,40,41] it can be hypothesized that the addition of 30 more minutes of intense match-play could further impair performance and increase injury risk. In fact, evidence of overtime-induced fatigue of central nervous system manifested as reductions of quadriceps' maximal voluntary force have been provided recently[14] which is likely to impair cognitive (e.g., reactions, decision-making) and muscular performance.[25] For instance, during domestic European top-league teams, the success rate of passes and dribbles during overtime is markedly impaired.[17] Similar observations have been reported for sprint performance and recovery of reserve teams and academy players during overtime.[16,40] Moreover, in some European federations, the schedule of the domestic league remains unaltered after games of UEFA Champions League thereby allowing players limited time for recovery. Congested schedules of match play are also associated with increased injury rates and underperformance.[10] The recovery of both performance and repletion of muscle glycogen after a 90-minute match may last 48 to 72 h[24] and may be associated with prolonged muscle damage and inflammatory responses.[9,11,21,29,36] Evidence suggests that when exercise duration exceeds 90-min, muscle glycogen stores are diminished drastically and fat is primarily used as an energy fuel.[46] Although no relevant data are available for football overtime, this could potentially explain the transient decline of physical performance during extra time.

There is also considerable disparity among coaching staffs in strategies used to prepare players and teams for 120-minute matches[16] mainly due to lack of knowledge on the effects of overtime on players' physiological, metabolic and performance responses. Furthermore, more information is needed in respect to the effects of overtime on technical/skill performance and mental fatigue of players. Evidence suggest that 67% of the football practitioners believe that overtime may determine tournament success.[16] Therefore, knowledge of the physiological, metabolic and mechanical strain induced by overtime would be beneficial in terms of adjusting strategies aiming to optimize match preparation, management of overtime, tactical options, and recovery aiming not only fatigue resistance and faster recovery kinetics but also to better protect players from injuries. This may be accomplished by studying and analysing the field activity (distance covered, i.e. high-intensity running, mechanical load, etc.) during 120-minute matches in combination with fatigue and inflammatory responses as well as performance recovery kinetics. As such, the present investigation aimed to address the following questions:

- What is the impact of overtime on the physiological, metabolic, physical and mental load imposed on the players compared to a 90-min match**?**
- What is the impact of two consecutive 120-min matches performed 72 hours apart on the physiological, metabolic, physical and mental load imposed on the players and what are the recovery kinetics of performance in this scenario**?**
- What is the effect of carbohydrate supplementation on performance recovery kinetics when players participate in two consecutive 120-min matches performed 72 hours apart**?**

4. METHODS

4.1. Experimental design

A randomized, two-trial (placebo vs. carbohydrate consumption), cross-over, double-blind, repeated measures design was applied in this study. The study was performed 5 days after completion of the participants' pre-season an before the commencement of the competitive season. Prior to the first trial (baseline), participants had their body mass, height, resting metabolic rate (RMR), body composition, daily dietary intake and performance measured [VO_{2max} and YO-YO (IE2 and IR2) testing, skill level].

Based on this preliminary dietary analysis, participants were given a dietary plan (taking into account the RMR and total daily physical activity related energy expenditure) over a 10-day period (adaptive period), providing a standard macronutrient and antioxidant intake (carbohydrates: ~50-55%, ~fat: 25-30%, protein: 15-20% or 1 g protein/kg/day). During this adaptive period, volunteers were also familiarized with experimental procedures and participated in very light training (at a local football facility) aimed at developing game tactics and team cohesion.

Each experimental trial included administration of either carbohydrate (CHO trial) or placebo (PLA trial). Each trial included two 120-min matches (M1 and M2) performed 3 days apart (days 1 and 4 at a local pitch, as a simulation of a typical schedule during an official tournament with two 120-min matches played within 72 hours). Participants were randomly assigned to two teams (equally representing all field positions) that played against each other in the experimental matches of both trials (organized according to official regulations). Participants participated in the full 120-min matches, i.e., there were no substitutions. Substitute players were used when a participant was injured during a match. On match days, volunteers participated only in morning testing sessions but not practices. Prior to each game, a standard breakfast and meal was consumed by all players as previously described[11] and then rested. A warm-up and a cool-down period were performed before and after each match, respectively. Scouts and coaches from professional clubs attended all matches in an attempt to increase the participants' motivation and competitiveness to a level corresponding to that of a formal competition. Matches were performed at 15.00 under normal conditions (20–23◦C, humidity ~60%). During the matches, participants consumed only water ad libitum. Field activity during matches was recorded using high time-resolution GPS instrumentation and heart-rate monitoring.

Two light practice sessions and testing were performed in between matches (days 2 and 3) of both trials. These practices were designed and implemented according to a training schedule usually adopted by national football teams during a UEFA EURO tournament. A 2-week washout period was adapted between trials during which players participated in regular training and in one official match (performed 6 days prior to the second trial). Light training was applied three days prior to the second trial. Each participant received carbohydrate or placebo supplementation in a random order (ingestion of supplements started immediately after M1 and until the commencement of M2). Fluid intake and weight loss was monitored throughout a match.

Performance [RSA, jumping ability, maximal isometric voluntary contraction (MVIC) of knee flexors (KF) and extensors (KE) of the dominant (DL) and non-dominant (ND) limb], delayed onset of muscle soreness (DOMS), perceived exertion, psychological measures (negative talk, ego depletion) were assessed before each match and at 90 and at 120 min of each match. Blood samples were collected at 90 and at 120 min of each match. Muscle biopsies were collected at rest and at 90 and 120 min of M1 and before M2. Technical performance (successful pass rate and duels won) was determined throughout each match. All testing and biological sampling sessions were performed at the same time of day in both trials to control for the effect of circadian variations. The experimental flow chart is shown in Figure 4.1.1.

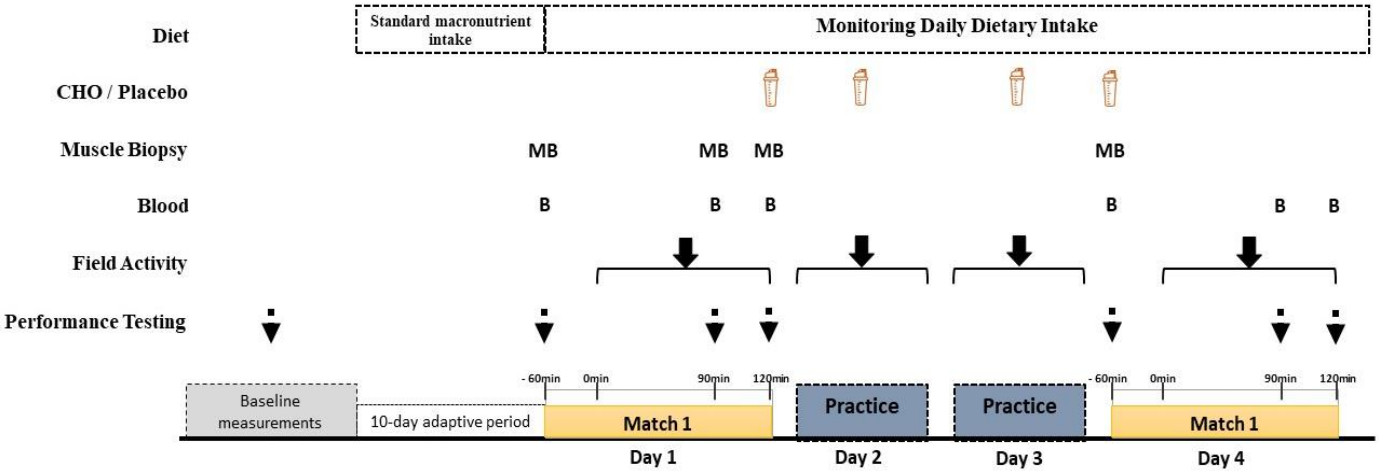

Figure 4.1.1. The experimental flow chart of the study

The CONSORT diagram of the study is shown in Figure 4.1.2.

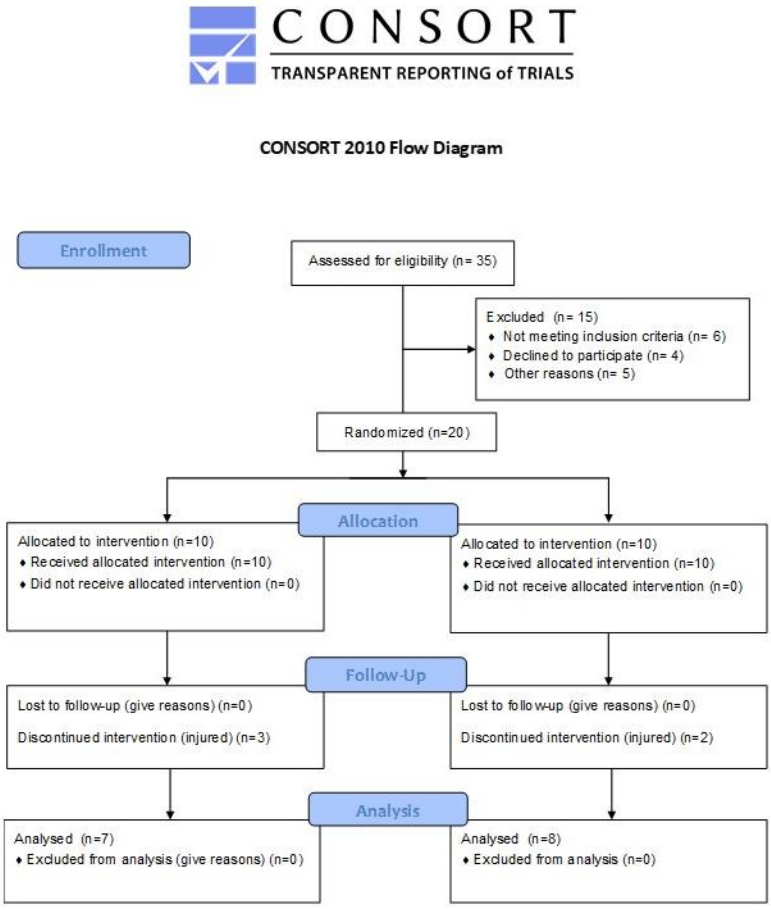

Figure 4.1.2. The CONSORT flow diagram of the study

The three main research questions of this study have been addressed in the following manner:

Question 1:

- What is the impact of overtime on the physiological, metabolic, physical and mental load imposed on the players compared to a 90-min match**?**

⇨ *In order to answer this question, data from M1 of each trial were used (N = 25 observations).*

Question 2:

- What is the impact of two consecutive 120-min matches performed 72 hours apart on the physiological, metabolic, physical and mental load imposed on the players and what are the recovery kinetics of performance in this scenario**?**

⇨ *In order to answer this question data from the PLA trial were used (N = 15 players).*

Question 3:

- What is the effect of carbohydrate supplementation on performance recovery kinetics when players participate in two consecutive 120-min matches performed 72 hours apart**?**

⇨ *In order to answer this question data from the CHO trial were used (N = 15 players)*

4.2. Participants

A preliminary power analysis (effect size > 0.55, probability error of 0.05, power of 0.90), indicated that a sample size of 16–18 subjects is necessary to detect a statistically meaningful treatment effect among serial measurements in response to repeated football games.[9,11,21,29] Accordingly, 35 competitive male football players were initially approached/interviewed but 20 participated (played in all four marches) in the study. Fifteen participants completed the entire study (5 participants were injured in M1). Participants' characteristics at baseline are shown in Table 4.2. Participation in this study was secured if volunteers (1) had played at a competitive level (top three divisions) for ≥4 years; (2) were free of any recent history of illnesses, musculoskeletal problems, and metabolic diseases; (3) had not used any supplements and medications (for ≤6 months prior to the study); (4) were non-smokers; and (5) participated in ≥5 training sessions/week and played ≥1 match/week. All volunteers signed an informed consent form after they were fully informed about all benefits, risks, and discomforts of this investigation. All procedures were applied in accordance with the Declaration of Helsinki, as revised in 2013, and approval was obtained from the Ethics Committee of School of Physical Education and Sport Science, University of Thessaly (1462/6-2-2019). The study is registered at ClinicalTrials.gov (identifier: NCT04159194).

Table 4.2. Participants' characteristics at baseline.

Age (years)	20.7 ± 2.1
Height (cm)	176 ± 5.8
Weight (kg)	68.8 ± 5.1
BMI (kg/m^2)	22.2 ± 1.2
Body fat (%)	14.9 ± 5.2
RMR (kcal/day)	1,711.1 ± 85.7
VO_{2max} (mL/kg/min.)	59.4 ± 3.6
Resting hearth rate (bpm)	62.1 ± 4.9
Maximal heart rate (bpm)	200.1 ± 3.7
Yo-Yo IE2 (m)	3,169.5 ± 333.6
Yo-Yo IR2 (m)	1,516.2 ± 154.3
Creative speed test (s)	16.2 ± 0.7
Short dribbling test (s)	12.0 ± 0.6
Type I muscle fibers (%)	37.9 ± 8.8
Type 2a muscle fibers (%)	53.7 ± 7.1
Type 2x muscle fibers (%)	8.4 ± 6.1

BMI, body mass index; RMR, resting metabolic rate; VO_{2max}, maximal oxygen consumption, bpm, beats per minute; IE, intermittent endurance; IR, intermittent recovery

4.3. Supplementation protocol and diet monitoring

Prior to the study, each participant was instructed to continue his normal diet and not to use any supplements. Participants were instructed by a registered dietitian how to record food/fluid servings and sizes and completed 7-day diet recalls evaluating their daily nutrient and energy intake during both trials to ensure that during the second trial they maintained the same dietary pattern that used during the first trial. Diet recalls were analyzed using the Science Fit Diet 200A (Science Technologies, Athens, Greece). Based on the dietary analysis, the RMR and total daily physical activity related energy expenditure, participants were given a dietary plan providing a carbohydrate intake of ~53%, a protein intake of ~17% and a fat intake of 30% of the total energy per day, when no match or training was scheduled for that day. Seven-day habitual physical activity level was determined via accelerometry (GT3X-BT, ActiGraph, FL, USA) as previously described.[6]

All dietary plans were isoenergetic, with a reduction in fat intake when the CHO supplement was administrated. Participants consumed either a CHO supplement or a placebo in a random order. Supplementation started immediately after MI and ended before M2. CHO and placebo drinks were isovolumetric (~450 mL), consumed along with water, and flavored chocolate drops to make the contents indistinguishable and non-transparent. In both trials, participants were instructed to follow a daily nutrition pattern consisting on three main meals (breakfast, pre-game or pre-training lunch, and post-game or post-training dinner), as well as two snacks (before lunch and post-match or post-training). Participants were given various food equivalents by a registered dietitian to provide the required intakes for match and training days.

On practice days (the two days between M1 and M2), in the "CHO trial", the dietary plan provided a carbohydrate intake of ~5.1 g/kg/day plus the CHO supplement (~150 g in three equal doses to achieve a total carbohydrate intake of ~76% of daily intake, consumed after each meal), a protein intake of ~1.2 g/kg/day (~12.5%) and fat intake of ~0.5 g/kg/day (~11.5%). Fat intake was reduced in order to achieve a total carbohydrate intake of >70% of the daily energy intake without changing the total energy intake. Each dose of CHO supplement contained

~48.3 g of maltodextrin (~0.7 g/kg). After M1 in the CHO trial, the dinner and the CHO supplement provided ~2.5 g/kg). During match days, the total CHO intake corresponded to 6.1 g/kg/day and the protein intake was 1.6 g/kg/day. Before M2 of the CHO trial, the dietary plan provided the nutrient intake, plus two doses of CHO supplement that were consumed after breakfast and after a light lunch before M2 (the two meals, two snacks and the two doses of CHO supplement provided about ~4 g/kg).

During the "Placebo trial" period, the dietary plan provided the dietary plan provided a carbohydrate intake of 53% (~5.1 g/kg/day), a protein intake of 12.5% (~1.2 g/kg/day) and a fat intake of 35% (~1.5 g/kg/day) of the total energy intake per day plus three dosages of placebo (consumed after each meal). During match days, the total CHO intake corresponded to 6.1 g/kg/day and the protein intake was 1.6 g/kg/day. After M1, of the PLA trial, the dietary plan provided the same nutrient intake, plus one dose of the placebo supplement. Before M2 of the PLA trial, the dietary plan provided the same nutrient intake, plus two doses of the placebo supplement that were consumed after breakfast and after a light lunch before the match.

Current nutritional recommendations for athletes engaged in moderate amounts of training suggest a protein and carbohydrate consumption of 1.0–1.5 and 4–6 g/kg/day, respectively.[22] Moreover, it has been recently shown that intermittent-type activity of similar nature, intensity, and duration as football activity used in this study results in an estimated average protein intake requirement of 1.20–1.40 g/kg/day,[34] which coincides with the daily protein intake levels used in this study. Diet intake was monitored daily during both trials and the wash-out period.

Participants were asked 4 times each (once per day) whether they realized if the drink (and the food) was the experimental supplement (or diet) or placebo (or diet). Out of a total of 60 responses, 31 times responded "I do not know", 19 times guessed incorrectly and 10 times guessed correctly (probably due to chance). Only 1 of 15 participants guessed correctly both CHO and PLA supplements. Similar responses were obtained for the consumed food. Therefore, we tend to believe that the players were well-blinded, and, as such, the placebo effect was eliminated. Participants' dietary energy intake and antioxidant profiles during the study are shown in Table 4.3.

Table 4.3. Participants' dietary energy intake and antioxidant profiles during the study.

	PLA			CHO		
	Diet	**Placebo**	**Total**	**Diet**	**Supplement**	**Total**
Daily energy intake (kcal/day)						
Adaptive period	1742.5±88.2	0.00±0.0	1742.5±88.2	1742.5±88.2	0.00±0.0	1742.5±88.2
M1 day (total)	2,863.5±79.8	0.00±0.0	2,863.5±79.8	2,553.0±81.5	193.2±0.0	2,746.2±85.4
M1 day (post-match)	954.5±24.6	0.00±0.0	954.5±24.6	768.2±29.8	193.2±0.0	961.4±29.8
M2 day (total)	2,863.5±79.8	0.00±0.0	2,863.5±79.8	2359.8±85.4	386.4±0.0	2,746.2±85.4
M2 day (pre-match)	1,889.9±63.7	0.00±0.00	1,889.9±63.7	1426.1±54.9	386.4±0.0	1,812.5±54.9
Practice days	2,670.3±91.8	0.00±0.0	2,670.3±91.8	2049.3±72.4	600±0.0	2649.3±72.4
Carbohydrate intake (% of total intake)						
Adaptive period		53.0±2.2			53.5±2.3	
Match days		54.0±2.8			63.3±1.9*	
Practice days		52.7±3.5			76.0±4.4*	
Protein intake (% of total intake)						
Adaptive period		19.0±1.2			19.0±1.2	
Match days		15.4±0.0			16.0±0.0	
Practice days		12.4±1.1			12.5±0.4	
Fat intake (g/kg) (% of total intake)						
Adaptive period		28.0±1.5			27.5±1.3	
Match days		30.6±2.1			20.7±1.3*	
Practice days		34.9±2.3			11.5±1.6*	
Selenium (µg/day)						
Adaptive period	63.5±9.1	0.0±0.0	63.5±9.1	63.5±9.1	0.0±0.0	63.5±9.1
Match days	59.7±12.4	0.0±0.0	59.7±12.4	60.3±9.7	0.0±0.0	60.3±9.7
Practice days	62.6±8.3	0.0±0.0	62.6±8.3	58.9±9.8	0.0±0.0	58.9±9.8
Zinc (mg/day)						
Adaptive period	12.2±2.5	0.0±0.0	12.2±2.5	12.2±2.5	0.0±0.0	12.2±2.5
Match days	12.8±2.8	0.0±0.0	12.8±2.8	13.2±3.2	0.0±0.0	13.2±3.2
Practice days	12.4±2.7	0.0±0.0	12.4±2.7	12.0±2.4	0.0±0.0	12.0±2.4
Vitamin C (mg/day)						
Adaptive period	121.8±8.6	0.0±0.0	121.8±8.6	121.8±8.6	0.0±0.0	121.8±8.6
Match days	119.8±8.4	0.0±0.0	119.8±8.4	120.6±9.3	0.0±0.0	120.6±9.3
Practice days	122.5±9.4	0.0±0.0	122.5±9.4	118.7±8.1	0.0±0.0	118.7±8.1
Vitamin E (mg/day, α-TE)						
Adaptive period	9.4±1.8	0.0±0.0	9.4±1.8	9.4±1.8	0.0±0.0	9.4±1.8
Match days	9.8±2.1	0.0±0.0	9.8±2.1	9.0±1.7	0.0±0.0	9.0±1.7
Practice days	9.6±2.0	0.0±0.0	9.6±2.0	9.7±2.4	0.0±0.0	9.7±2.4

PLA, the placebo trial; CHO, the carbohydrate trial; BM, body mass; FFM, fat-free mass; * statistical differences detected between trials at P<0.05.

4.4. Measurements

4.4.1. Measurement of locomotor activity pattern and technical performance during a match

Field locomotor activity during match-play was monitored using high time resolution global positioning GPS (10 Hz, Viper Units; STATSports, Newry, Ireland) (Beato, 2018). Field activity was classified as total distance covered during a match, distance covered with high speed running (HSR, distance covered at speeds >21 km/h), sprinting (Sprinting, distance covered at speeds >24 km/h), number of high-accelerations (>2 m/s), and number of high-decelerations (>-2 m/s).[29] Intensity during match-play and practices was monitored using continuous heart rate measurements (Team Polar Pro system, Polar, Kempele, Finland). All matches were videotaped and two experienced football video analysts performed a notational analysis of individual skill-related performance variables, i.e. total number of passes and duels, on video recordings. Here we report the rate of successful passes and duels won. There was a 91% inter-rater reliability

4.4.2. Descriptives

Body mass and height were measured on a beam balance with a stadiometer (Beam Balance-Stadiometer, SECA, Vogel & Halke, Hamburg, Germany) as previously described.[11] Body mass index was calculated as mass per height squared. Dual-emission X-ray absorptiometry (GE Healthcare, Lunar DPX-NT) was used for body composition assessment as previously published.[6] Open-circuit spirometry was utilized for assessment of maximal oxygen consumption (VO_{2max}) using an automated online pulmonary gas exchange system (Vmax Encore 29, BEBJO296, Yorba Linda, CA, USA) via breath-by-breath analysis during a graded exercise testing on a treadmill (Stex 8025 T, Korea) as previously described.[29] For RMR assessment, resting VO_2/CO_2 was measured in the morning (07.00–09.00) after an overnight fast using an open-circuit indirect calorimeter with a ventilated hood system (Vmax Encore 29, BEBJO296, Yorba Linda, CA, USA), and the 24-h RMR was calculated as previously described.[11] Football-specific conditioning was measured using the Yo-Yo intermittent endurance test 2 (Yo-Yo IE2) and the Yo-Yo intermittent recovery test 2 (Yo-Yo IR2) with procedures previously described.[1] Yo-Yo and VO_{2max} testing were performed on separate days. Participants' level of technical performance was determined using the creative speed and short dribbling tests as previously described.[44]

4.4.3. Performance and muscle damage measurements

Countermovement jump height (CMJ) was measured on an Ergojump contact platform (Chronojump, Bosco-System, Barcelona, Spain) as previously described.[11] For repeated sprinting ability testing (RSA), participants performed of five 30-m sprints on grass, separated by a 25-s period of active recovery, during which the players jogged back to the starting line. Thus, each test lasted approximately 2 min. The sprint times were recorded by infrared light sensors with a precision of 0.01 s (Chronojump, Bosco-System, Barcelona, Spain) and the fatigue index was calculated by substracting the worst time from the best time, divided by the best time and then multiplied by 100.[24] MVIC of KE and KF of both limbs was measured on an isokinetic dynamometer (Cybex Norm, Ronkonkoma, New York, USA) as previously described.[9] DOMS in the KF and KE of both limbs was evaluated as previously described.[29]

4.4.4. Fluid loss and intake

To determine sweat loss during the match, the players were weighed wearing dry shorts immediately before the match as well as at 90 and 120 min using a beam balance with a stadiometer (Beam Balance-Stadiometer, SECA, Vogel & Halke, Hamburg, Germany) as previously described.[11] The

water intake of each participant was recorded throughout the match.

4.4.5. Psychometric assessment

The Borg scale was used to examine differences in perceived exertion following the completion of the regular time and the overtime in all matches as it was previously described.[18] Furthermore, two psychometric self-reported measures were obtained to assess the impact of the physical loads on ego-depletion and negative self-talk. Ego depletion reflects low levels of energy for mental activity that results in reduced states of self-control, and has a negative impact on subsequent performance, in particular when performance requires demanding cognitive processes and it was measured as previously described.[15] Negative self-talk refers to spontaneous statements, mostly worry and self-evaluative statements, athletes experience while performing and it was measured as previously described.[19]

4.4.6. Blood sampling and assays

Fasting blood samples were collected by venipuncture using a disposable needle (20-gauge) and a Vacutainer tube holder from an antecubital arm vein with the participants sitting. Plasma was prepared by centrifugation (1370 g, 4 ◦C, 10 min) from blood samples collected into tubes containing ethylenediaminetetraacetic acid (EDTA) to measure glycerol (CK), ammonia and TBARS. Serum [to measure glucose, creatine kinase activity (CK), TAC and protein carbonyls] was prepared by centrifugation (1370 g, 4◦C, 10 min) from blood samples containing SST-Gel/clot activator that were first allowed to clot at room temperature. Packed erythrocytes (RBC) were prepared after lysis of plasma samples[5] to measure reduced glutathione (GSH), a powerful antioxidant. All samples were stored at -75◦C to - 80◦C in multiple aliquots until assayed. A small portion (2 mL) of whole blood was collected in tubes containing ethylenediaminetetraacetic acid to assess WBC, haemoglobin (Hb) and hematocrit using an automated hematology analyzer (Mythic 18, Orphee SA, Geneva, Switzerland). All samples were thawed only once before being analyzed and were protected from light and auto-oxidation. All assays were performed in duplicate, and samples collected after a match were corrected for plasma volume changes as described.[8]

CK was measured using a Clinical Chemistry Analyzer Z 1145 (Zafiropoulos Diagnostica, Greece) with commercially available kits (Zafiropoulos, Greece). For measuring PC,[42] 20% trichloroacetic acid (TCA, 50 μL) was added to RBC lysates (diluted 1/10), and the mixture was incubated (ice bath, 15 min) and centrifuged (15,000 g, 4◦C, 5 min). The supernatant was then discarded and 2,4-dinitrophenylhydrazine (10 mM in 2.5 N HCl) or HCL (2.5 N) was added to the tube of sample or blank solution, respectively. Following incubation (dark room, 1 h) and mixing (every 15 min), samples were centrifuged (15,000 g, 5 min, 4◦C), TCA (10%, 1 mL) was added to the supernatant, and the samples were then vortexed and centrifuged (15,000 g, 5 min, 4 ◦C). Subsequently, ethanol–ethyl acetate (1:1 v/v) was added to the new pellet, and the samples were centrifuged again (15,000 g, 4◦C, 5 min). The samples were then washed two more times, centrifuged once more, and urea (5 M, pH 2.3) was added to the pellet. The samples were then mixed and incubated (37 ◦C, 15 min). The samples were then centrifuged again (15,000 g, 4◦C, 3 min) and their absorbance was read spectrophotometrically at 375 nm. TBARS were analyzed as described.[42] Briefly, plasma samples were mixed with TCA (35%, 200 mM) and Tris–HCl (pH 7.4) and incubated (room temperature, 10 min). Na2SO4 (2M) and thiobarbituric acid (55 mM) were then added, and the resultant solution incubated (95◦C, 45 min). The samples were left to cool (5 min), had TCA (70%) added, were mixed and centrifuged (15,000 g, 3 min), and the absorbance of the supernatant was then read at 530 nm. For GSH measurement, RBC lysates were treated with 5% TCA, mixed with sodium potassium phosphate (67 mM, pH 8.0) and 5,5-dithio-bis-2 nitrobenzoate (1 mM), incubated (in the dark, 45 min, room temperature), and had their absorbance read spectrophotometrically at 412 nm.[42] For TAC analysis, sodium-potassium phosphate (10 mM, pH 7.4) and 2,2-diphenyl-1 picrylhydrazyl (0.1 mM) were added to serum samples, which were then

incubated (in the dark, room temperature, 30 min), centrifuged (20,000 g, 3 min), and had their absorbance read spectrophotometrically at 520 nm.[42] Ammonia was measured using a commercially available kit (Cobas Substrates, USA) using a spectrophotometer (Integra 400PUSS, Ross) at 340 nm. Glycerol is currently being measured using a commercially available ELISA kit (Sigma-Aldrich, glycerol assay kit). Due to the restrictions applied by the Hellenic Government on University operations (lockdown), this measurement has been interrupted since February 2020. We are anticipating that University operations will be restored in mid-May 2020. As such, these measurements will be available in late May 2020. Inter- and intra-assay coefficients in all assays performed ranged from 2.8 to 8.1% and from 3.4 to 8.1%, respectively.

4.4.7. Muscle biopsy sampling and analyses

The muscle biopsies (~70–120 mg w.w.) were obtained from m. vastus lateralis of the DL using the needle biopsy technique with suction. All biopsies were obtained with the subjects lying in the supine position on one of four beds standing 2 m from the sideline. Muscle biopsies were collected shortly after cessation of match-play. The muscle tissue was immediately frozen in liquid N_2 and stored at -80°C. The frozen sample was weighed before and after freeze drying to determine water content. After freeze drying, the muscle samples were dissected free of blood, fat, and connective tissue, and about 1 mg d.w. tissue was extracted in a solution of 0.6 M perchloric acid (PCA) and 1 mM EDTA, neutralized to pH 7.0 with 2.2 M KHCO3 and stored at -80°C until analyzed for lactate by a flurometric assay.[26] Another, 1–2 mg d.w. muscle tissue was extracted in 1M HCl and hydrolyzed at 100°C for 3 h, and the glycogen content was determined by the hexokinase method.[26] A part of each biopsy obtained before and after the game was mounted in an embedded medium (OCT Compound Tissue-Tek, Sakura Finetek, Zoeterwoude, The Netherlands) and frozen in isopentane that was cooled to the freezing point in liquid nitrogen. These samples were stored at -80°C until analyzed for fiber-type distribution and fiber-type–specific glycogen content by histochemical analysis as previously described.[24] The analysis of fiber-type–specific glycogen content is currently being measured. Due to the restrictions applied by the Danish Government on University operations (lockdown), this measurement has been interrupted since February 2020. We are anticipating that University operations will be restored in May 2020. As such, these measurements will be available in late May 2020.

4.5. Statistics

Data are presented as mean ± SD. The Shapiro-Wilk test was used to determine data normality. To assess the different time point changes between the CHO and PLA group (according to absolute values and Δ changes), a two-way (time x group) repeated measures analysis of variance (ANOVA) with planned contrasts on different time points was used, if data were normally distributed. When a significant interaction was noted, a Bonferroni correction analysis was performed for pairwise comparisons. In case of non-normal distribution, non-parametric tests were applied. To identify the time-effect in each trial, the Friedman analysis of variance by ranks test was utilized accompanied by the Wilcoxon signed-rank test for pairwise comparisons. Differences between the trials were examined by using the Kruskal-Wallis analysis of variance accompanied by the Mann-Whitney U test for pairwise comparisons. Statistical significance was accepted at $P < 0.05$. The SPSS (IBM SPSS for Windows, Version 20.0, Chicago, IL, USA) was used for all analysis.

5. RESULTS

The results of the present investigation are shown in the figures shown in the sections that follow. These figures illustrate the percent (%) change of each variable from its baseline value. The absolute values of each variable at each time point of measurement are provided in the form of tables in Appendix 1 at the end of this report.

5.1. Participant profile

Data from five players were used only in the evaluation of overtime effects due to injury (three during the first trial and two during the second trial). Baseline values (prior to each trial) of all variables examined were comparable (see Tables and Figures of the results section), suggesting that the wash-out period was effective to eliminate any systemic inflammation, and muscle damage manifestations developed in response to the first trial so that the two trials were performed under the same conditions. No adverse side effects were reported by the participants due to CHO supplementation.

5.2. The effects of overtime on the physiological, metabolic, physical and mental load of the players compared to a 90-min match

To address this research question, data from M1 of each trial were pooled together resulting in a total of 25 observations. All variables demonstrated comparable changes in the first matches of each trial). In this section, results recorded during the 1-90 minutes of the match were compared to those recorded during the 90-120 minutes of the overtime.

5.2.1. The profile of internal and external load of overtime compared to a 90-min match

External load

During the 90-min match play of the first matches of each trial, players run a total distance of 10870.3 (± 755.3) to 10993 (± 953.8) m of which 203.0 (± 125.9) to 213.7 (± 121.2) m were covered by high intensity running and 114.1 (± 92.2) to 118.0 (± 92.2) m by sprinting.

The average speed was 7.2 (±overtime (ranged from 27.8 ± 8.1 to 28.2 ± 6.2 mL/min). overtime (ranged from 27.8 ± 8.1 to 28.2 ± 6.2 mL/min).0.5) to 7.4 (± 0.6) km/h whereas the maximal speed reached 32.2 (± 2.6) to 32.5 (± 2.6) km/h. In addition they performed 98.1 (± 18.7) to 103.1 (± 18.7) and 95.4 (± 21.7) to 96.9 (± 20.4) hard accelerations and decelerations, respectively.

During the 30-min match-play of overtime of the first matches of each trial, players run a total distance of 3296.1 (± 320.1) to 3461.3 (± 499.0) m of which 89.5 (± 65.2) to 96.9 (± 46.1) m were covered by high intensity running and 54.4 (± 58.0) to 57.5 (± 63.0) m by sprinting. The average speed was 6.6 (± 0.6) to 6.9 (± 1.0) km/h whereas the maximal speed reached 29.2 (± 2.4) to 29.3 (± 1.6) km/h. In addition, they performed 28.1 (± 8.6) to 28.5 (± 11.9) and 26.14 (± 10.6) to 27.1 (± 8.1) hard accelerations and decelerations, respectively. In absolute terms, these numbers represent a marked reduction ($p < 0.05$) of total distance by 68.2%, of high intensity running by 56.1%, of average speed by ~4.2%, of maximal speed by ~9.3%, of the total number of accelerations by ~70.9% ad of the total number of deccelerations by ~72.6% during overtime compared to the first 90 min of the match. It must be noted, however, that the decline in distance covered by sprinting (by ~52.3%) during overtime (compared to the first 90 min) was not statistically meaningful, probably because of its high inter-individual variability.

Internal load

The mean heart rate was ranged from 167.1 (± 6.4) to 168.4 (± 9.4) beats/min during the 90-min match play and remained elevated during overtime, i.e. from 162.1 (± 7.8) to 163.0 (± 8.1) beats/min. As soon as the University resumes its operations, the consortium will complete the analyses of muscle lactate. Sweat loss exceeded 2 L (ranged from 2019.8 ± 504.7 to 2048.3 ± 655.5) during the first 90 min of the match and it was further increased ($p < 0.05$) during overtime when it approached (2862.5 ± 551.8) or even exceeded (3389.4 ± 748.4) 3 L. The sweat rate reached 22.7 ± 7.2 mL/min during the first 90 min and increased ($p < 0.05$) further during

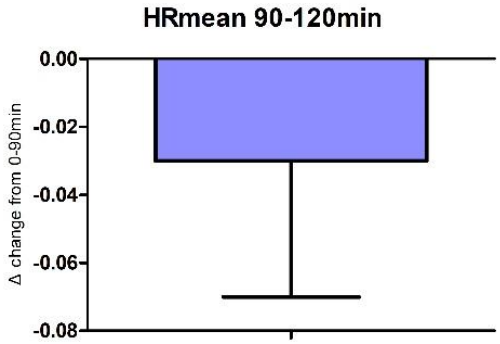

Figure 5.2.1.1. Δ change of mean heart rate from 0-90 min to 90-120 min, in match 1, in PLA trial 1

Technical performance

A significant effect of overtime was also observed on technical performance, which was substantially deteriorated. Specifically, the % of successful passes and duels won were reduced ($p < 0.05$) by 9% to 27% during the overtime.

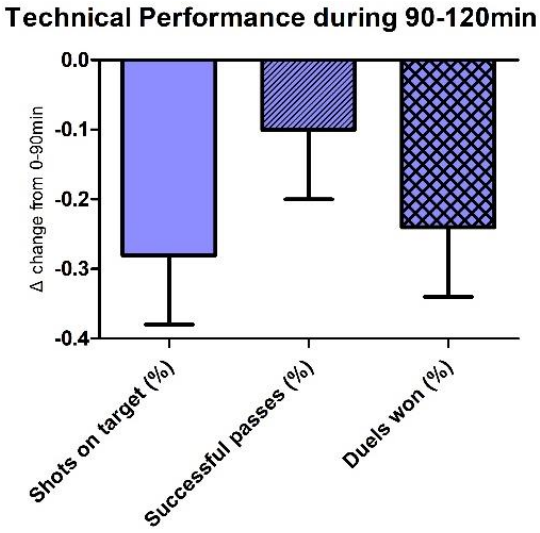

Figure 5.2.1.2. Δ change of shots on target, successful passes and duels won, from 0-90 min to 90-120 min in match 1, in PLA trial.

5.2.2. The effects of overtime on performance compared to a 90-min match

CMJ performance was reduced ($p < 0.05$) by 18% at 90 min and by 27% at the end of overtime, i.e. a decline ($p < 0.05$) of 11% due to overtime. RSA performance was reduced ($p < 0.05$) by 40% at 90 min and by 51% at the end of overtime, i.e. a decline ($p < 0.05$) of 11% due to overtime.

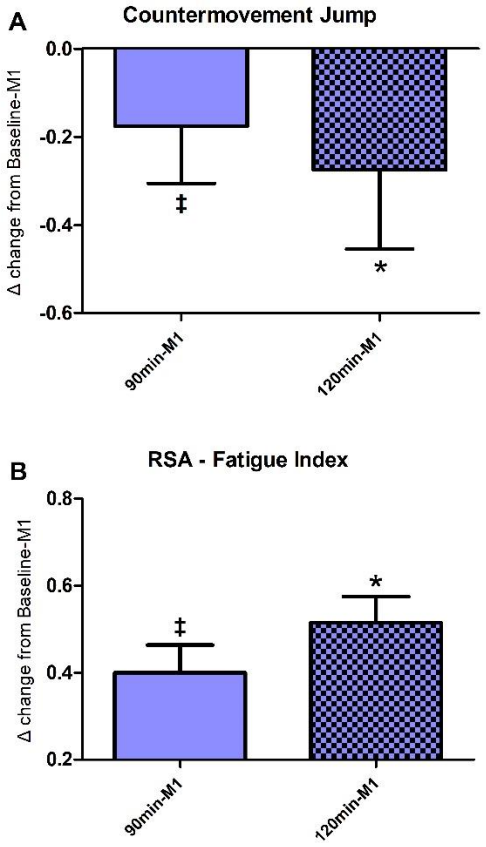

Figure 5.2.2. Δ change of countermovement jump (**A**) and RSA (**B**) performance, from baseline (Baseline-M1) in match 1, in PLA trial. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

5.2.3. The metabolic profile of overtime compared to a 90-min match

Metabolic indicators were also affected by overtime. Specifically, plasma glucose levels remained unaltered after the 90-min match, but they were reduced ($p < 0.05$) at 120 min by ~13% compared to baseline and by ~14% compared to 90 min suggesting that overtime had a moderate hypoglycemic impact. The concentration of ammonia in plasma was considerably elevated ($p < 0.05$) both at 90 min (by 56%) and at 120min (by 194%) compared to baseline, with overtime inducing an additional 89% increase compared to the 90-min match-play. Furthermore, skeletal muscle glycogen stores were considerably reduced ($p < 0.05$) both at 90 min (by 35%) and at 120 min (by 48%) compared to baseline, with overtime inducing an additional 13% decline compared to the 90-min match-play.

As soon as the University resumes its operations, the consortium will complete the analyses of plasma glycerol which will provide a clearer picture in respect to fat metabolism during overtime.

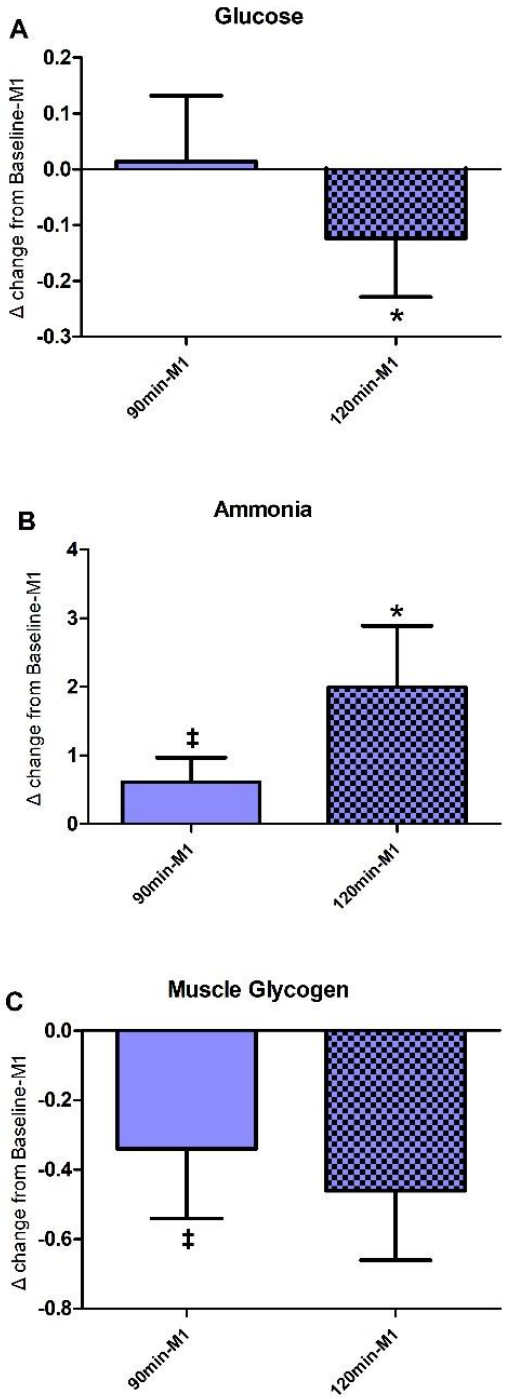

Figure 5.2.2. Δ change of glucose (**A**), ammonia (**B**) and muscle glycogen (**C**) levels from baseline (Baseline-M1), in match 1, in PLA trial. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

5.2.4. The inflammatory response induced by overtime compared to a 90-min match

Muscle damage markers

Overtime resulted in a pronounced elevation of muscle damage indices and provoked a greater inflammatory response compared to the 90-min match.

In fact, CK increased ($p < 0.05$) both at 90 and at 120 min by 168% and 314%, respectively, with the rise induced by overtime being greater (by 146%) than the 90-min match (90 min: 635.4 ± 219 U/L vs 120 min: 983 ± 346.3 U/L).

DOMS of knee extensors increased ($p < 0.05$) by 3.8 fold at 90 min and by 5.6 fold following overtime, i.e. overtime exacerbated ($p < 0.05$) KE DOMS by almost 2-fold. Similarly, DOMS of knee flexors increased ($p < 0.05$) by 4.8-fold at 90 min and by 7.6-fold following overtime, i.e. overtime exacerbated ($p < 0.05$) KE DOMS by almost 2.8 fold. DOMS was more pronounced ($p < 0.05$) in knee flexors compared to knee extensors both at 90 and 120 min.

Maximal isometric voluntary contraction (MVIC) was assessed at baseline and at the end of the extra time, and therefore, the comparison between the 90-min match and overtime is unfeasible here. Nonetheless, it should be highlighted that the 120-min match play resulted in deterioration ($p < 0.05$) of MVIC by 9% and 12%, in knee extensors and flexors, respectively, in the dominant limb and by 9% and 10%, in knee extensors and flexors, respectively, in the non-dominant limb.

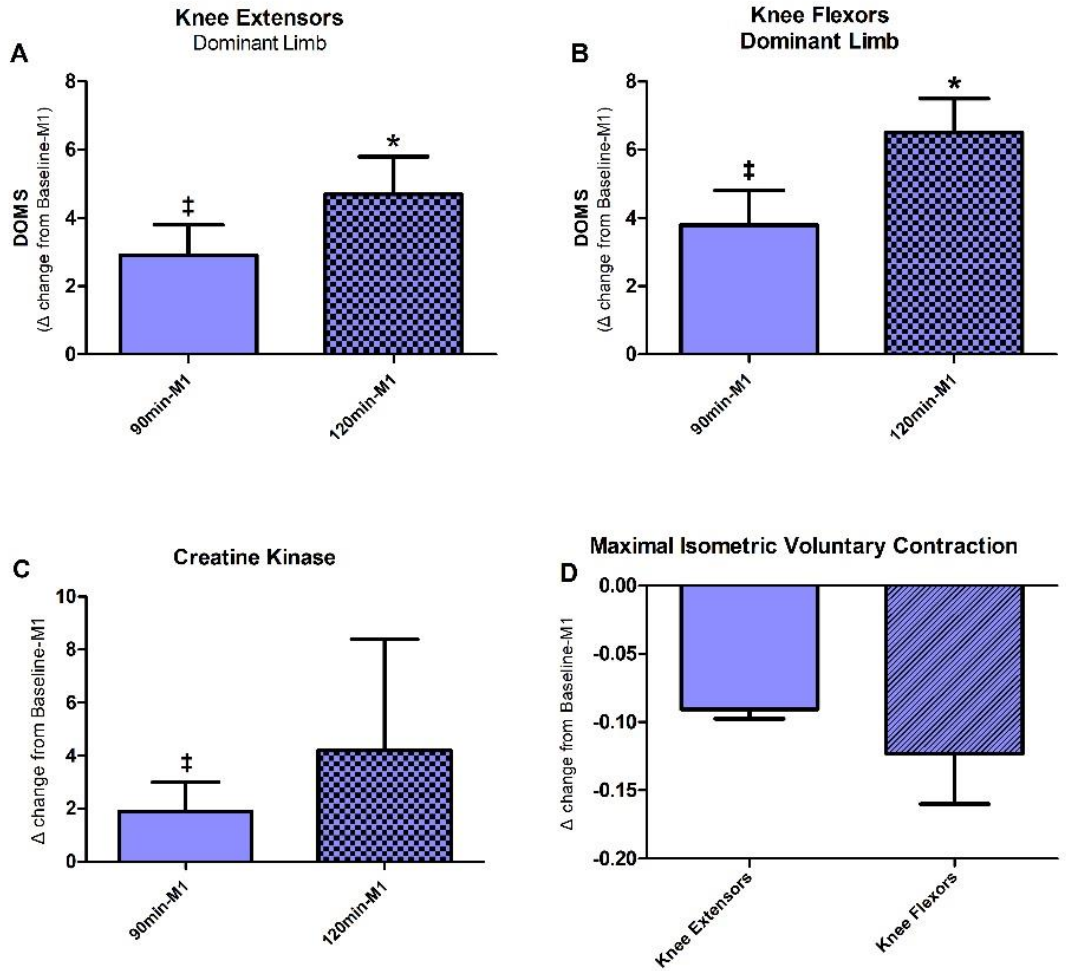

Figure 5.2.4.1. Δ change of DOMS in knee extensors (**A**), DOMS in knee flexors (**B**), creatine kinase (**C**) and maximal isometric voluntary contraction of knee extensors and flexors (dominant limb) (**D**), from baseline (Baseline-M1) in match 1, in PLA trial. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

Inflammatory markers

WBC count (+103% at 90 min; +127% at 120 min), monocyte count (+92% at 90 min; +128% at 120 min) and granulocyte count (+172% at 90 min; +208% at 120 min) increased ($p < 0.05$) at 90 min and 120 min. Although the additional effect of overtime was not found to be statistically meaningful, it must be noted that overtime did cause a 24% to 36% extra rise of this immune response which may be of physiological relevance. In contrast, no meaningful alterations were noted in lymphocytes, red blood cells, hematocrit and haemoglobin at any time-point.

Overtime induced a greater ($p < 0.05$) rise in protein carbonyls (90 min: +127% vs 120 min: +245%), TBARS (90 min: +25% vs 120 min: +43%) and TAC (90 min: +10% vs 120 min: +20%) as well as a greater ($p < 0.05$) reduction of GSH (90 min: -23% vs 120 min: -42%) compared to the 90-min match suggesting that the addition of 30 more minutes of match-play could cause greater redox perturbations.

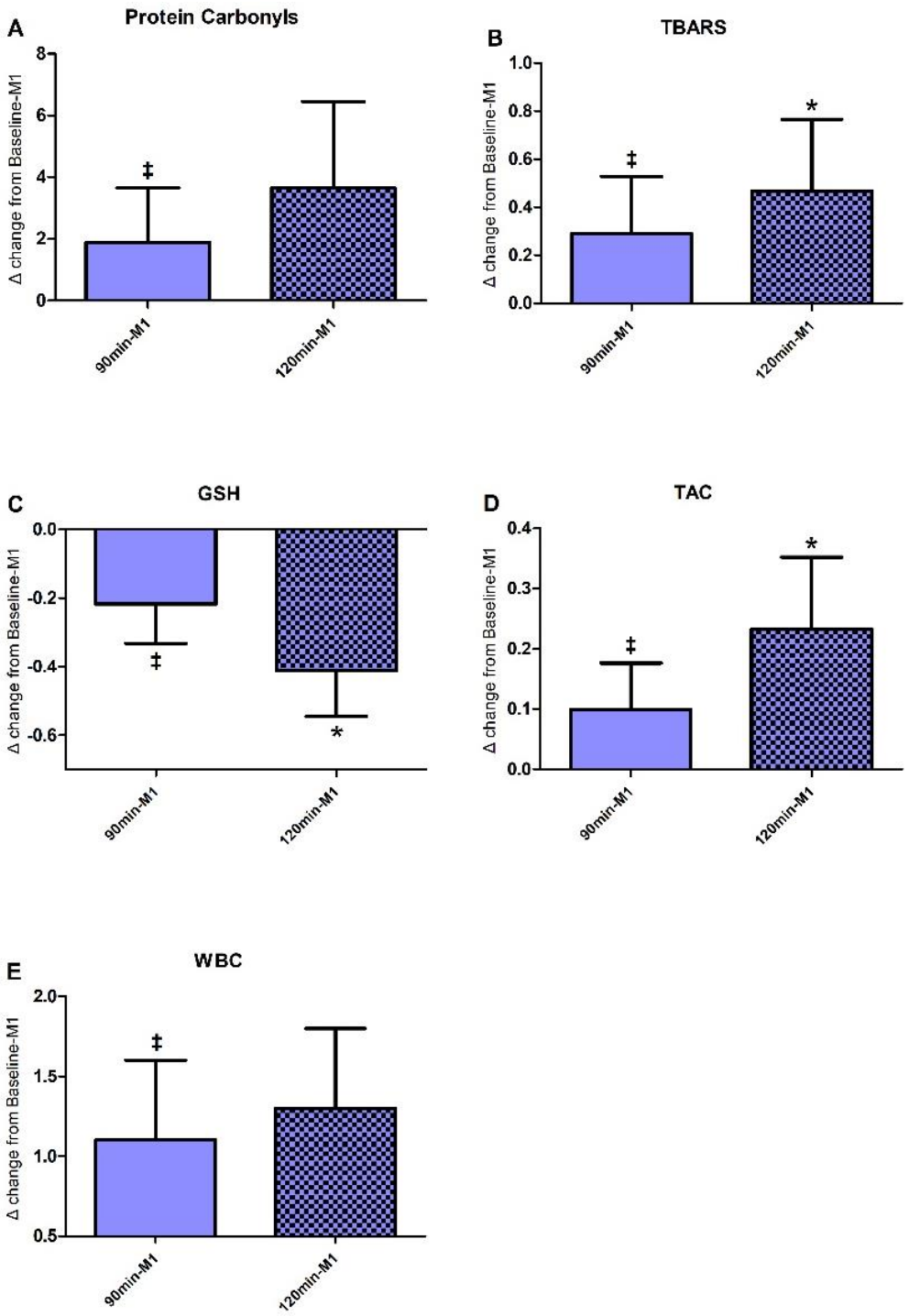

Figure 5.2.4.2. Δ change of protein carbonyls (**A**), TBARS (**B**), GSH (**C**), total antioxidant capacity (**D**) and white blood cells (**E**) from baseline (Baseline-M1) in match 1, in PLA trial. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

5.2.5. The effects of overtime on the psychometric profile of players compared to a 90-min match

Perceived exertion, negative self-talk and ego depletion increased ($p < 0.05$) by 2.5-, 2-, and 4-fold, respectively, at 90 min and by 3-, 3.8-, and 5-fold, respectively, at 120 min with overtime inducing a greater change of these variables compared to the 90 min match-play.

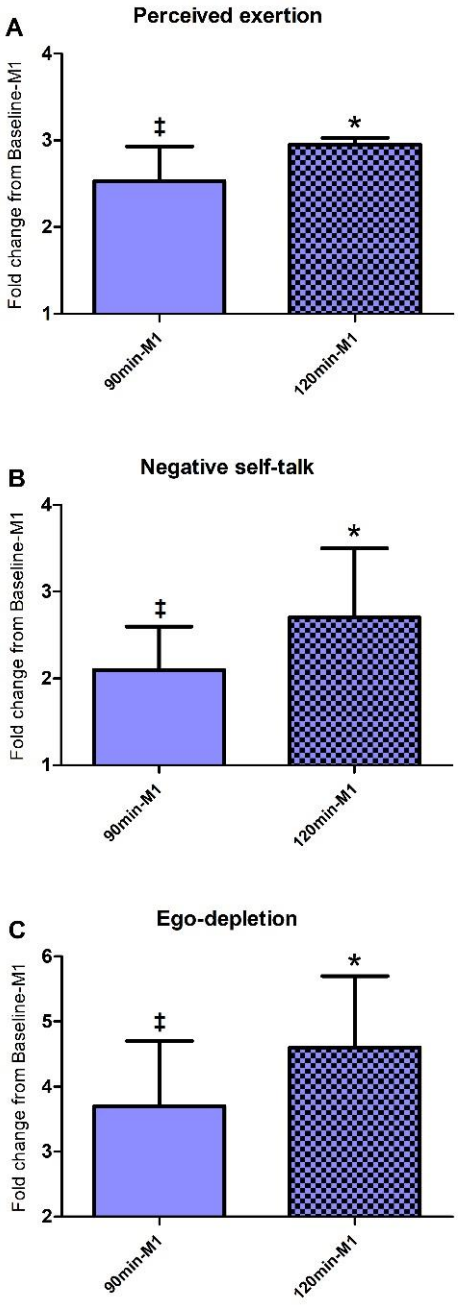

Figure 5.2.5. Fold-change of perceived exertion (**A**), negative self-talk (**B**) and ego-depletion(**C**) from baseline (Baseline-M1) in match 1, in PLA trial. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

5.3. The recovery kinetics of performance following a 120-min match – performance responses during the second match

To address this research question, data from the two matches of the PLA trial were used. In this section, we evaluated (i) the recovery rate from the end of MI to baseline before M2 and (ii) whether the responses during M2 were different than those during M1.

5.3.1. The recovery kinetics of performance following a 120-min match and responses during the second match

Did performance recover before M2?

Prior to M2, CMJ and RSA performance were reduced ($p < 0.05$) by 3% and 5%, respectively, compared to baseline of M1, suggesting that physical performance is not fully recovered at 72h following a 120-min match.

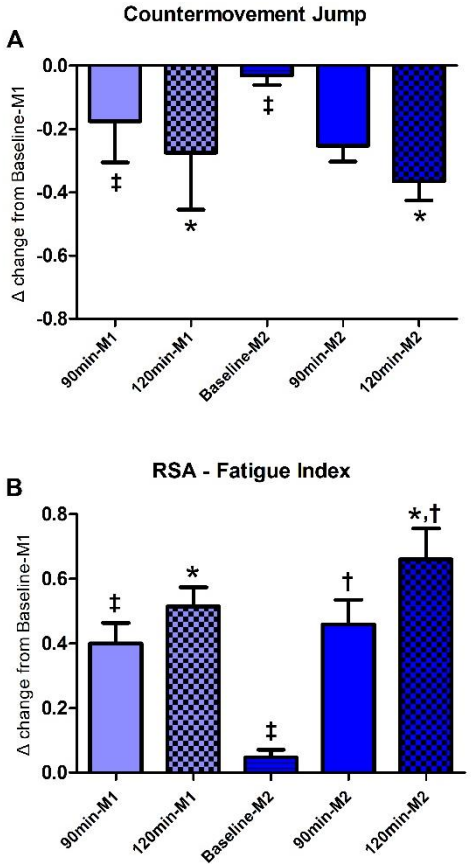

Figure 5.3.1.1. Δ change of countermovement jump (**A**) and RSA (**B**) performance, from baseline (Baseline-M1) in match 1 and match 2, in PLA trial. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

Was performance affected during M2 compared to M1?

Although total distance in M2 remained comparable to that measured in M1 during the 90 min match-play, it was reduced during overtime in M2 compared to the overtime values recorded in M1 (3461 vs. 3089 m). Participants covered similar distances with high intensity running and sprinting during the 90 min of the two matches, but smaller ($p < 0.05$) distances during overtime of M2 compared to that of M1 by 15% and 22%, respectively. Similarly, average and peak speed were reduced ($p < 0.05$) during overtime of M2 compared to but not during the first 90 min. Furthermore, participants performed less ($p < 0.05$) hard accelerations and decelerations during the entire M2 compared to M1. Average heart rate remained comparable during the 90 min match-play between the two marches but it declined ($p < 0.05$) in overtime of M2 compared to that of M1. Sweat loss was similar among the two matches during the first 90 min of match-play, but it declined ($p < 0.05$) in overtime of M2 compared to that of M1.

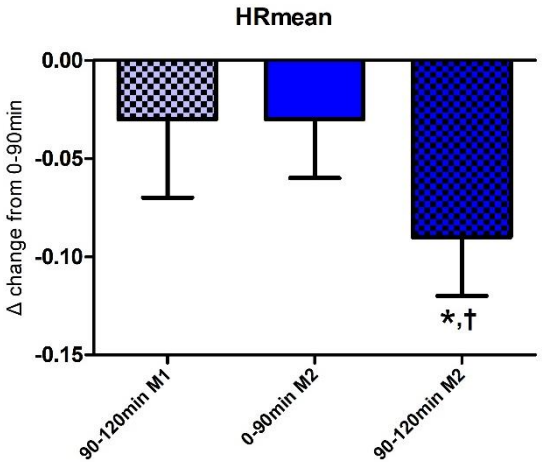

Figure 5.3.1.2. Δ change of HRmean, from 0-90min of match 1 in match 1 and match 2, in PLA trial. * Indicates significant difference between 0-90min and 90-120min in the same match ($p < 0.05$); †

Although passing success was similar among the two matches, duels won were reduced ($p < 0.05$) in overtime, but not during the first 90 min, of M2 compared to that of M1. CMJ in M2 was reduced ($p < 0.05$) to similar extent with M1 (i.e. a 23% and 35 decline at 90 min and at 120 min, respectively. In contrast, the reduction in RSA performance was more pronounced during M2 compared to M1, whereby fatigue index increased by 44% at 90 min (i.e. a 4% greater reduction than M1) and by 65% at 120 min (i.e. a 14% greater reduction than M1).

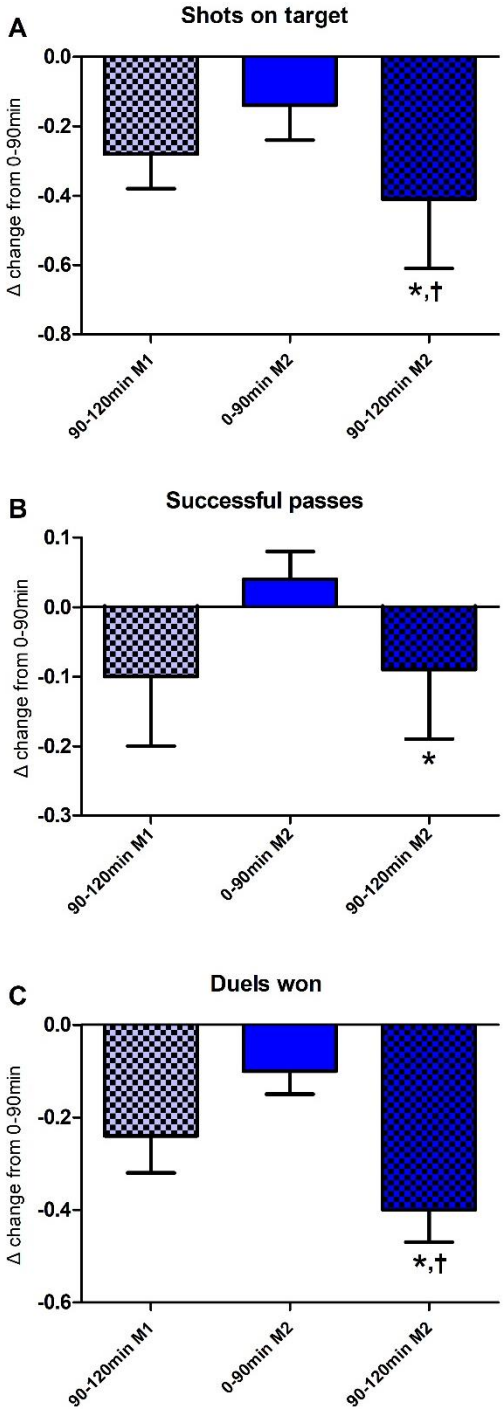

Figure 5.3.1.3. Δ change of shots on target (**A**), successful passes (**B**) and duels won (**C**), from 0-90min of match 1 in match 1 and match 2, in PLA trial. * Indicates significant difference between 0-90min and 90-120min in the same match ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$).

5.3.2. The recovery kinetics of metabolic responses following a 120-min match and responses during the second match

Did metabolic changes recover before M2?

Although glucose levels were restored before M2, participants started the second match with elevated (by 31%, $p < 0.05$) plasma ammonia levels and reduced (by 39%, $p < 0.05$) skeletal muscle glycogen stores compared to their respective values at baseline before M1.

Were metabolic responses altered during M2 compared to M1?

During M2, plasma glucose levels remained unaltered in response to the 90-min match, but they were reduced ($p < 0.05$) at the end of overtime (by 10%, $p < 0.05$), i.e. at the same extent with M1, further corroborating the hypoglycemic effect of overtime. The 90-min match play in M2 provoked a greater ($p < 0.05$) rise in plasma ammonia concentration by 47% compared to the 90-min match in M1 (90 min M1: +56% vs 90 min M2: +103%), whereas the extra 30-min match play in M2 induced a rise in ammonia concentration similar to that observed in M1 (120 min M1: +194% vs 120 min M2: +203%).

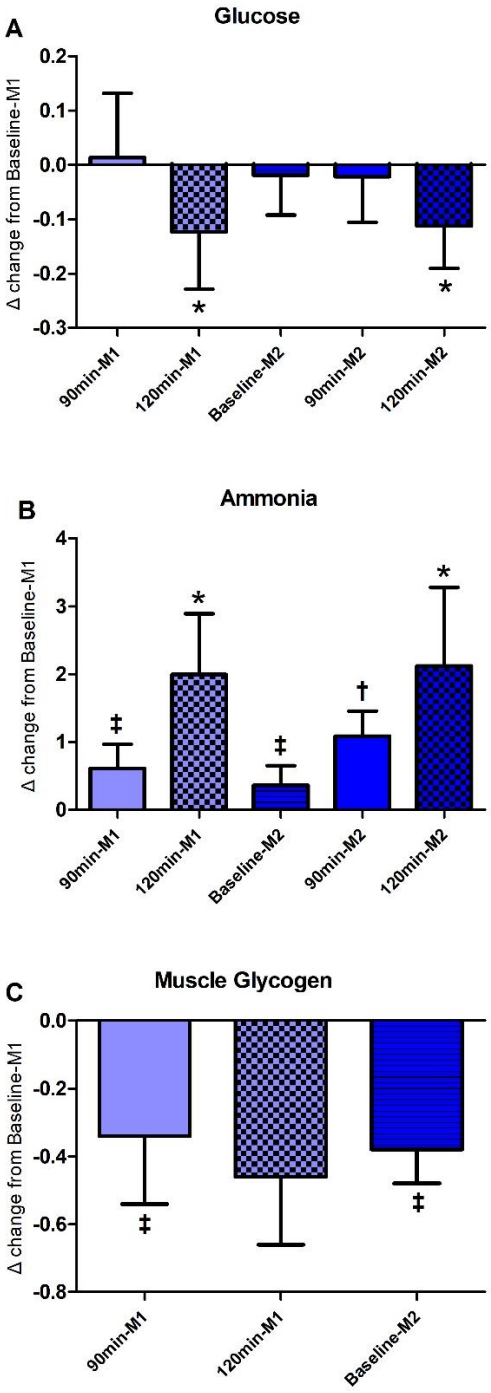

Figure 5.3.2. Δ change of glucose (**A**), ammonia (**B**) and muscle glycogen (**C**) levels from baseline (Baseline-M1), in match 1 and match 2, in PLA trial. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

5.3.3. The recovery kinetics of inflammatory responses following a 120-min match and responses during the second match

Did inflammatory responses recover before M2?

Soreness of KF and KE of both limbs and CK did not subside after 72 h of recovery. Furthermore, MVIC of quadriceps was not restored after 72 h of recovery. Specifically, before M2 CK was increased ($p < 0.05$) by 111% whereas DOMS of knee extensors and flexors was increased ($p < 0.05$) by 2-fold and 2.9-foled, respectively, while MVIC of knee extensors and flexors was reduced ($p < 0.05$) by 6% and 9%, respectively, compared to baseline values of M1.

However, immune responses were normalized before M2. However, an unexpected reduction ($p < 0.05$) was noted in lymphocytes by 13% (2.4 ± 0.4 vs 2.1 ± 0.3 10^3/uL) as well as in hematocrit and haemoglobin by 3% (43.8 ± 1.9 % vs Baseline of M2: 42.3 ± 1.7 %) and 7% (15.3 ± 0.7 vs M2: 14.3 ± 0.8 g/dL), respectively, before M2 compared to the values obtained before M1. Furthermore, protein carbonyls, TBARS and TAC were all normalized before M2, except GSH levels that remained below the values recorded before M1 by 8% (2.6 ± 0.8 vs 2.4 ± 0.8 μmol/g Hb)

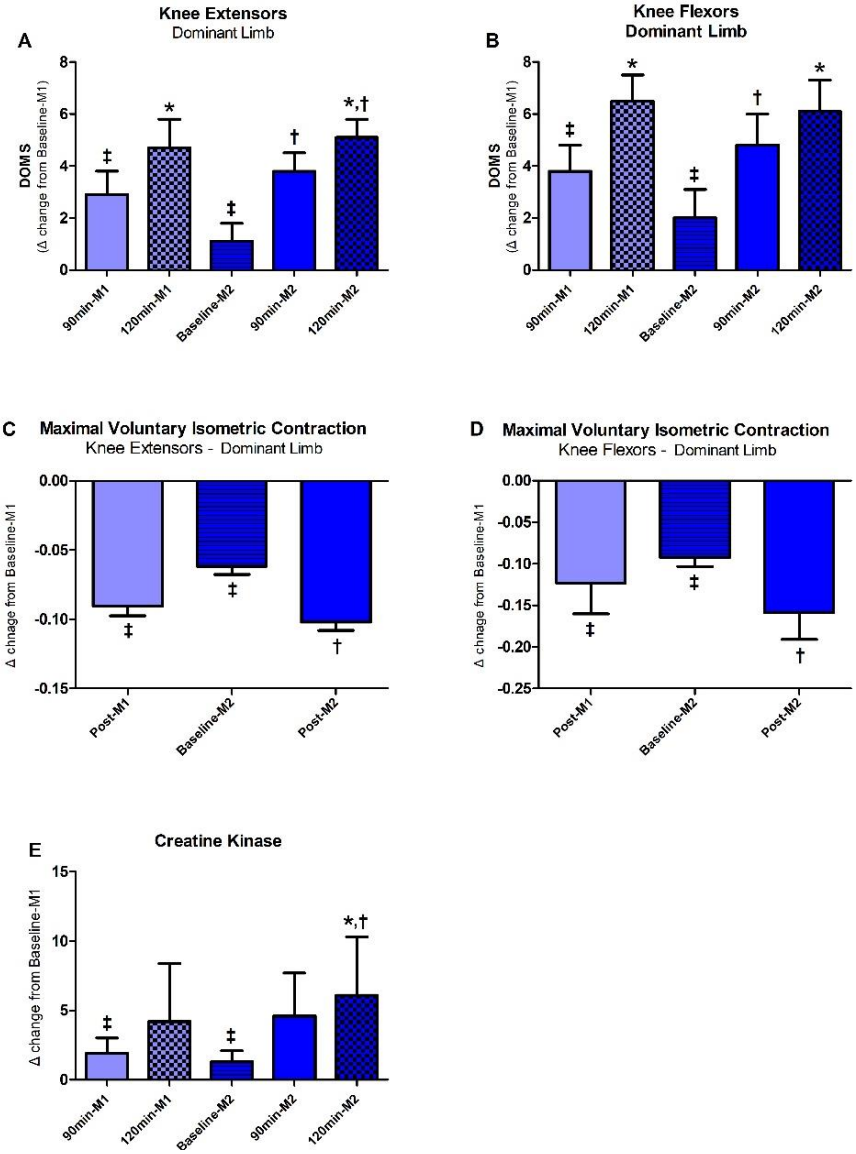

Figure 5.3.3.1. Δ change of DOMS in knee extensors (**A**), DOMS in knee flexors (**B**), maximal isometric voluntary contraction of knee extensors (**C**), maximal isometric voluntary contraction of knee flexors (**D**) and creatine kinase (**E**) from baseline (Baseline-M1) in match 1 and match 2, in PLA trial. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

Were inflammatory responses altered during M2 compared to M1?

During M2, CK demonstrated higher ($p < 0.05$) absolute values ($p < 0.05$) at 90 (635.4 ± 219.0 vs 1115.8 ± 225.4 U/L) and at 120 min (983.0 ± 346.30 vs 1439.5 ± 441.7 U/L) compared to the corresponding values of M1, with the % change observed during the 30-min extra time being greater ($p < 0.05$) by 193% in M2 compared to M1 (+314% vs +507%). Likewise, DOMS of knee extensors displayed greater absolute values ($p < 0.05$) at 90 (3.8 ± 1.1 vs 4.8 ± 0.7) and 120 min (5.6 ± 1.2 vs 6.1 ± 0.7) with the rise observed during the 90 min match and the 30-min extra time being greater ($p < 0.05$) by 100% and 50% in M2 compared to M1, respectively. In knee flexors, although M2 resulted in a greater ($p < 0.05$) elevation of DOMS at 90 min by 1-fold (4.8 ± 0.9 vs 5.8 ± 1.2), the rise at 120 min was comparable with that in M1. MVIC of knee extensors and flexors displayed a greater ($p < 0.05$) reduction after the end of the 120-min match play in M2 compared to M1 (by 1% and 4%), respectively, but their values were comparable among matches at 90 min.

WBC, lymphocyte and monocyte counts responded similarly at 90- and 120-min of M1 and M2. However, M2 resulted in a smaller rise ($p < 0.05$) of granulocytes at 90- (9.8 ± 2.5 vs 7.5 ± 2.1 10^3/uL) and 120-min (11.1 ± 2.8 vs 10.1 ± 2.4 10^3/uL). A similar ($p < 0.05$) response was observed in hematocrit with M2 demonstrating higher values than M1 at 90- (45.0 ± 2.0 vs 42.5 ± 1.8%) and 120-min (44.4 ± 2.1 vs 42.2 ± 1.9%). Haemoglobin exhibited lower ($p < 0.05$) values in M2 compared to M1 both at 90-min (15.6 ± 0.8 vs 14.5 ± 0.8 g/dL) and 120-min (15.4± 0.9 vs 14.4 ± 0.8 g/dL). RBC demonstrated a greater ($p < 0.05$) decline in M2 compared to M1 both at 90-min (5.1 ± 0.2 vs 4.8 ± 0.2 10^6/uL) and 120-min (5.0 ± 0.2 vs 4.8 ± 0.2 10^6/uL). Protein carbonyls, TBARS and TAC revealed higher ($p < 0.05$) absolute values and GSH lower values in M2 compared to M1. Additionally, M2 induced a greater ($p < 0.05$) rise in TBARS than M1 only following overtime by 10% (+43% vs +53%).

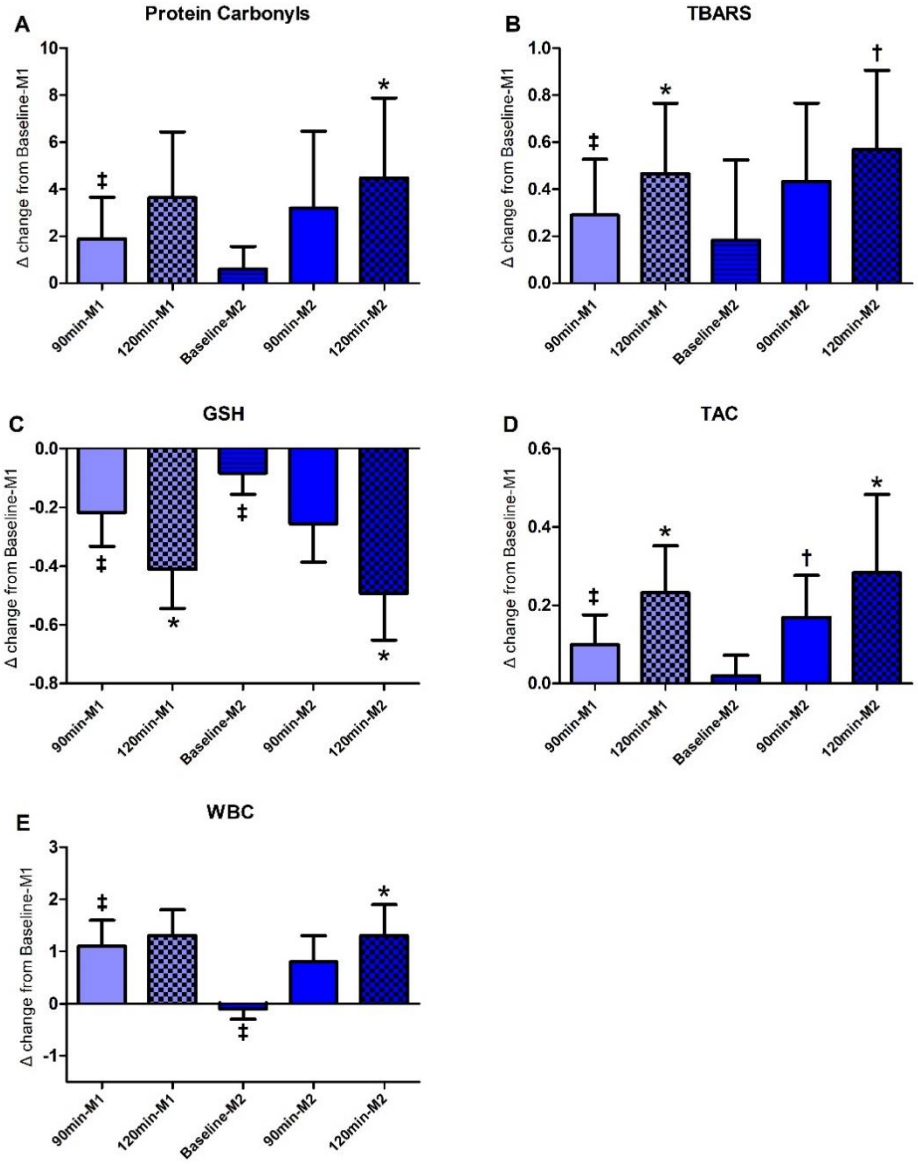

Figure 5.3.3.2. Δ change of protein carbonyls (**A**), TBARS (**B**), GSH (**C**), total antioxidant capacity (**D**) and white blood cells (**E**) from baseline (Baseline-M1) in match 1 and match 2, in PLA trial. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

5.3.4. The recovery kinetics of psychometric responses following a 120-min match and during the second match

Did mental fatigue related responses recover before M2?

Recovery was adequate to restore all mental fatigue related measures.

Were mental fatigue related altered during M2 compared to M1?

Perceived exertion was more pronounced ($p < 0.05$) in M2 compared to M1 at 90 min (15.1 ± 2.5 vs 18.0 ± 1.7) but not at 120 min. Negative self-talk demonstrated similar responses in M2 compared to M1 both at 90- and 120-min of match-play However, ego depletion exhibited a greater ($p < 0.05$) rise in M2 compared to M1 at 120-min (4.9 ± 1.2 vs 5.3 ± 0.8) but not at 90-min.

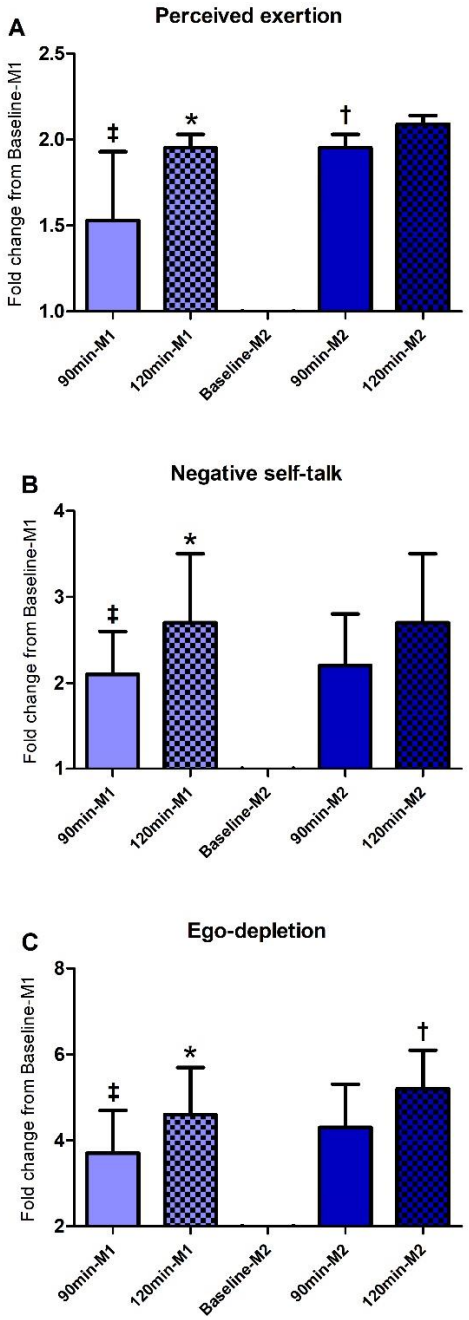

Figure 5.3.4. Fold-change of perceived exertion (**A**), negative self-talk (**B**) and ego-depletion(**C**) from baseline (Baseline-M1) in match 1 and match 2, in PLA trial. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

5.4. The effects of carbohydrate supplementation on recovery kinetics.

In order to answer this question we compared (i) the recovery kinetics from the end of M1 before M2 of the PLA and CHO trials and (ii) the responses during M2 to those of M1 of the PLA and CHO trials. Although a total of 15 participants participated in both trials, muscle data were derived from 10 participants.

5.4.1. The effects of carbohydrate supplementation on the recovery kinetics of performance following a 120-min match and during the second match

Did carbohydrate supplementation helped the recovery of performance following a 120 min match?

CHO supplementation did not enhance the recovery of CMJ and RSA before the second match as their values remained below their baseline levels ($p < 0.05$) in the CHO trial (i.e. CMJ was reduced by 4% and RSA by 5%).

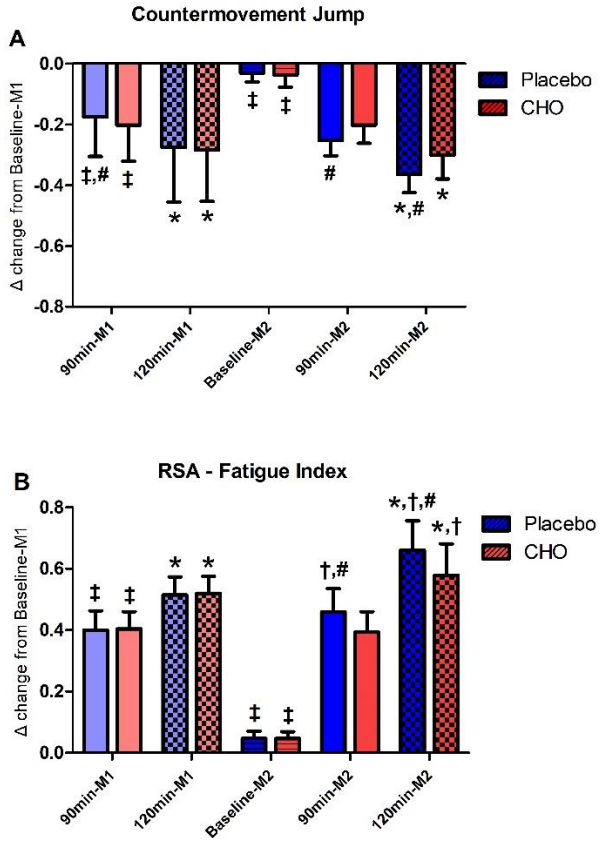

Figure 5.4.1.1. Δ change of countermovement jump (**A**) and RSA (**B**) performance, from baseline (Baseline-M1) in match 1 and match 2, in PLA and CHO trials. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$); # Indicates significant difference between CHO and PLA trial ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

Did carbohydrate supplementation helped the performance during a second match performed 72 h following a first 120 min match?

Participants covered a similar total distance during the entire second match of both trials. High-intensity running distance was greater ($p < 0.05$) in the CHO trial during the 90 min match-play (251.6 ± 97.3 vs. 177.8 ± 77.5) of the second match in the CHO trial but not during the overtime. However, sprinting distance was similar in the second match of the two trials. Average speed remained higher ($p < 0.05$) only during overtime (6.5 ± 0.7 vs 6.2 ± 0.4) of the second match in the CHO trial but not during the first 90 min of the match. On the other hand, no differences were noted between trials for the maximal speed, accelerations, decelerations and mean heart rate. However, fluid loss was greater ($p < 0.05$) during the first 90 min (2207.1 ±444.5 vs 1988.4) of the second match in the CHO trial compare to the PLA trial.

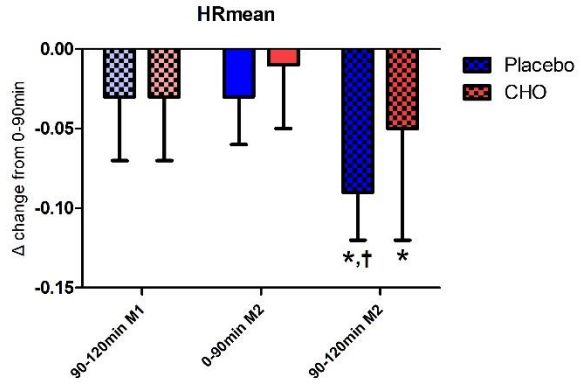

Figure 5.4.1.2. Δ change of HRmean, from 0-90min of match 1 in match 1 and match 2, in PLA and CHO trials. * Indicates significant difference between 0-90min and 90-120min in the same match ($p < 0.05$); † Indicates significant difference between match 1 and match

CHO supplementation mitigated the decline in CMJ and RSA performance during the second match compared to PLA, by inducing an attenuate ($p < 0.05$) decline in CMJ at 90 min and 120 min by 3% and 5%, respectively and also a rise of a smaller magnitude ($p < 0.05$) in RSA fatigue index by 7% and 9% at 90 min and 120 min, respectively. Although successful passing remained unaffected by CHO supplementation, the rate of duels won was greater ($p < 0.05$) during overtime (but not during the first 90 min) of the second match (31.7 ± 4.4 vs 27.0 ± 4.8) in the CHO trial compared to the PLA trial.

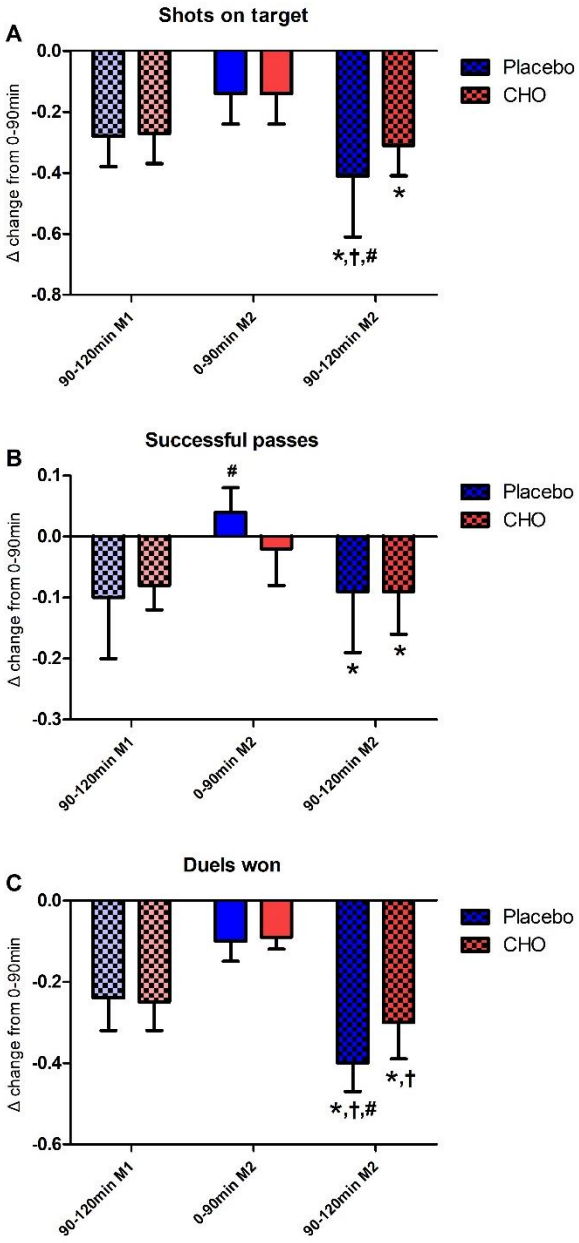

Figure 5.4.1.3. Δ change of shots on target (**A**), successful passes (**B**) and duels won (**C**), from 0-90min of match 1 in match 1 and match 2, in PLA and CHO trials. * Indicates significant difference between 0-90min and 90-120min in the same match ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$), # Indicates significant difference between PLA and CHO trial ($p < 0.05$).

5.4.2. The effects of carbohydrate supplementation on the recovery kinetics of metabolic responses following a 120-min match and during the second match

Did carbohydrate supplementation enhanced the recovery of metabolic responses following a 120 min match?

Metabolic responses were considerably affected by CHO supplementation. Specifically, prior to M2 plasma glucose levels were elevated by 9% ($p < 0.05$) compared to its pre-M1 values in the CHO trial and were also higher than those measured in the PLA trial before M2 by 12% ($p < 0.05$). No differences were noted between the two trials in ammonia values before M2. Of note, CHO supplementation did efficiently accelerated ($p < 0.05$) the restoration of muscle glycogen following the first 120-min match in the CHO trial but not in the PLA trial, as muscle glycogen reached its baseline vales prior to M2 in CHO trial, whereas in PLA it was reduced ($p < 0.05$) by 39%.

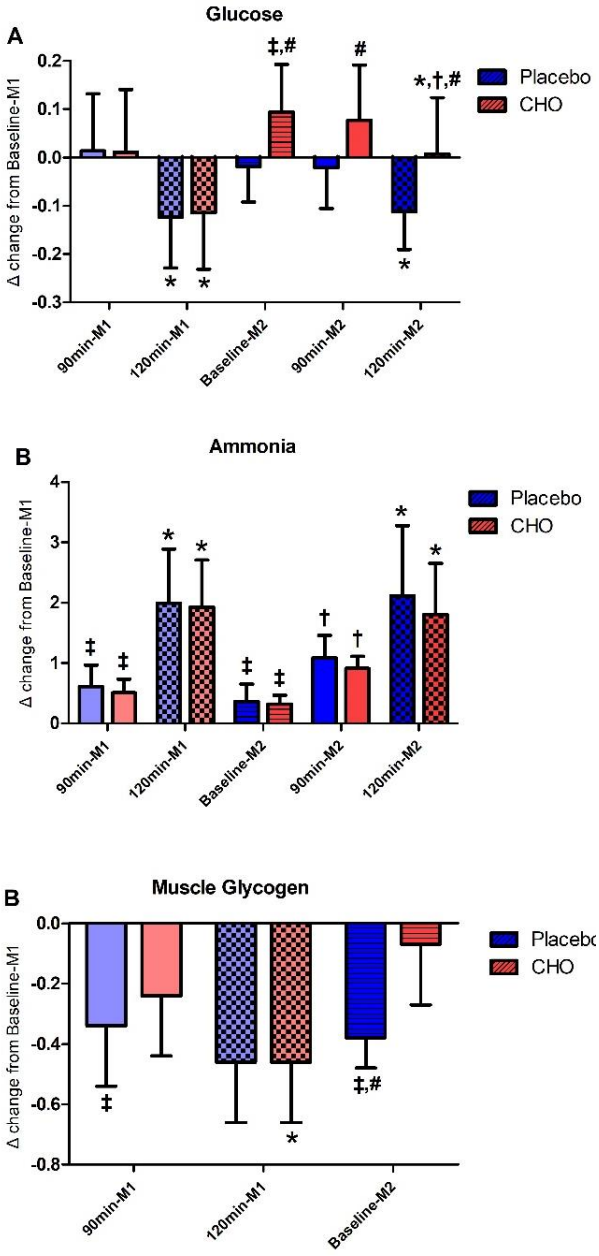

Figure 5.4.2. Δ change of glucose (**A**), ammonia (**B**) and muscle glycogen (**C**) levels from baseline (Baseline-M1), in match 1 and match 2, in PLA and CHO trials. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$); # Indicates significant difference between CHO and PLA trial ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

Did carbohydrate supplementation altered the metabolic responses during a second match performed 72 h following a first 120 min match?

Although not statistically meaningful, glucose levels at the of 90-min match play of M2 in the CHO trial were 12% higher than those in the PLA trial while at the end of overtime of M2 in the CHO trial, glucose levels were well above ($p < 0.05$) those in the PLA trial (93.0 ± 4.8 vs $81.7 \pm 5.$). In total, the decline in glucose was prevented during the entire M2 in the CHO trial whilst in PLA it was declined ($p < 0.05$) at the end of the overtime by 9%. However, in the CHO trial ammonia displayed a rise of smaller magnitude ($p < 0.05$) during the entire M2 compared to the PLA trial (by 8% and 10% at 90 min and 120 min, respectively).

5.4.3. The effects of carbohydrate supplementation on the recovery kinetics of inflammatory responses following a 120-min match and during the second match

Did carbohydrate supplementation facilitated the recovery of inflammatory responses following a 120 min match?

CK and DOMS of knee extensors and flexors in the CHO trial remained elevated prior to M2, with no differences noted among trials except for that of DOMS of knee extensors in the NDL that was lower ($p < 0.05$) in the CHO trial by 26%. Similarly, MVIC of knee extensors and flexors remained unaffected by CHO supplementation prior to M2, suggesting that supplementation was insufficient to accelerate the recovery of muscle strength.

Interestingly, the WBC and granulocyte counts in CHO trial were higher ($p < 0.05$) prior to M2 compared to the PLA trial (WBC count was higher by 26% and the granulocyte count by 37%). There was no impact of CHO supplementation on lymphocytes, monocytes, hematocrit, hemoglobin and red blood cells before M2. No significant differences were detected between trial in terms of protein carbonyls, TBARS, GSH and TAC, suggesting that CHO supplementation did not interfere with redox status perturbations.

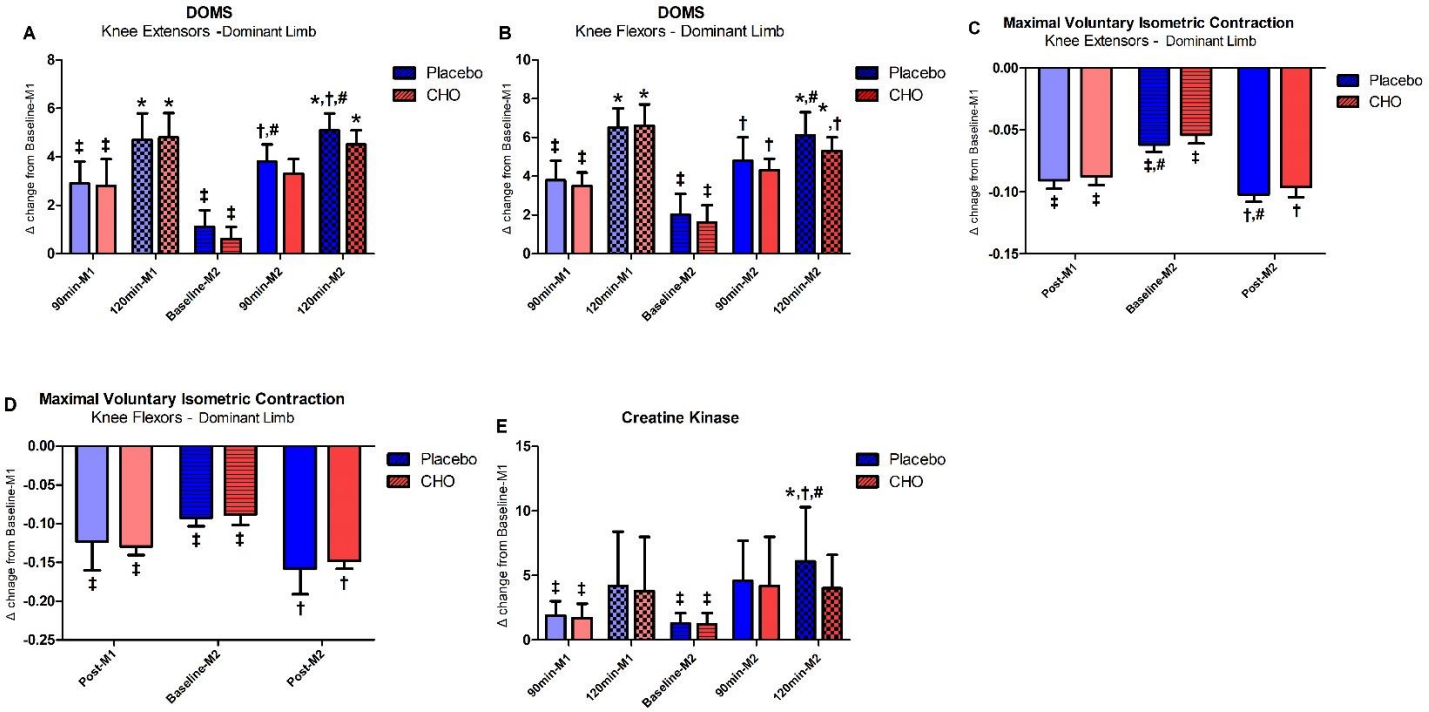

Figure 5.4.3.1. Δ change of DOMS in knee extensors (**A**), DOMS in knee flexors (**B**), maximal isometric voluntary contraction of knee extensors (**C**), maximal isometric voluntary contraction of knee flexors (**D**) and creatine kinase (**E**) from baseline (Baseline-M1) in match 1 and match 2, in PLA and CHO trials. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$); # Indicates significant difference between CHO and PLA trial ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

Did carbohydrate supplementation altered the inflammatory responses during a second match performed 72 h following a first 120 min match?

The rise in CK was lower ($p < 0.05$) by 9% at 90 min and by 27% at 120 min of M2 in the CHO trial compared to the PLA trial. Likewise, DOMS of knee extensors increased to a lesser extent ($p < 0.05$) at 90 min and after overtime in the CHO trial (10% less in DL and 9% less in NDL), while in knee flexors there was a rise of smaller ($p < 0.05$) magnitude in DOMS in the CHO trial compared to the PLA trial at 120 min for the DL (11%, $p < 0.05$) and at 90 min for the NDL (12%, $p < 0.05$). The decline in MVIC of knee extensors DL: 3.48 ± 0.6 in PLA vs 3.53 ± 0.5 in CHO; NDL: 3.52 ± 0.5 in PLA vs 3.55 ± 0.6 in CHO) was prevented ($p < 0.05$) after overtime of M2 but no effect of CHO supplementation were seen in MVIC of knee flexors.

The immune response was lower ($p < 0.05$) following overtime of M2 (the WBC count by 15% and the granulocyte count by 18%) compared to the PLA trial. CHO supplementation had no effects on redox status markers during the entire M2.

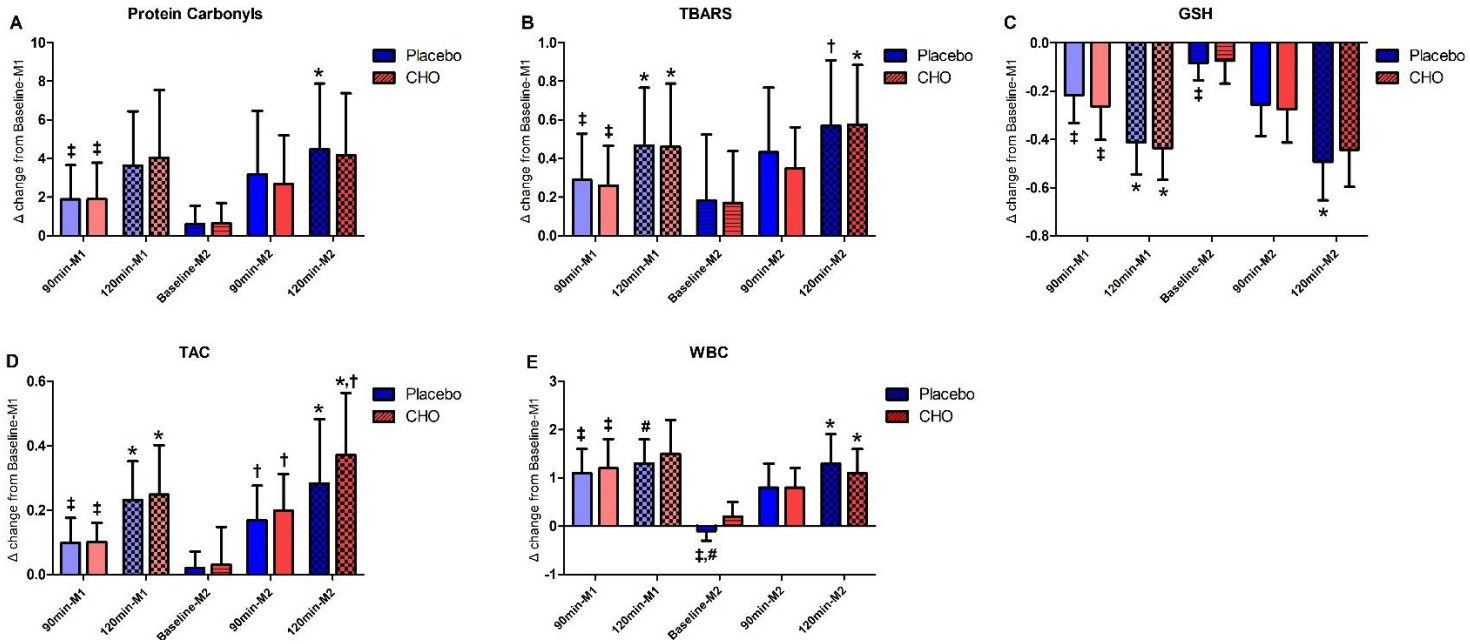

Figure 5.3.3.2. Δ change of protein carbonyls (**A**), TBARS (**B**), GSH (**C**), total antioxidant capacity (**D**) and white blood cells (**E**) from baseline (Baseline-M1) in match 1 and match 2, in PLA and CHO trials. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$); # Indicates significant difference between CHO and PLA trial ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

5.4.4. The effects of carbohydrate supplementation on the recovery kinetics of psychometric responses following a 120-min match and during the second match

Did carbohydrate supplementation relieved mental fatigue-related responses following a 120 min match?

All mental fatigue related variables and perceived exertion remained unaffected by CHO supplementation before M2.

Did carbohydrate supplementation altered the mental fatigue related responses during a second match performed 72 h following a first 120 min match?

Perceived exertion was lower ($p < 0.05$) in the CHO trial following overtime (17.4 ± 1.3 vs 18.6 ± 1.4), but not during the first 90 min of match-play) compared to the PLA trial. Ego depletion values were lower ($p < 0.05$) during the entire M2 in the CHO trial compared to the PLA trial (90 min: 3.9 ± 1 vs 4.4 ± 0.9 - 120 min: 4.7 ± 0.9 vs 5.3 ± 0.8). Negative self-talk increased similarly in both trials during the entire M2.

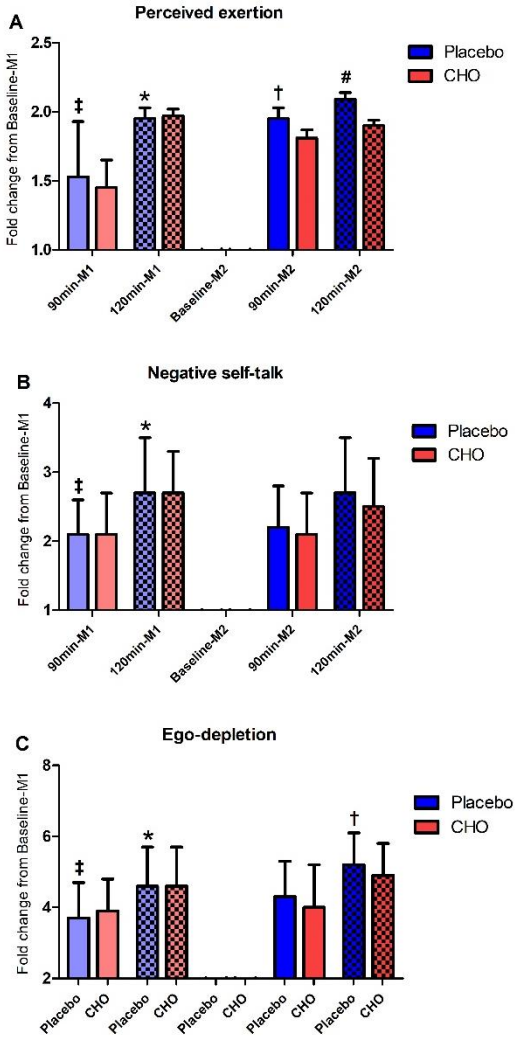

Figure 5.4.4. Fold-change of perceived exertion (**A**), negative self-talk (**B**) and ego-depletion(**C**) from baseline (Baseline-M1) in match 1 and match 2, in PLA and CHO trials. * Indicates significant difference between 90 min and 120 min ($p < 0.05$); † Indicates significant difference between match 1 and match 2 ($p < 0.05$); # Indicates significant difference between CHO and PLA trial ($p < 0.05$); ‡ Indicates significant difference from baseline (according to absolute values) ($p < 0.05$).

6. CONCLUSIONS & PERSPECTIVES

This section of the final report is organized based on the main research questions that this study attempted to answer.

6.1. The effects of overtime on the physiological, metabolic, physical and mental load of the players

Compared to a 90-min match-play, the overtime induces the following:

External load	✓ Causes a reduction in meters covered per min by the athletes by ~8 m/min. ✓ Reduces the average speed of the players by ~11%. ✓ Reduces the number of hard accelerations per min by ~18%. ✓ Reduces the number of hard decelerations per min by ~19%.
Internal load	✓ Maintained the mean heart rate at the same level. ✓ Increases sweat loss and sweat rate by approximately 48% and 19%, respectively.
Technical performance	✓ Reduces the rate of successful passing by 6%. ✓ Reduces the rate of duels won by 10-12%.
Performance	✓ Reduces jumping ability by 11%. ✓ Reduces the ability to perform repeated sprints by 11%.
Metabolic overload	✓ Causes a moderate hypoglycemia. ✓ Further promotes the deamination of AMP, an indication of increased energy turnover rate. ✓ Caused a substantial reduction of muscle glycogen stores with values falling below those considered as critical (~200 mM/kg d.w.).
Muscle damage	✓ Induced a marked elevation of CK (a known muscle damage marker) by ~150%. ✓ Resulted in a more pronounced sensation of soreness of both knee flexors and extensors, with the former being more affected than the later. ✓ Overtime was associated with a ~10 decline in quadriceps' isometric force.
Inflammation	✓ Exacerbates the acute inflammatory response by inducing an additional rise of ~30% of white blood cells associated with the local muscle damage. ✓ Does not affect natural immunity. ✓ Induces a more pronounced redox perturbation.
Psychometric profile	✓ Promotes negative self-talk and ego depletion probably due to a greater perceived exertion.

Conclusion

Therefore, the answer to the first question is that overtime substantially augments both the internal and the external load of the players, resulting in a deterioration of physical and technical performance as well as in mental fatigue related measures compared to a 90 min match-play. These findings suggest that the rise in overload may be attributed to a reduction in energy stores (mainly skeletal muscle glycogen) and an associated hypoglycemic response as well as to an induced muscle damage that causes a marked inflammatory response. However, it appears that a football match with overtime does not affect natural immunity and the ability to fight infections although more in depth analysis is warranted in this area. It must also be noted that overtime did exacerbate fluid loss suggesting the onset of dehydration despite that participants received adequate amounts of water during the entire match. Fluid loss could have also

contributed to the deterioration of performance in overtime compared to the 90 min match-play.

6.2. The recovery kinetics following a 120-min match

Following evaluation of (i) the recovery rate from the end of MI to baseline of M2 and (ii) the differences of responses between M2 and those of M1, we concluded that:

A. Following a 120 min match, a 72 h recovery:

Performance	✓ Does not fully restore jumping performance and repeated sprint ability.
Metabolic overload	✓ Restores euglycemia. ✓ Does not restore plasma ammonia levels. ✓ Cannot restore muscle glycogen stores.
Muscle damage	✓ Does not restore CK levels. ✓ Does not fully restore muscle soreness of knee flexors and extensors. ✓ Does not restore quadriceps' isometric force.
Inflammation	✓ Normalizes immune responses but lymphocytes demonstrate a decline. ✓ Hematocrit and hemoglobin are reduced. ✓ Restores protein oxidation and lipid peroxidation but on the expense of GSH which remains reduced.
Psychometric profile	✓ Restores perceived exertion all mental fatigue related variables.

Conclusion

Therefore, the answer to this question is that performance is not fully recovered even after 72 h of recovery. This persistence of performance deterioration may be associated with an increased state of energy turnover rate, inadequate restoration of skeletal muscle glycogen stores as well as to a continuous inflammatory response due to an ongoing muscle damage. Unexpectedly, a decline of lymphocytes was note despite a normalization of the other immune cell subpopulations. This observation should be further explored in relation to the player's ability to fight various upper respiratory tract infections. It appears that redox responses are restored due to an active antioxidant system as evidenced by the continuous decline of GSH, a powerful antioxidant located in the muscle and other tissues. These observations should be taken into account when scheduling knock-out matches during tournaments.

B. When a second 120 min match is played 72 h after a first one of similar durations, the following adaptations are seen in the second match compared to the first one:

External load	✓ TD, high-intensity running, sprinting, average speed and peak speed exhibits a similar decline during the first 90 min of match-play but they are more reduced following overtime. ✓ Hard accelerations and decelerations are more reduced during the entire match.
Internal load	✓ Average heart rate and fluid loss exhibits a similar increase during the first 90 min of match-play but they are less increased following overtime.
Technical performance	✓ The rate of successful passing exhibits a similar decline during the entire match. ✓ The rate of duels won exhibits a similar decline during the first 90 min of match-play but it is more reduced following overtime.
Performance	✓ Jumping performance exhibits a similar decline during the entire match. ✓ Repeated sprint ability is more reduced during the entire match.
Metabolic overload	✓ Plasma glucose levels exhibit a similar decline during the first 90 min of match-play but they are more reduced following overtime. ✓ Ammonia demonstrates a greater rise during the first 90 min of match-play but a similar increase following overtime.
Muscle damage	✓ CK levels and muscle soreness were far more pronounced during the entire duration. ✓ Isometric force (as an index of fatigue) demonstrated a similar decline during the first 90 min of match-play but it was more reduced following overtime.
Inflammation	✓ White blood cell counts exhibited a similar rise during the entire match. Only the granulocytes demonstrated a smaller rise. ✓ Hemoglobin and red blood cell counts exhibited a greater decline during the entire match. ✓ Protein carbonyls, TBARS and total antioxidant capacity increased more during the entire match but lipid peroxidation demonstrated a great rise only after overtime. ✓ GSH was more consumed during the entire match.
Psychometric profile	✓ Perceived exertion was more pronounced during the first 90 min of match-play but not after overtime. ✓ Ego depletion was more pronounced only after overtime. ✓ Negative self-talk demonstrated similar responses.

Conclusions

When two matches with overtime are played 72 h apart, the second match is characterized by a reduced external and internal load, reduced physical and technical performance, and potentially be greater mental fatigue. This deterioration of performance during the second match is probably associated with (i) the reduced initial glycogen stores, (ii) the more pronounced drop of blood glucose and energy turnover rate (iii) a reduced antioxidant capacity which may augment oxidative stress, especially followingovertime and (iv) alter hemoglobin and red blood cell homeostasis and (v) a greater muscle damage. These results suggest that knock-out matches should be interspersed by more than 72 h recovery to allow performance and physiological restoration.

6.3. The effects of carbohydrate supplementation on the recovery kinetics following a 120-min match

Following comparison between the CHO and the PLA trial of the (i) the recovery rate from the end of MI to baseline of M2 and (ii) the differences of responses between M2 and those of M1, we concluded that:

A. The carbohydrate supplementation following a 120 min match:

Performance	✓ Failed to restore jumping performance and repeated sprint ability prior to the second match.
Metabolic overload	✓ Maintained higher blood glucose levels throughout recovery before the second match. ✓ Did not affect resting ammonia levels throughout recovery before the second match. ✓ Restored muscle glycogen stores back to baseline levels before the second match.
Muscle damage	✓ Did not accelerate muscle damage resolution before the second match. ✓ Does not restore quadriceps' isometric force.
Inflammation	✓ It did not affect immune responses except of a rise that was seen in WBC and granulocyte counts before the second match. ✓ Did not affect hematocrit and hemoglobin levels throughout recovery. ✓ Did not change redox status markers throughout the entire recovery period.
Psychometric profile	✓ It did not alter mental fatigue related measures and perceived exertion prior to the second match.

Conclusion

CHO supplementation restored skeletal muscle glycogen stores but it had no effect on the recovery kinetics of the other metabolic, inflammatory, psychometric and performance after a 120 min match and before a second 72 h later.

B. When a second 120 min match is played 72 h after a first one of similar duration, the following adaptations are seen in the second match compared to the first one in response to CHO supplementation:

External load	✓ High-intensity running was greater during the first 90 min match-play but not overtime of the second match. ✓ Average speed was higher during overtime only of the second match. ✓ It did not affect total distance, sprinting distance, peak speed, hard accelerations and decelerations during the entire second match.
Internal load	✓ Average heart rate remained unaffected during the entire second match. ✓ Fluid loss was greater during the first 90 min of match-play, but not overtime, of the second match.
Technical performance	✓ The rate of successful passing exhibits a similar decline during the entire match. ✓ The rate of duels won exhibits a similar decline during the first 90 min of match-play but it is more reduced following overtime.
Performance	✓ Mitigated the decline in jumping performance and repeated sprint ability during the entire second match. ✓ It did not affect passing but it improved the rate of duels won during overtime but not during the first 90 min of match-paly of the second match.
Metabolic overload	✓ Prevented a drop of plasma glucose levels during the entire second match. ✓ It attenuated the rise of ammonia during the entire second match.
Muscle damage	✓ It attenuated muscle damage responses during the entire second match. ✓ It prevented the deterioration of knee extensor strength, but not that of flexors, following overtime of the second match.
Inflammation	✓ It attenuated the immune response following overtime of the second match but it did not affect the redox responses.
Psychometric profile	✓ It attenuated the perceived exertion reported by participants following overtime but not after the first 90 min of match-play of the second match. ✓ It attenuated the rise of ego depletion during the entire second match. ✓ It did not affect negative self-talk.

Conclusions

It appears that CHO supplementation helps to help players maintain a higher intensity during match-play, enhance both physical and technical performance and reduce mental fatigue during a second 120 min match performed only 72 h after a first one of the same duration. This improvement is likely to be related to a greater carbohydrate availability and reduced inflammatory response as a result of less muscle damage. These results suggest carbohydrate supplementation may offer an attractive nutritional strategy for knock-out matches which may lead to overtime, especially when these matches follow a congestive schedule.

7. LITERATURE

1. Bangsbo J, Mohr M. Fitness Testing in Football. Fitness Training in Soccer II; *Bangsbosport*: Copenhagen, Denmark, 2012.
2. Bangsbo J, Iaia FM, Krustrup P. Metabolic response and fatigue in soccer. *Int J Sports Physiol Perform*, 2(2):111–127, 2007.
3. Bangsbo J, Mohr M, Krustrup P. Physical and metabolic demands of training and match-play in the elite football player. *J Sports Sci*, 24(7): 665-674, 2006.
4. Bangsbo J. The physiology of soccer with special reference to intense intermittent exercise. *Acta Physiol Scand Suppl*, 619:1–155, 1994.
5. Barbas I, Fatouros IG, Douroudos II, et al. Physiological and performance adaptations of elite Greco-Roman wrestlers during a one-day tournament. *Eur J Appl Physiol*, 111(7):1421–1436, 2011.
6. Batrakoulis A, Jamurtas AZ, Georgakouli K, et al. High intensity, circuit-type integrated neuromuscular training alters energy balance and reduces body mass and fat in obese women: A 10-month training-detraining randomized controlled trial. *PLoS ONE*, 13(8):e0202390, 2018.
7. Bradley PS, Sheldon W, Wooster B, et al. High-intensity running in English FA Premier League soccer matches. *J Sports Sci*, 27(2):159-168, 2009.
8. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol*, 37(2), 247–248, 1974.
9. Draganidis D, Chatzinikoloau A, Avloniti A, et al. Recovery kinetics of knee flexor and extensor strength after a football match. *PLoS One*, 10(6):e0128072, 2015.
10. Ekstrand J, Waldén M, Hägglund M. A. A congested football calendar and the wellbeing of players: correlation between match exposure of European footballers before the World Cup 2002 and their injuries and performances during that World Cup. *Br J Sports Med*, 38(4):493–497, 2003.
11. Fatouros IG, Chatzinikolaou A, Douroudos II, et al. Time-course of changes in oxidative stress and antioxidant status responses following a soccer game. *J Strength Cond Res*, 24(12): 3278-86, 2010.
12. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev*, 8(4):1725-1789, 2001.
13. Girard O, Millet GP. Neuromuscular fatigue in racquet sports. *Phys Med Rehabil Clin N Am*, 20(1):161-173, 2009.
14. Goodall S, Thomas K, Harper LD, et al. The assessment of neuromuscular fatigue during 120 min of simulated soccer exercise. *Eur J Appl Physiol*, 117(4):687-697, 2017.
15. Gregersen J, Hatzigeorgiadis A, Galanis E, et al. Countering the Consequences of Ego Depletion: The Effects of Self-Talk on Selective Attention. *J Sport Exerc Psychol*, 39(3):161-171, 2017.
16. Harper LD, Fothergill M, West DJ, et al. Practitioners' Perceptions of the Soccer Extra-Time Period: Implications for Future Research. *PLoS One*, 11(7):e0157687, 2016.
17. Harper LD, West DJ, Stevenson E, et al. Technical performance reduces during the extra-time period of professional soccer match-play. *PLoS One*, 9(10), e110995, 2014.
18. Hatzigeorgiadis, A, Bartura K, Argiropoulos C, et al. Beat the Heat: Effects of a Motivational Self-Talk Intervention on Endurance Performance. *J Appl Sport Psychol*, 30(4):388-401, 2018.
19. Hatzigeorgiadis A, Theodorakis Y, Zourbanos N. Self-talk in the swimming pool: The effects of self-talk on thought content and performance on water-polo tasks. *J Appl Sport Psychol*, 16(2):138-150, 2004.
20. Hostrup M, Bangsbo J. Limitations in intense exercise performance of athletes - effect of speed endurance training on ion handling and fatigue development. *J Physiol*, 595(9):2897–2913, 2017.
21. Ispirlidis I, Fatouros IG, Jamurtas AZ, et al. Time-course of changes in inflammatory and performance responses following a soccer game. *Clin J Sport Med*, 18(5):423–431, 2008.
22. Kreider RB, Wilborn CD, Taylor L, et al. ISNN exercise & sport nutrition review: Research & recommendations. *J Int Soc Sports Nutr*, 2010, 7(1):5, 2010.

23. Krustrup P, Ortenblad N, Nielsen J, et al. Maximal voluntary contraction force, SR function and glycogen resynthesis during the first 72 h after a high-level competitive soccer game. *Eur J Appl Physiol*, 111(12):2987–2995, 2011.
24. Krustrup P, Mohr M, Nybo L, et al. The Yo-Yo IR2 test: physiological response, reliability, and application to elite soccer. *Med Sci Sports Exerc*, 38(9):1666–1673, 2006.
25. Lorist MM, Boksem MM, Ridderinkhof KR. Impaired cognitive control and reduced cingulate activity during mental fatigue. *Brain Res Cogn Brain Res*, 24(2):199-205, 2005.
26. Lowry OH, Passonneau JV. A flexible system of enzymatic analysis. *New York: Academic*, 237– 249, 1972.
27. Marshall PW, Lovell R, Jeppesen GK, et al. Hamstring muscle fatigue and central motor output during a simulated soccer match. *PLoS ONE* 9(7):e102753, 2014.
28. Millet GY, Lepers R. Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. *Sports Med*, 34(2):105-116, 2004.
29. Mohr M, Draganidis D, Chatzinikolaou A, et al. Muscle damage, inflammatory, immune and performance responses to three football games in 1 week in competitive male players. *Eur J Appl Physiol*, 116(1):179–193, 2016.
30. Mohr M, Nybo L, Grantham J, et al. Physiological responses and physical performance during football in the heat. *PLoS One*, 7(6):e39202, 2012.
31. Mohr M, Krustrup P, Bangsbo J. Fatigue in soccer: a brief review. *J Sports Sci*, 23(6):593–599, 2005.
32. Mohr M, Krustrup P, Nybo L, et al. Muscle temperature and sprint performance during soccer matches-beneficial effects of re-warm-up at half time. *Scand J Med Sci Sports*, 14(3):156-162, 2004.
33. Mohr M, Krustrup P, Bangsbo J. Match performance of high-standard soccer players with special reference to development of fatigue. *J Sports Sci*, 21(7):519-528, 2003.
34. Packer JE, Wooding DJ, Kato H, et al. Variable-intensity simulated team-sport exercise increases daily protein requirements in active males. *Front. Nutr*, 4:64, 2017.
35. Peñas CL, Dellal A, Owen AL, et al. The influence of the extra-time period on physical performance in elite soccer. *Int J Perform Anal Sport*, 15(3):830-839, 2015.
36. Poulios A, Fatouros IG, Mohr M, et al. Post-game high protein intake may improve recovery of football-specific performance during a congested game fixture: results from the PRO-FOOTBALL study. *Nutrients*, 10(4):494, 2018.
37. Rampinini E, Bosio A, Ferraresi I, et al. Match-Related Fatigue in Soccer Players. *Med Sci Sports Exerc*, 43(11):2161-2170, 2011.
38. Rampinini E, Impellizzeri FM, Castagna C, et al. Technical performance during soccer matches of the Italian Serie A league: Effect of fatigue and competitive level. *J Sci Med Sport*, 12(1):227-233, 2009.
39. Rampinini E, Impellizzeri FM, Castagna C, et al. Effect of match-related fatigue on short-passing ability in young soccer players. *Med Sci Sports Exerc*, 40(5):934-942, 2008.
40. Russell M, Sparkes W, Northeast J, et al. Responses to a 120 min reserve team soccer match: a case study focusing on the demands of extra time. *J Sports Sci*, 33(20): 2133-2139, 2015.
41. Silva JR, Ascensão A, Marques F, et al. Neuromuscular function, hormonal and redox status and muscle damage of professional soccer players after a high-level competitive match. *Eur J Appl Physiol*, 113(9):2193-2201, 2013.
42. Theodorou AA, Nikolaidis MG, Paschalis V, et al. Comparison between glucose-6-phosphate dehydrogenase-deficient and normal individuals after eccentric exercise. *Med Sci Sports Exerc*, 42(6):1113–1121, 2010.
43. Thomas K, Dent J, Howatson G, et al. Etiology and recovery of neuromuscular fatigue after simulated soccer match play. *Med Sci Sports Exerc*, 49(5):955–964, 2017.
44. Tzatzakis T, Papanikolaou K, Draganidis D, et al. Recovery kinetics after speed-endurance training in male soccer players. *Int J Sports Physiol Perform*, 1–14, 2020.
45. Varley MC, Aughey RJ. Acceleration profiles in elite Australian soccer. *Int J Sports Med*, 34(1):34–39, 2013.
46. Watt MJ, Heigenhauser GJ, Dyck DJ, et al. Intramuscular triacylglycerol, glycogen and acetyl group metabolism during 4 h of moderate exercise in man. *J Physiol*, 541(3):969-978, 2002.

47. Winder N, Russell M, Naughton RJ, et al. The Impact of 120 Minutes of Match-Play on Recovery and Subsequent Match Performance: A Case Report in Professional Soccer Players. *Sports (Basel)*, 6(1):22, 2018

APPENDICES

8. APPENDIX 1

8.1. Match performance

	Match Performance			
	0 - 90min M1	90 – 120 min M1	0 – 90 min M2	90 – 120 min M2
Total distance (m)				
Placebo	10870.3 ± 755.3	3461.3 ± 499.0*	10685.1 ± 748.9‡	3089.3 ± 224.1*,‡
CHO	10993.9 ± 953.8	3296.1 ± 320.1*	10799.1 ± 576.3‡	3261.5 ± 352.1*,‡
High intensity running (m)				
Placebo	203.9 ± 125.9	89.5 ± 65.2*	177.8 ± 77.5‡,#	75.9 ± 36.0*,‡
CHO	213.7 ± 121.2	96.9 ± 46.1*	251.6 ± 97.3‡	87.4 ± 30.3*,‡
Sprinting (m)				
Placebo	114.1 ± 92.2	54.4 ± 58.0	94.9 ± 64.1	42.6 ± 37.1*,‡
CHO	118.0 ± 105.4	57.5 ± 63.0	105.1 ± 73.1	52.7 ± 43.6‡
Average speed (km/h)				
Placebo	7.2 ± 0.5	6.9 ± 1.0	7.1 ± 0.5	6.2 ± 0.4*,‡,#
CHO	7.4 ± 0.6	6.6 ± 0.6*	7.2 ± 0.4‡	6.5 ± 0.7*,‡
Maximal speed (km/h)				
Placebo	32.2 ± 2.6	29.2 ± 2.4*	30.2 ± 2.2*	28.0 ± 2.1*,‡
CHO	32.5 ± 2.2	29.3 ± 1.6*	30.8 ± 2.4*,‡	28.6 ± 2.3*,‡
Accelerations (total number)				
Placebo	98.1 ± 18.7	28.5 ± 11.9*	88.9 ± 17.4‡	21.5 ± 5.4*,‡
CHO	103.1 ± 26.0	28.1 ± 8.6*	96.9 ± 24.3‡	25.7 ± 7.7*,‡
Deccelerations (total number)				
Placebo	95.4 ± 21.7	26.1 ± 10.6*	85.9 ± 16.5‡	19.1 ± 4.7*,‡
CHO	96.9 ± 20.4	27.1 ± 8.1*	90.8 ± 22.1‡	22.3 ± 6.6*,‡
Mean heart rate (beats/min)				
Placebo	168.4 ± 6.4	163.0 ± 7.8	163.7 ± 5.6	152.6 ± 6.3*,‡
CHO	167.1 ± 9.4	162.1 ± 8.1	165.7 ± 8.6	158.5 ± 6.7‡
Sweat loss (mL)				
Placebo	2048.3 ± 655.5	3389.4 ± 748.4*	1988.4 ± 265.9#	3143.3 ± 877.8*,‡
CHO	2019.8 ± 504.7	3162.5 ± 551.8*	2207.1 ±444.5‡,#	2984.3 ± 562.9*,‡
Sweat rate (mL/min)				
Placebo	22.7 ± 7.2	28.2 ± 6.2*	22.1 ± 2.9	26.1 ± 7.3*,#
CHO	22.4 ± 5.6	27.8 ± 8.1*	24.5 ± 4.9	24.9 ± 4.6‡,#
Successful passes (%)				
Placebo	70.5 ± 9.7	64.5 ± 11.2*	72.3 ± 7.4	63.9 ± 8.0*
CHO	71.8 ± 7.0	66.0 ± 8.0*	70.4 ± 8.1	65.8 ± 8.7*
Duels won (%)				
Placebo	47.3 ± 9.5	36.9 ± 10.4*	40.5 ± 6.4‡	27.0 ± 4.8*,‡,#
CHO	45.1 ± 3.7	33.9 ± 3.9*	41.2 ± 3.0	31.7 ± 4.4*,#

***** indicates significant difference from 0-90min M1; **‡** indicates significant difference from previous time-point; **#** indicates significant difference among trials.

8.2. Performance changes

	Performance					
	Baseline-M1	90 min-M1	120 min-M1	Baseline-M2	90 min-M2	12O min-M2
CMJ (cm)						
Placebo	53.4 ± 8.4	44.0 ± 9.7*,#	38.8 ± 12.0*,‡	51.8 ± 8.2*,‡	40.0 ± 7.5*,‡,#	33.9 ± 6.1*,‡,#
CHO	53.9 ± 7.9	43.0 ± 9.6*	38.6 ± 11.4*,‡	51.8 ± 7.3*,‡	43.0 ± 6.7*,‡	37.5 ± 5.4*,‡
RSA – Fatigue index						
Placebo	4.3 ± 0.2	6.0 ± 0.1*	6.5 ± 0.1*,‡	4.5 ± 0.1*,‡	6.2 ± 0.2*,‡,#	7.1 ± 0.2*,‡,#
CHO	4.3 ± 0.1	6.0 ± 0.1*	6.5 ± 0.1*,‡	4.5 ± 0.1*,‡	5.9 ± 0.2*,‡	6.7 ± 0.3*,‡

CMJ: Countermovement jump; RSA: Repeated sprint ability; ***** indicates significant difference from Baseline-M1; **‡** indicates significant difference from previous time-point; **#** indicates significant difference among trials.

8.3. Changes of maximal isometric voluntary contraction

	Maximal Isometric Voluntary Contraction (Nm/kg)			
	Baseline-M1	Post-M1	Baseline-M2	Post-M2
KE - Dominant limb				
Placebo	3.87 ± 0.6	3.53 ± 0.6*	3.64 ± 0.6*,‡,#	3.48 ± 0.6*,‡,#
CHO	3.90 ± 0.6	3.56 ± 0.5*	3.69 ± 0.6*,‡	3.53 ± 0.5*,‡
KE - Non Dominant limb				
Placebo	3.90 ± 0.6	3.55 ± 0.6*	3.68 ± 0.6*,‡	3.52 ± 0.5*,‡,#
CHO	3.91 ± 0.6	3.58 ± 0.6*	3.70 ± 0.6*,‡	3.55 ± 0.6*,‡
KF – Dominant limb				
Placebo	2.63 ± 0.3	2.31 ± 0.3*	2.39 ± 0.3*,‡	2.22 ± 0.3*,‡
CHO	2.63 ± 0.2	2.29 ± 0.2*	2.40 ± 0.3*,‡	2.24 ± 0.2*,‡
KF - Non Dominant limb				
Placebo	2.60 ± 0.2	2.33 ± 0.3*	2.42 ± 0.3*,‡	2.25 ± 0.3*,‡
CHO	2.60 ± 0.2	2.31 ± 0.2*	2.41 ± 0.3*,‡	2.26 ± 0.2*,‡

KE: Knee Extensors; KF: Knee Flexors; Post denotes immediately post exercise; ***** indicates significant difference from Baseline-M1; **‡** indicates significant difference from previous time-point; **#** indicates significant difference among trials.

8.4. Changes in muscle damage markers

	Muscle damage markers					
	Baseline-M1	90 min-M1	120 min-M1	Baseline-M2	90 min-M2	12O min-M2
CK (U/L)						
Placebo	237.3 ± 92.0	635.4 ± 219.0*	983.0 ± 346.30*,‡	501.2 ± 188.7*,‡	1115.8 ± 225.4*,‡,#	1439.5 ± 441.7*,#
CHO	241.0 ± 88.5	600.9 ± 232.5*	919.3 ± 298.3*,‡	485.5 ± 174.9*,‡	1016.2 ± 202.7*,‡	1049.2 ± 424.0*
DOMS						
KE – Dominant limb						
Placebo	1.0 ± 0.0	3.8 ± 1.1*	5.6 ± 1.2*,‡	2.0 ± 0.6*,‡	4.8 ± 0.7*,‡,#	6.1 ± 0.7*,‡,#
CHO	1.0 ± 0.0	3.6 ± 0.7*	5.8 ± 1.0*,‡	1.6 ± 0.5*,‡	4.3 ± 0.6*,‡	5.5 ± 0.6*,‡
KE - Non Dominant limb						
Placebo	1.0 ± 0.0	3.6 ±0.7*	5.2 ± 0.9*,‡	1.9 ± 0.5*,‡,#	4.4 ± 0.5*,‡,#	5.8 ± 0.7*,‡,#
CHO	1.0 ± 0.0	3.4 ± 0.6*	5.3 ± 0.8*,‡	1.4 ± 0.5*,‡	4.0 ± 0.5*,‡	5.3 ± 0.6*,‡
KF – Dominant limb						
Placebo	1.0 ± 0.0	4.8 ± 0.9*	7.6 ± 1.1*,‡	2.9 ± 1.1*,‡	5.8 ± 1.2*,‡	7.1 ± 1.2*,‡,#
CHO	1.0 ± 0.0	4.5 ± 0.7*	7.6 ± 1.1*,‡	2.6 ± 0.9*,‡	5.3 ± 0.6*,‡	6.3 ± 0.7*,‡
KF - Non Dominant limb						
Placebo	1.0 ± 0.0	4.3 ± 0.8*	7.1 ± 1.0*,‡	2.6 ± 0.9*,‡	5.2 ± 0.8*,‡,#	6.4 ± 1.0*,‡
CHO	1.0 ± 0.0	4.1 ± 0.3*	7.1 ± 0.8*,‡	2.3 ± 0.5*,‡	4.6 ± 0.5*,‡	5.7 ± 0.9*,‡

CK: Creatine kinase; KE: Knee Extensors; KF: Knee Flexors; ***** indicates significant difference from Baseline-M1; **‡** indicates significant difference from previous time-point; **#** indicates significant difference among trials.

8.5. Changes in oxidative stress markers

	Oxidative stress					
	Baseline-M1	90 min-M1	120 min-M1	Baseline-M2	90 min-M2	12O min-M2
PC (nmol/ml)						
Placebo	25.2 ± 14.2	57.3 ± 23.1*	86.9 ± 31.6*,‡	32.2 ± 13.8‡	72.6 ± 24.1*,‡	101.7 ± 31.7*,‡
CHO	26.2 ± 15.3	58.0 ± 25.8*	93.6 ± 33.2*,‡	34.5 ± 16.9‡	71.0 ± 36.6*	100.5 ± 34.1*
TBARS (µmol/L)						
Placebo	4.0 ± 1.0	5.0 ± 1.0*	5.7 ± 1.2*,‡	4.5 ± 0.8‡	5.5 ± 0.9*,‡	6.1 ± 1.4*
CHO	4.1 ± 0.9	5.1 ± 0.8*	5.9 ± 1.3*,‡	4.7 ± 0.7‡	5.5 ± 0.9*,‡	6.4 ± 1.3*
GSH (µmol/g Hb)						
Placebo	2.6 ± 0.8	2.0 ± 0.6*	1.5 ± 0.4*,‡	2.4 ± 0.8*,‡	1.9 ± 0.5*,‡	1.3 ± 0.5*,‡
CHO	2.7 ± 0.9	1.9 ± 0.8*	1.4 ± 0.4*,‡	2.4 ± 0.9‡	1.9 ± 0.6*,‡	1.4 ± 0.4*
TAC (mmol DPPH/L)						
Placebo	1.0 ± 0.1	1.1 ± 0.1*	1.2 ± 0.1*,‡	1.0 ± 0.2	1.1 ± 0.1*,‡	1.3 ± 0.2*
CHO	1.0 ± 0.1	1.1 ± 0.2*	1.2 ± 0.2*,‡	1.0 ± 0.1	1.2 ± 0.2*,‡	1.3 ± 0.2*,‡

PC: Protein carbonyls; TBARS: Thiobarbituric acid reactive substances; GSH: Reduced glutathione; TAC: Total antioxidant capacity; ***** indicates significant difference from Baseline-M1; **‡** indicates significant difference from previous time-point; **#** indicates significant difference among trials.

8.6. Changes in blood chemistry

	Blood Counts					
	Baseline-M1	90 min-M1	120 min-M1	Baseline-M2	90 min-M2	12O min-M2
WBC (10^3/uL)						
Placebo	6.2 ± 1.3	12.6 ± 2.7*	14.1 ± 3.3*	5.4 ± 0.8‡,#	10.8 ± 2.2*,‡	13.7 ± 2.6*,‡,#
CHO	5.8 ± 1.2	12.8 ± 3.6*	14.1 ± 3.9*	6.8 ± 2.1‡	10.2 ± 2.2*,‡	11.7 ± 1.7*,‡
Lymphocytes (10^3/uL)						
Placebo	2.4 ± 0.4	2.6 ± 0.8	2.6 ± 0.7	2.1 ± 0.3*	2.8 ± 0.6‡	3.0 ± 0.7
CHO	2.3 ± 0.5	2.5 ± 0.6	2.6 ± 0.6	2.3 ± 0.7	2.5± 0.7	2.8 ± 0.7
Monocytes (10^3/uL)						
Placebo	0.25 ± 0.1	0.48 ± 0.1*	0.57 ± 0.2*	0.27 ± 0.1‡	0.42 ± 0.1*,‡	0.55 ± 0.2*
CHO	0.27 ± 0.1	0.48 ± 0.1*	0.53 ± 0.1*	0.35 ± 0.1‡	0.46 ± 0.1*	0.56± 0.1*,‡
Granulocytes (10^3/uL)						
Placebo	3.6 ± 1.0	9.8 ± 2.5*	11.1 ± 2.8*	3.0 ± 0.6‡,#	7.5 ± 2.1*,‡	10.1 ± 2.4*,‡,#
CHO	3.3 ± 0.9	9.5 ± 3.8*	11.0 ± 3.7*	4.1 ± 1.6‡	7.2 ± 2.2*,‡	8.3 ± 1.6*
Hematocrit (%)						
Placebo	43.8 ± 1.9	45.0 ± 2.0*	44.4 ± 2.1	42.3 ± 1.7*,‡	42.5 ± 1.8	42.2 ± 1.9*
CHO	43.6 ± 1.4	44.9 ± 2.1	44.2 ± 2.1	43.0 ± 1.6	43.0 ± 2.4	42.6 ± 2.0
Haemoglobin (g/dL)						
Placebo	15.3 ± 0.7	15.6 ± 0.8	15.4± 0.9	14.3 ± 0.8*,‡	14.5 ± 0.8*	14.4 ± 0.8*
CHO	14.9 ± 0.7	15.3 ± 0.9	15.0 ± 0.9	14.6 ± 0.5	14.5 ± 0.8	14.4 ± 0.8
Red Blood Cells (10^6/uL)						
Placebo	4.9 ± 0.2	5.1 ± 0.2	5.0 ± 0.2	4.8 ± 0.2	4.8 ± 0.2	4.8 ± 0.2
CHO	4.9 ± 0.2	5.1 ± 0.2	5.0 ± 0.2	4.9 ± 0.2	4.8 ± 0.2	4.8 ± 0.2

WBC: White blood cells; ***** indicates significant difference from Baseline-M1; **‡** indicates significant difference from previous time-point; **#** indicates significant difference among trials.

8.7. Changes in psychometric variables

	Psychometric Variables					
	Baseline-M1	90 min-M1	120 min-M1	Baseline-M2	90 min-M2	12O min-M2
Borg scale						
Placebo	6.0 ± 0.0	15.1 ± 2.5	18.0 ± 1.7*	6.0 ± 0.0	18.0 ± 1.7‡	18.6 ± 1.4#
CHO	6.0 ± 0.0	14.6 ± 1.8	17.8 ± 1.3*	6.0 ± 0.0	17.0 ± 1.5‡	17.4 ± 1.3#
Negative self-talk						
Placebo	0.0 ± 0.0	2.2 ± 0.7	2.8 ± 0.7*	0.0 ± 0.0	2.1 ± 0.6	2.6 ± 0.8*
CHO	0.0 ± 0.0	2.1 ± 0.6	2.7 ± 0.5*	0.0 ± 0.0	2.0 ± 0.6	2.5 ± 0.7*
Ego depletion						
Placebo	0.0 ± 0.0	4.1 ± 1.1	4.9 ± 1.2*	0.0 ± 0.0	4.4 ± 0.9#	5.3 ± 0.8*,‡,#
CHO	0.0 ± 0.0	3.8 ± 0.8	4.7 ± 1.0*	0.0 ± 0.0	3.9 ± 1.0#	4.7 ± 0.9*,#

WBC: White blood cells; ***** indicates significant difference from Baseline-M1; **‡** indicates significant difference from previous time-point; **#** indicates significant difference among trials.