# Extended Match Time Exacerbates Fatigue and Impacts Physiological Responses in Male Soccer Players

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## ABSTRACT

MOHR, M., G. ERMIDIS, A. Z. JAMURTAS, J. F. VIGH-LARSEN, A. POULIOS, D. DRAGANIDIS, K. PAPANIKOLAOU, P. TSIMEAS, D. BATSILAS, G. LOULES, A. BATRAKOULIS, A. SOVATZIDIS, J. L. NIELSEN, T. TZATZAKIS, C. K. DELI, L. NYBO, P. KRUSTRUP, and I. G. FATOUROS. Extended Match Time Exacerbates Fatigue and Impacts Physiological Responses in Male Soccer Players. *Med. Sci. Sports Exerc.*, Vol. 55, No. 1, pp. 80–92, 2023. **Purpose:** This study evaluated how extended match time (90 + 30 min) affected physiological responses and fatigue in male soccer players. **Methods:** Twenty competitive players (mean ± SD: age, 20 ± 1 yr; maximal oxygen uptake, 59 ± 4 mL·min$^{-1}$·kg$^{-1}$) completed an experimental match with their activity pattern and heart rate assessed throughout the game, whereas countermovement jump performance and repeated sprint ability were tested and quadriceps muscle biopsies and venous blood samples were taken at baseline and after 90 and 120 min of match play. **Results:** Less high-intensity running (12%) was performed in extra time in association with fewer intense accelerations and decelerations per minute compared with normal time. Peak sprint speed was 11% lower in extra time compared with normal time, and fatigue also manifested in impaired postmatch repeated sprint ability and countermovement jump performance (all $P < 0.05$). Muscle glycogen declined from 373 ± 59 mmol·kg$^{-1}$ dry weight (dw) at baseline to 266 ± 64 mmol·kg$^{-1}$ dw after 90 min, with a further decline to 186 ± 56 mmol·kg$^{-1}$ dw after extra time ($P < 0.05$) and with single-fiber analyses revealing depleted or very low glycogen levels in ~75% of both slow and fast twitch fibers. Blood glucose did not change during the first 90-min but declined ($P < 0.05$) to 81 ± 8 mg·dL$^{-1}$ after extra time. Plasma glycerol and ammonia peaked at 236 ± 33 mg·dL$^{-1}$ and 75 ± 21 μmol·L$^{-1}$ after the extra period. **Conclusions:** These findings demonstrate exacerbated fatigue after extra time compared with normal time, which seems to be associated with muscle glycogen depletion, reductions in blood glucose levels, and hyperammonemia. Together, this points to metabolic disturbances being a major part of the integrated and multifaceted fatigue response during extended soccer match play. **Key Words:** FOOTBALL OVERTIME, MUSCLE GLYCOGEN, FATIGUE, PERFORMANCE, CENTRAL FATIGUE, HYPERAMMONEMIA

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The intensity and physiological demands in international competitive male soccer have increased markedly over the last 50 years, and this trend is expected to continue in the upcoming decade (1). In addition to intensified matches, more frequent participation in European club tournaments and national team tournaments is contributing to a further acceleration of the overall physiological demands of modern male soccer, as will the proposed expansion of the number of teams in future World Cups (1). In this context, extended match time is an important but often ignored factor, with very little scientific evaluation of its importance for fatigue development and performance (fatigue herein defined as a disabling symptom in which physical and cognitive function is limited by interactions between performance fatigability and perceived fatigability [2]).

At the men's Union of European Football Associations (UEFA) EURO 2020 tournament, more than half of the games in the knockout stages went to extra time (8 of 15), and more than 85% of the medalists in the men's EUROs and World Cups since 1992 have participated in games going to extra time. Thus, extended game scenarios frequently occur and are almost mandatory to go through to final cup stages in modern tournaments. The establishment of a new European club tournament, that is, the UEFA Europa Conference League; the addition of the Nations League for national teams; and the decision to expand the number of teams in future World Cups are likely to increase the occurrence of 120-min soccer games. Furthermore, the recent rule change about abolishing the advantage of away goals in international and national soccer will potentially contribute to this development and further increase the frequency of games going into extra time.

It is well established that fatigue develops during an elite soccer game and peaks in the last 15 min, which compromises high-intensity performance (3). Undeniably, repeated sprint ability (RSA) (4,5), muscle strength (6), and muscle function (7) are markedly impaired after a normal game. Muscle physiological mechanisms leading to muscle fatigue toward the end of a game are likely to be multifactorial but may in part originate from altered muscle metabolism and partially depleted muscle glycogen stores (8). Indeed, it has been reported in male (9) and female (5) players that a substantial fraction of both slow twitch (ST) and fast twitch (FT) fibers are fully or nearly depleted of glycogen after a normal game. Likewise, in both of the aforementioned studies, correlations were found between the drop in muscle glycogen and impairment in post-game repeated sprint performance (5,9). Thus, pronounced muscle glycogen degradation during a soccer game is likely to be associated with end-game fatigue, especially if the depletion of single fibers becomes pronounced and the mixed muscle glycogen content declines to 250 mmol·kg$^{-1}$ dry weight (dw) or less, as this has been associated with impaired muscle function (10). Specifically, it has been demonstrated that glycogen stored in subcellular pools in direct association with the main steps in the excitation–contraction (E–C) coupling (e.g., $Ca^{2+}$ handling, $Na^+$ and $K^+$ regulation, and crossbridge cycling) (10) is affected already as consequence of an ordinary 90-min soccer game (11), and this impairment of muscle excitability and contractility could

be further aggravated if players are exposed to extended match play (10). Finally, degradation of the glycogen stores can reduce the glycolytic flux and increase the reliance on fat oxidation as well as exacerbate metabolic disruptions of the muscle and/or systemic homeostasis (10–12), which may provoke fatigue via peripheral or central mechanisms. Extended match play in soccer implies that players will be exposed to a total of 2-h intense intermittent exercise, which we hypothesized would challenge depletion of single fibers and mixed muscle glycogen stores. Specifically, that in comparison to an ordinary 90-min soccer game, more players would reach glycogen levels less than 250 mmol·kg$^{-1}$ dw that has been proposed as critical for maintained muscle function and performance (10). For the first time with parallel measures of performance and physiological measures of muscle metabolism, we wanted to explore how extended match time affected fatigue with special focus on its proposed association with glycogen depletion.

A few studies have examined the physiological effects of extra time in soccer. For example, Field et al. (13) reported decreased mechanical efficiency and increased fat oxidation during extra time using a simulated soccer protocol. Others, using simulated models and both peripheral nerve and transcranial magnetic stimulations, showed an exacerbated neuromuscular fatigue response to extra "playing" time (14). Moreover, a systematic review based on 11 studies reported decreased technical and physical performance after simulated 120-min soccer games (15), although the authors also concluded that there is currently limited knowledge on the impact of extra time on muscle metabolism and physiological responses. In fact, to date, there are no studies of extra time using real soccer matches. Therefore, the limited information that exist so far regarding the physiology and metabolism of overtime comes from simulated soccer conditions and not actual soccer match play.

Along these lines, the purpose of this study was to investigate the physiological, biochemical, and performance responses during a 120-min soccer game in well-trained competitive male players with the main emphasis on the impact of extra time in relation to muscle glycogen depletion. We hypothesized that the additional 30 min of playing time would markedly exacerbate muscle physiological fatigue responses, in part because of exacerbated muscle glycogen depletion below the proposed threshold of 250 mmol·kg$^{-1}$ dw and in both main muscle fiber types after an extra-time period.

## METHODS

**Experimental design.** This study is part of the 120-min project funded by the UEFA to investigate the physiological, performance, recovery, and psychological effects of extra time in competitive soccer using a randomized, repeated-measures, crossover, double-blind design. The research design of the 120-min project is shown in Figure 1. In this study, we report the physiological, metabolic, and performance effects of a 30-min extra-time period based on the analysis of the pooled data obtained from the first two games of each trial ($n = 40$ observations, i.e., two trials with 20 observations (players) per trial), because the diet intervention
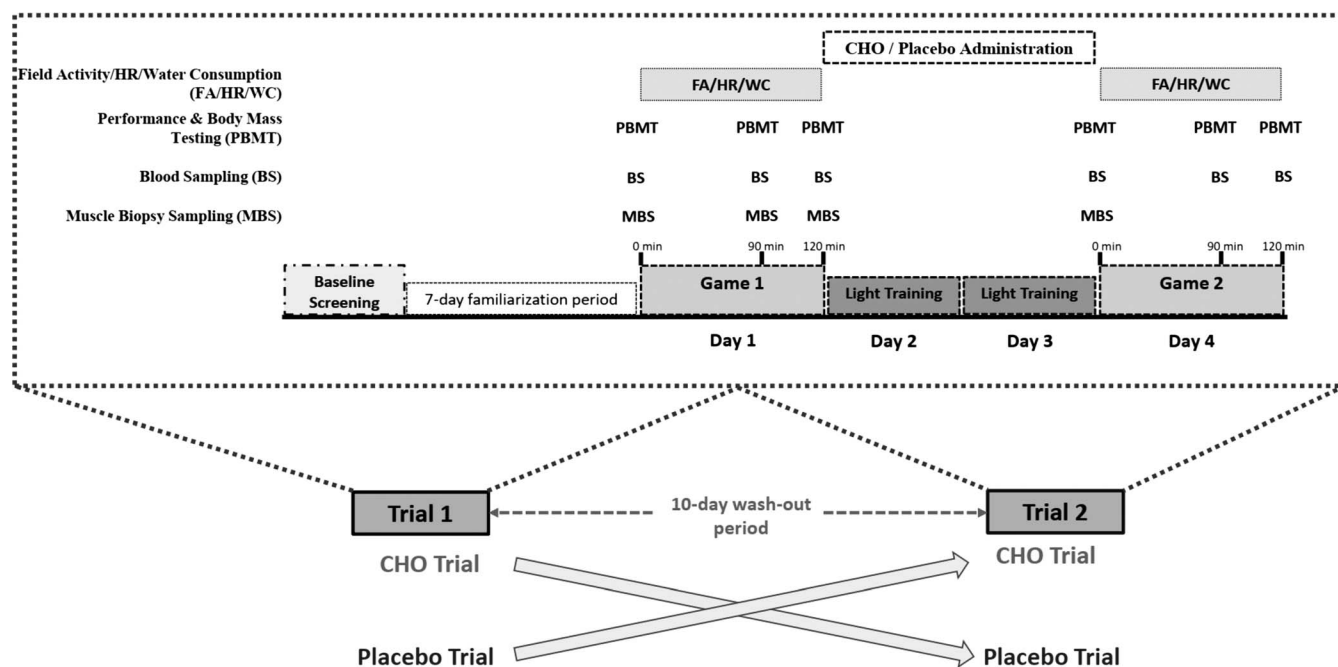
FIGURE 1—Experimental design of the study.

was not initiated at this point (supplementation took place after game 1), thereby allowing us to study only the net effect of extra time. Therefore, the first two games of the placebo and carbohydrate trial were performed under identical conditions (data following the subsequent diet interventions will be presented in a companion paper). This project was preregistered at ClinicalTrials. gov (ID: NCT04159194). Participants ($n = 20$) underwent a thorough baseline screening including assessment of body composition, technical skills and physical performance, resting metabolic rate, daily dietary intake, and physical activity level, followed by a familiarization period during which they were accustomed to the experimental procedures and participated in light training to develop team cohesion over a 7-d period. Subsequently, they completed two trials (placebo vs increased carbohydrate intake during the postgame recovery period) separated by a 10-d wash-out period. Each trial included two 120-min official games (two teams played against each other) performed 3 d apart according to UEFA official schedules for national team tournaments. Field activity (using global positioning system instrumentation), heart rate, technical performance, and water consumption were monitored continuously during matches, as previously done in 90-min games (7). Body mass measurements (to estimate sweat loss), performance testing, and blood sampling were performed before each game, at baseline, and after 90 and 120 min of play according to procedures used multiple times by our research group (4–9). Muscle biopsies were collected before games 1 and 2 of each trial and at 90 and 120 min of game 1 of each trial. There were multiple biopsy stations and performance testing stations to be able to perform the biopsies and testing as fast as possible. Players were taken out of the game in groups of five to approximately comply with the 5-min interval time allowed in official games after the game and before the

initiation of extra time. Players were taken out of the game in the interval 87–94 and 117–124 min after the 90 and 120 min, respectively. During the testing, they were replaced by substitute players and then entered the game immediately after testing. The rationale for applying broad-spectrum testing enables us to link game performance, fatigue development, and both physiological alterations at both systemic and muscular levels. Participants were asked to follow a standardized meal plan during the entire experimental period.

**Participants.** A total of $n = 27$ competitive male soccer players (representing all outfield positions) were initially approached and underwent medical screening. The participants provided written consent to participate after they were fully informed about the experimental procedures and potential risks/discomforts associated with the study. The experimental procedures were in accordance with the World Medical Association Code of Ethics (Helsinki Declaration, as revised in 2013), and approval was obtained from the Institutional Ethics Committee (protocol number: 1462). Participation in the study was secured if participants (i) had played at a competitive level (top three divisions) for ≥4 yr, (ii) had practiced at least five times a week and participated in at least 1 game a week in the last five seasons, (iii) were free of recent injuries and/or illnesses (≥8 months before the study), (iv) were not consuming ergogenic supplements/medications (≥8 months before the study), and (v) were not smokers. From the initial pool of 27 players, there were 25 outfield players that met the inclusion criteria, whereas 2 players declined to participate in the study. Subsequently, 20 players were randomly selected and participated in the study (8 defenders, 6 midfielders and 6 attackers), whereas the remaining 5 players (not selected during randomization) were appointed as substitutes for the main participants (in the event of injury). The participants' physical

TABLE 1. Participants' characteristics at baseline ($n$ = 20).

| Variable | Mean ± SD |
|---|---|
| Age (yr) | 20.1 ± 1.4 |
| Body height (m) | 1.76 ± 0.06 |
| Body mass (kg) | 69.6 ± 4.7 |
| BMI (kg·m$^{-2}$) | 22.4 ± 0.9 |
| Fat (%) | 14.9 ± 5.1 |
| $\dot{V}O_{2max}$ (mL·kg$^{-1}$·min$^{-1}$) | 59.4 ± 3.7 |
| Maximum treadmill speed (km·h$^{-1}$) | 19.3 ± 1.3 |
| HRmax (bpm) | 200.1 ± 3.7 |
| Resting HR (bpm) | 61.4 ± 4.8 |
| Lactate threshold (%) | 79.5 ± 3.2 |
| Yo-Yo IE2 | |
|    Speed level | 18.7 ± 0.7 |
|    Distance (m) | 3144 ± 350 |
|    HRmax (bpm) | 196.0 ± 3.0 |
| Yo-Yo IR2 | |
|    Speed level | 23.2 ± 0.5 |
|    Distance (m) | 1517 ± 168 |
|    HRmax (bpm) | 196.6 ± 3.6 |
| Skill level | |
|    Creative speed test (s) | 16.3 ± 0.7 |
|    Short dribbling test (s) | 12.2 ± 0.6 |

BMI, body mass index; HR, heart rate; Yo-Yo IE2, Yo-Yo intermittent endurance test level 2; Yo-Yo IR2, Yo-Yo intermittent recovery test level 2.

characteristics are shown in Table 1. Thirty observations were included in the analysis (only cases without any missing measurement time points were selected from the 40 observations that were examined in total in this study). Figure 2 provides the CONSORT diagram of the study. According to a power analysis (effect size of 0.3, power of 0.85, $\alpha$ probability error of 0.05), a sample size of 11 participants is required for a within-between factor, repeated-measures ANOVA with two trials and three measurement time points (per trial).

**Baseline measurements.** Body mass and height were measured on a beam balance with a stadiometer (Beam Balance Stadiometer, SECA; Vogel & Halke, Hamburg, Germany), as previously described, and body mass index was calculated (16). Body composition was assessed using dual-emission x-ray absorptiometry (GE Healthcare, Lunar DPX-NT), as previously described (17). Open-circuit spirometry was utilized for maximal oxygen uptake ($\dot{V}O_{2max}$) assessments using an automated online pulmonary gas-exchange system (Vmax Encore 29, BEBJO296; CareFusion, San Diego, CA) via breath-by-breath analysis during a graded exercise test on a treadmill (Stex 8025 T, Hwaseong, Korea), as described elsewhere (7). Soccer-specific conditioning was tested using the Yo-Yo intermittent endurance test level 2 and the Yo-Yo intermittent recovery test level 2 following standard procedures previously described (18). The Yo-Yo and $\dot{V}O_{2max}$ tests were performed on separate days. The participants' level of technical skill performance was evaluated using the creative speed and short dribbling tests, as described before (18).

**Performance measurements.** Countermovement jump (CMJ) height was measured on an Ergojump contact platform (Newtest Oy, Oulu, Finland) (16). The average of the three trials was calculated and used as the test result. To measure RSA, the participants performed five consecutive 30-m sprints separated by 25 s of active recovery during which they jogged back to the starting line (5). Sprint times were measured with infrared photocells with a precision of 0.01 s (Newtest Oy). Peak isometric force was determined at baseline and after 120 min in knee extensors and flexors in the dominant and nondominant legs, as previously described (6). The CMJ and RSA tests were performed after biopsy sampling. The CMJ was always performed before the RSA test.

**Measurement of field activity pattern and hydration status.** Field locomotor activity during match play was monitored using a high-resolution global positioning system (10 Hz, Viper Units; STATSports, Newry, Ireland) (19). Field activity was classified as total distance covered, mean speed, high-speed running (distance covered at speeds >21 km·h$^{-1}$), sprinting (distance covered at speeds >24 km·h$^{-1}$), number of high accelerations (>2 m·s$^{-1}$), and number of high decelerations (−2 m·s$^{-1}$) (7). Intensity during match play was monitored using continuous heart rate measurements (Team Polar Pro system; Polar, Kempele, Finland). Individual skill-related performance variables (rates of successful passes and duels won) were examined by experienced soccer analysts. To determine sweat loss during the 120-min match, players were weighed wearing dry shorts before each match and at 90 and 120 min using a beam balance with a stadiometer as previously described (16). The water intake of each participant was recorded throughout the match.

**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 to measure glucose, glycerol, and ammonia. All samples were stored at −80°C in multiple aliquots until assayed. A small portion (2 mL) of whole blood was collected into tubes containing ethylenediaminetetraacetic acid to assess white blood cells (WBCs), red blood cells (RBC), lymphocytes, monocytes, granulocytes, hemoglobin, 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 autooxidation. All assays were performed in duplicate, and samples collected after a match were corrected for plasma volume changes as described (20). Blood lactate concentration was measured using a handheld portable analyzer (Lactate Plus; Nova Biomedical, Waltham, MA) as described (21). Plasma glucose concentration was determined using the Clinical Chemistry Analyzer Z1145 (Zafiropoulos Diagnostica S.A., Koropi, Greece) and a commercially available kit (Zafiropoulos Diagnostica S.A.). Glycerol concentration was determined by a commercially available kit (Glycerol Assay Kit, MAK117; Sigma-Aldrich®, St. Louis, IL) using a coupled enzyme assay involving glycerol kinase and glycerol phosphate oxidase, resulting in a colorimetric/fluorometric product proportional to the glycerol present. Plasma ammonia ($NH_3$) concentration was quantitatively determined on a Cobas Integra 400 plus (Roche Diagnostics, Rotkreuz, Switzerland) analyzer using a commercially available kit ($NH_3L$, 0–168, Cobas® substrates) in accordance with the manufacturer's instructions. Interassay and intra-assay coefficients of variation for the aforementioned variables ranged from 2.2% to 7.1% and from 3.2% to 7.8%, respectively.

## CONSORT 2010 Flow Diagram



**Enrolment**

Assessed for eligibility (n=27)

Excluded (n=2)
• Declined to participate (n=2)

Included (n=25)

**Allocation**

Allocated to placebo trial (n=25)
• Received allocated intervention (n=20)
• Did not receive allocated intervention (n=5*)
  ○ were not selected during randomization (n=5)

Allocated to CHO trial (n=25)
• Received allocated intervention (n=20)
• Did not receive allocated intervention (n=5*)
  ○ were not selected during randomization (n=5)

**Follow-up**

Lost to follow-up (n=5)
Discontinued intervention (n=5)
  ○ Injured (n=2)
  ○ Incomplete assessment (n=1)
  ○ Unwilling to continue (n=2)

Lost to follow-up (n=5)
Discontinued intervention (n=5)
  ○ Injured (n=2)
  ○ Incomplete assessment (n=1)
  ○ Unwilling to continue (n=2)

**Analysis**

Analysed (n=15)
  ○ Excluded from analysis (n=0)

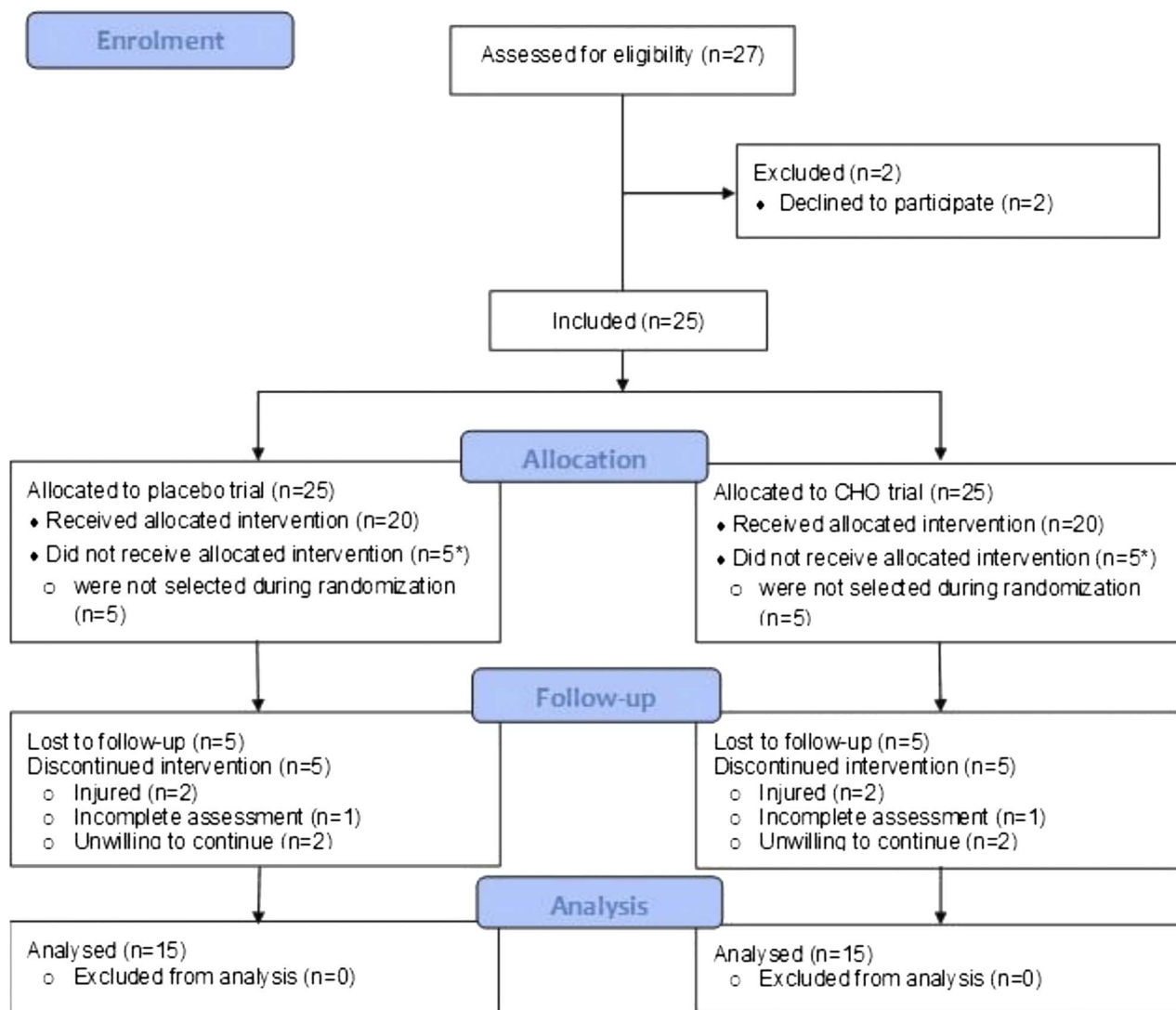Analysed (n=15)
  ○ Excluded from analysis (n=0)

FIGURE 2—CONSORT flow diagram of the study.

**Muscle biopsy sampling and assays.** Muscle biopsies (~70–100 mg wet weight) were obtained from *musculus vastus lateralis* in the dominant leg using the needle biopsy technique with suction, with the participants in supine position. Before the game, two incisions were made in the medial part of the muscle, as previously described (5,9), to be able to take the 90- and 120-min biopsies fast (within 30–40 s). The baseline biopsy was obtained from the more distal incision, and the 90- and 120-min biopsies were collected from the more proximal incision. 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. After extraction with $HClO_4$, neutralized extracts were analyzed for lactate, as previously described (22). Muscle glycogen content

was determined spectrophotometrically (Beckman DU 650), as previously described (23). Freeze-dried muscle tissue (1.5 mg) was boiled in HCl (1 M, 0.5 mL) for 150 min before it was quickly cooled, whirl-mixed, and centrifuged (3500$g$, 10 min, 4°C). Boiled muscle sample (40 µL) and reagent solution (1 mL) containing Tris buffer (1 M), distilled water, ATP (100 mM), $MgCl_2$ (1 M), NADP+ (100 mM), and G-6-PDH were mixed before the process was initiated by adding diluted hexokinase (10 µL). Absorbance was recorded for 60 min before the glycogen content was calculated. Muscle glycogen was expressed as millimoles per kilogram of dry weight. Analysis of glycogen content was performed on cross sections (8 µm) cut from tissue-tec embedded muscle samples. A section was stained with periodic acid–Schiff stain and myosin heavy chain fast for visualization of glycogen content and FT myofibers. Individual myofiber relative glycogen content was based on the average glycogen density of the 25 most full (100%) and empty (0%) ST and FT myofibers, from which the fiber-type–specific relative glycogen content of individual myofibers was calculated (9).

**Statistical analysis.** Data normality was verified using the Shapiro–Wilk test. Because a vast majority of our data sets were normally distributed, we applied parametric statistics. A repeated-measures ANOVA was utilized to detect different time-point changes, accompanied by a Bonferonni *post hoc* analysis test when a significant main effect for time was observed. For statistically meaningful differences, effect sizes (ES) and confidence intervals were calculated according to the corrected-for-bias Hedges' *g* method. ES was considered none, small, medium, and large for values 0.00–0.19, 0.20–0.49, 0.50–0.79, and ≥0.8, respectively. The between-day (baseline data of the first games in trials 1 and 2) intraindividual coefficients of variation for the main outcome variables (muscle glycogen, muscle lactate, blood lactate, plasma ammonia, plasma glycerol, blood glucose, RSA fatigue index, and CMJ performance) have been calculated (using the formula (SD/mean) × 100) and incorporated into the Results section. The level of statistical significance was set at $P < 0.05$. Data are presented as means ± SD. Analysis was performed using the SPSS 20.0 software (IBM SPSS Statistics, Armonk, NY).

# RESULTS

**Match locomotor activity, heart rate, and sweat loss.** During the 30-min extra-time period, total distance covered, sprinting, and high-intensity running expressed as distance per minute were 9.7% ($P < 0.01$; ES = 1.15 (0.60 to 1.70)), 0.8% ($P < 0.01$; ES = 0.01 (−0.50 to 0.51)), and 11.6% ($P < 0.01$; ES = 0.19 (−0.31 to 0.70)), respectively, lower compared with the 90-min normal-time period (Table 2). Also, average locomotion speed and maximal running speed were 10.9% ($P < 0.01$; ES = 1.06 (0.52 to 1.60)) and 10.1% ($P < 0.01$; ES = 1.51 (0.94 to 2.09)), respectively, lower in extra time compared with normal time (Table 2). Intense accelerations and decelerations per minute were 18.3% ($P < 0.01$; ES = 0.69 (0.17 to 1.21)) and 16.8% ($P < 0.01$; ES = 0.70 (0.18 to 1.22)), respectively, lower in extra time than in normal time (Table 2).

TABLE 2. Match performance, HR, and sweat rate during the 90-min match (0–90 min) and extra time (90–120 min).

| | 0–90 min | 90–120 min | % Change |
|---|---|---|---|
| **Match performance** | | | |
| Total distance (m·min⁻¹) | 121.5 ± 9.4 | 109.7 ± 10.8* | −9.7 |
| Sprinting (m·min⁻¹) | 1.29 ± 1.08 | 1.28 ± 1.19 | −0.8 |
| HIR (m·min⁻¹) | 2.32 ± 1.35 | 2.05 ± 1.4 | −11.6 |
| Average speed (km·h⁻¹) | 7.3 ± 0.6 | 6.6 ± 0.7* | −10.9 |
| Maximal speed (km·h⁻¹) | 32.4 ± 2.3 | 29.1 ± 2.0* | −10.1 |
| ACC/min | 1.15 ± 0.3 | 0.94 ± 0.3* | −18.3 |
| DEC/min | 1.07 ± 0.2 | 0.89 ± 0.3* | −16.8 |
| Successful passes (%) | 71.4 ± 7.4 | 65.8 ± 8.4* | −5.3 |
| Duels won (%) | 45.4 ± 4.8 | 34.7 ± 5.4* | −10.7 |
| **HR** | | | |
| Mean HR (bpm) | 168.8 ± 8.6 | 162.7 ± 7.7* | −3.6 |
| **Sweat rate** | | | |
| Sweat rate (mL·min⁻¹) | 22.7 ± 6.4 | 28.6 ± 5.3* | +25.9 |

ACC, accelerations (>2 m·s⁻²); DEC, decelerations (>2 m·s⁻²); HIR, high-intensity running; HR, heart rate.
*Significant difference with 0–90 min ($P < 0.05$).

To compare the last part of normal time with extra time, the last 15-min period of normal time (75–90 min) was tested against the two 15-min periods of extra time (90–105 and 105–120 min). Total distance covered, high-intensity running, and sprinting distance were 5%–12%, 19%–36%, and 27%–47% lower (all $P < 0.05$) in the two 15-min periods of extra time compared with the last 15-min period of normal time. Moreover, peak sprinting speed declined ($P < 0.05$) from 31.1 ± 1.6 km·h⁻¹ in the 75- to 90-min period to 30.1 ± 2.0 and 28.1 ± 2.1 km·h⁻¹ in the two 15-min periods of extra time. Finally, the frequency of intense accelerations and decelerations was lower ($P < 0.05$) in extra time than at the end of normal time (data not shown). Successful passes and duels won also declined by 5.3% ($P < 0.01$; ES = 0.70 (0.18 to 1.22)) and 10.7% ($P < 0.01$; ES = 2.07 (1.44 to 2.69)), respectively, during extra time (Table 2). Average heart rate was 169 ± 9 bpm during the first 90 min but declined to 163 ± 8 bpm during extra time ($P < 0.01$; ES = 0.74 (0.21 to 1.26); Table 2). The total sweat loss rate rose by 67.8% ($P < 0.01$; ES = 2.24 (−2.89 to −1.59)) during extra time compared with the previous 90 min, resulting in a 25.9% higher ($P < 0.01$; ES = 0.99 (−1.53 to −0.45)) sweat rate in extra time compared with normal time (Table 2).

**Sprint ability, jump performance, and muscle function.** The between-day intracoefficients of variation for RSA fatigue index and CMJ were 0.4% and 1.4%, respectively. The RSA fatigue index was increased by 1.7% at the end of the 90-min game compared with baseline ($P < 0.01$; ES = 16.8 (−19.8 to −13.7)) and had risen a further 6.6% by the end of extra time ($P < 0.01$; ES = 4.94 (−5.95 to −3.92); Fig. 3A). Likewise, CMJ performance declined by 19% and 27% at 90 min ($P < 0.01$; ES = 1.15 (0.60 to 1.69)) and 120 min ($P < 0.01$; ES = 1.49 (0.92 to 2.07)), respectively, compared with baseline, with CMJ performance at 120 min 11% lower ($P < 0.01$; ES = 0.45 (−0.06 to 0.96)) compared with 90 min (Fig. 3B). Peak isometric force, as a marker of muscle function, declined ($P < 0.05$) from baseline to 120 min. Knee extensor strength decreased from 3.9 ± 0.6 and 3.9 ± 0.6 to

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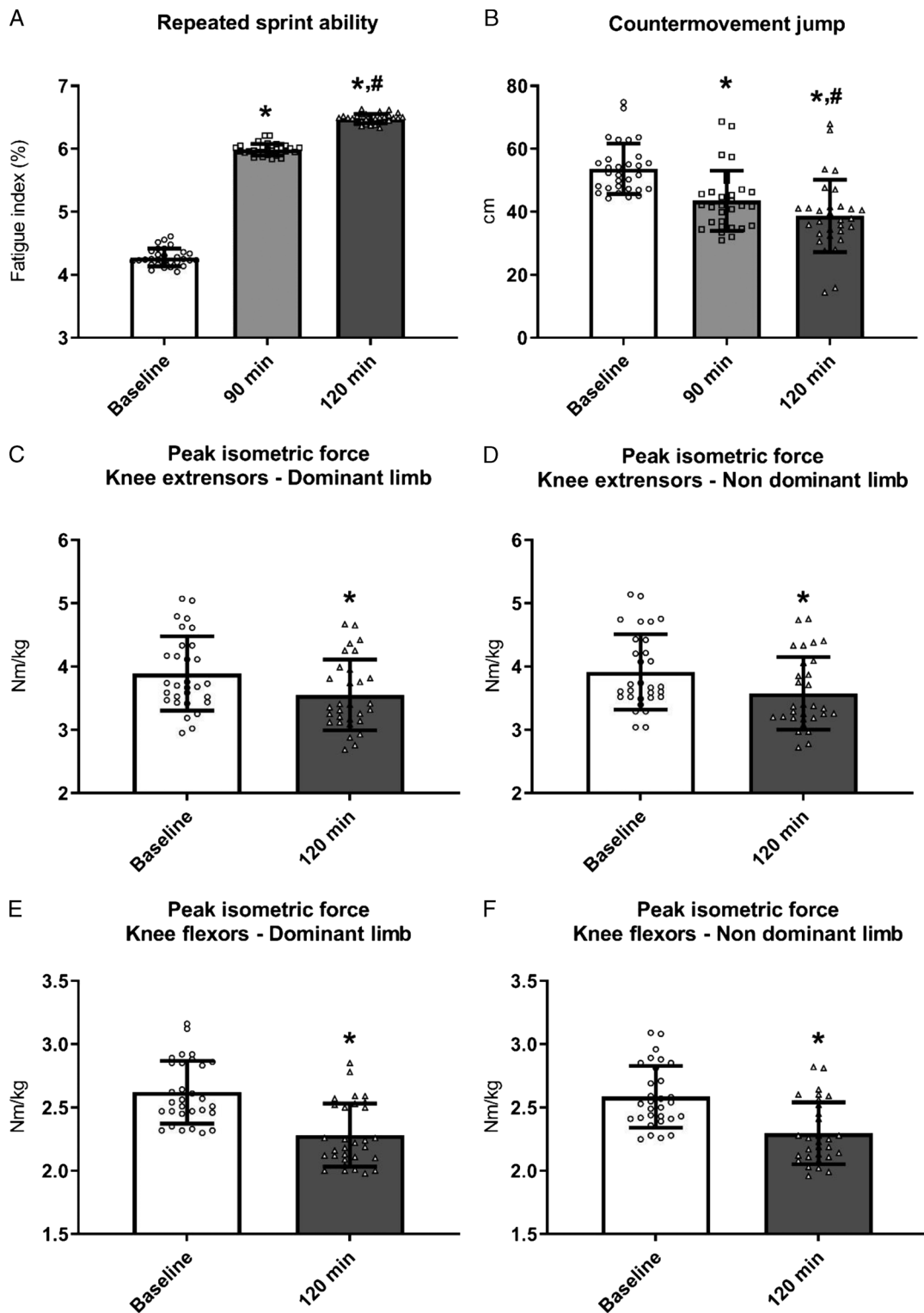
**FIGURE 3—RSA expressed as a fatigue index (A), CMJ performance (B), peak isometric force development in the dominate (C) and non-dominate (D) knee extensors, as well as dominate (E) and non-dominate (F) knee flexors during the 120-min game protocol ($n = 30$). * indicates significant difference to baseline; # indicates significant difference compared with 90 min.**

$3.6 \pm 0.6$ and $3.6 \pm 0.6$ $(N \cdot m) \cdot kg^{-1}$ in the dominant and non-dominant legs, respectively (dominant: $P < 0.05$, ES = 0.55 ($-0.18$ to $1.28$); nondominant: $P < 0.05$, ES = 0.54 ($-0.19$ to $1.26$); Figs. 3C, D). Knee flexor strength declined from $2.6 \pm 0.3$ and $2.6 \pm 0.2$ to $2.3 \pm 0.3$ and $2.3 \pm 0.2$ $(N \cdot m) \cdot kg^{-1}$

in the dominant and nondominant legs, respectively (dominant: $P < 0.05$, ES = 1.65 ($0.82$ to $2.48$), nondominant: $P < 0.05$, ES = 1.36 ($0.57$ to $2.16$); Figs. 3E, F).

**Blood metabolite response.** The between-day intracoefficients of variation for blood glucose, plasma glycerol,

ammonia, and blood lactate were 3.6%, 8.2%, 7.3%, and 14.1%, respectively. Blood glucose was unchanged at 90 min compared with baseline, but after the 30-min extra-time period, there was a 13% reduction compared with baseline ($P < 0.01$; ES = 1.41 (0.85 to 1.98)) and 90 min ($P < 0.01$; ES = 1.40 (0.84 to 1.96); Fig. 4A). Plasma glycerol doubled from baseline to 90 min ($P < 0.01$; ES = 3.20 (−3.96 to −2.43)), and at 120 min, it had further increased by 137% ($P < 0.01$; ES = 5.26 (−6.33 to −4.19)) and 22% ($P < 0.01$; ES = 1.18 (−1.73 to −0.63)) compared with baseline and 90 min, respectively (Fig. 4B). Likewise, plasma ammonia was increased both at 90 min (+53%, $P < 0.01$; ES = 3.36 (−4.14 to −2.57)) and 120 min (+191%,
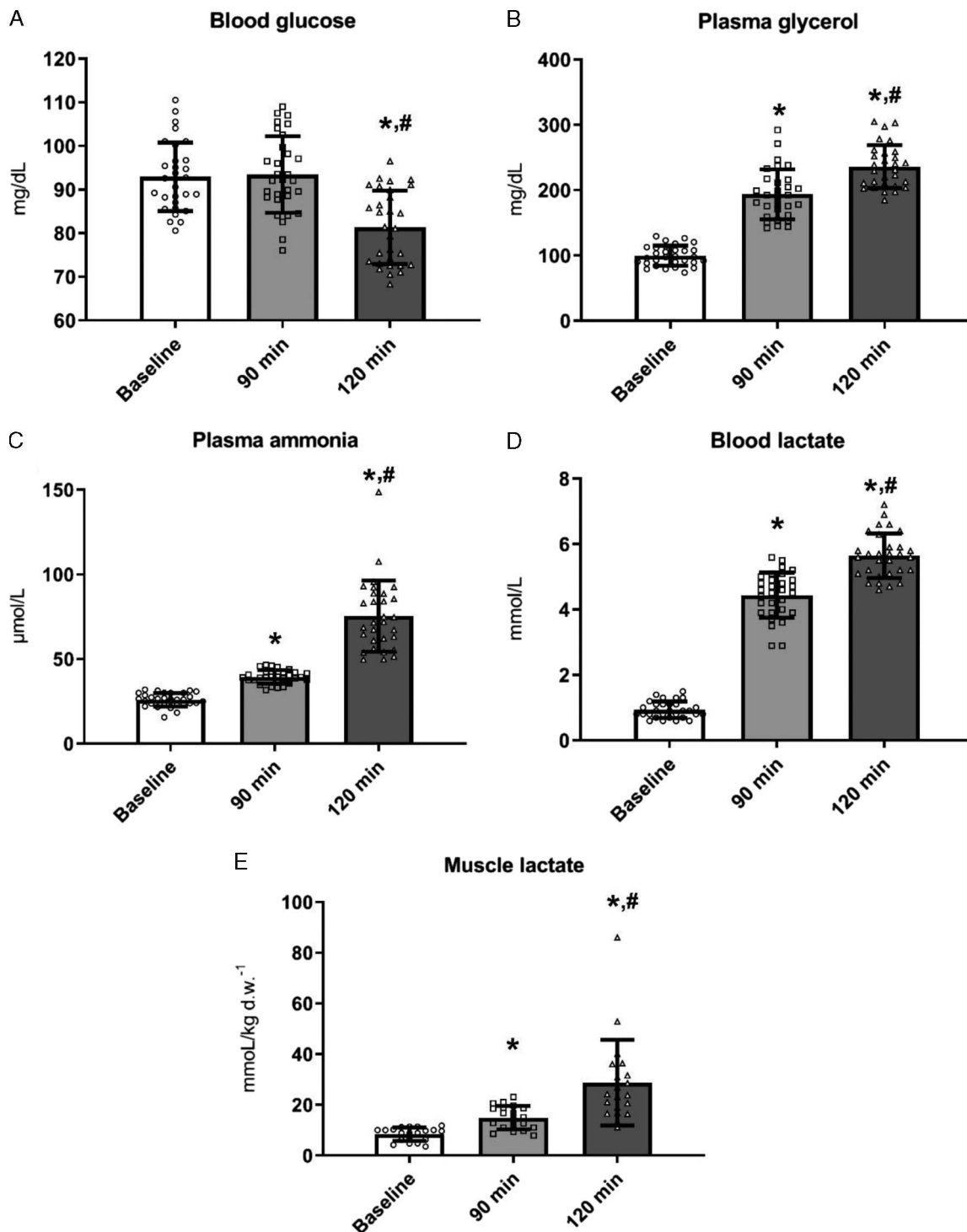


FIGURE 4—Blood glucose ($n = 30$; A), plasma glycerol ($n = 30$; B), plasma ammonia ($n = 30$; C), blood lactate ($n = 30$; D), and muscle lactate ($n = 25$; E) concentrations at baseline, after normal time (90 min), and after extra time (120 min). * indicates significant difference compared with baseline; # indicates significant difference compared with 90 min.

$P < 0.01$; ES = 3.23 (−3.99 to −2.46)), with a 90% higher concentration at 120 min compared with 90 min ($P < 0.01$; ES = 2.34 (−2.99 to −1.68); Fig. 4C). Blood lactate increased ~5-fold at 90 min ($P < 0.01$; ES = 6.41 (−7.67 to −5.16)) and ~6-fold at 120 min ($P < 0.01$; ES = 8.61 (−10.24 to −6.99)), with a significant effect of extra time compared with normal time (90 min: 4.4 ± 0.7 vs 120 min: 5.6 ± 0.7 mmol·L$^{-1}$; $P < 0.01$; ES = 1.69 (−2.28 to −1.10); Fig. 4D).

**Muscle metabolic responses.** The between-day intra-coefficients of variation for muscle lactate and glycogen were 19.5% and 11.1%, respectively. Muscle lactate increased by almost twofold and fourfold at 90 min ($P < 0.01$; ES = 1.78 (−2.44 to −1.13)) and 120 min ($P < 0.01$; ES = 1.66 (−2.30 to −1.01)), respectively, compared with baseline, with extra time inducing a further rise of 103% compared with normal time ($P < 0.01$; ES = 1.13 (−1.73 to −0.53); Fig. 4E). Muscle glycogen declined by 29% at 90 min ($P < 0.01$; ES = 1.70 (0.96 to 2.44)) and by 50% at 120 min ($P < 0.01$; ES = 3.20 (2.24 to 4.15)) compared with baseline (Fig. 5A). Extra time induced a further decline of 30% compared with 90 min (90 min: 266.1 ± 64.0 mmol·kg$^{-1}$ dw vs 120 min: 185.6 ± 55.7 mmol·kg$^{-1}$ dw; $P < 0.01$; ES = 1.31 (0.61 to 2.01)), with several individual values falling below the proposed critical level of 250 mmol·kg$^{-1}$ dw (Fig. 5A). The muscle glycogen concentrations at 120 min correlated inversely ($r = -0.56$) to the fatigue index during the RSA test after the extra-time interval. The glycogen degradation rate almost doubled during extra time compared with the first 90 min ($P < 0.05$; ES = 0.83 (−1.49 to 0.17)) of the game (Fig. 5B). The analyses of the fiber-type–specific glycogen depletion pattern revealed that 69% and 66% of ST and FT fibers, respectively, were completely or partly full of glycogen at baseline (Fig. 5C). At 90 min, this had dropped to 29% for ST ($P < 0.01$; ES = 2.10 (1.06 to 3.14)) and 37% for FT fibers ($P = 0.05$; ES = 1.29 (0.37 to 2.21)), and at 120 min, it had further declined to 18% ($P < 0.01$; ES = 2.82 (1.64 to 4.01)) and 22% ($P < 0.05$; ES = 1.89 (0.88 to 2.89)) for ST and FT, respectively (Fig. 5C), with a significant effect of extra time compared with normal time (ST: $P < 0.05$, ES = 0.96 (0.08 to 1.84); FT: $P < 0.05$, ES = 0.75 (−0.11 to 1.62)). Accordingly, the percentage of ST and FT fibers that were completely or almost empty increased progressively after 90 min (ST: $P < 0.01$, ES = 2.10 (−3.14 to −1.06) vs FT: $P = 0.05$, ES = 1.29 (−2.21 to −0.37)) and 120 min (ST: $P < 0.01$, ES = 2.82 (−4.01 to −1.64) vs FT: $P < 0.05$, ES = 1.89 (−2.90 to −0.89)) compared with baseline values, with a significant effect of extra time compared with normal time (ST: $P < 0.05$, ES = 0.96 (−1.84 to −0.08); FT: $P < 0.05$, ES = 0.76 (−1.62 to 0.11)). Specifically, 72% of ST and 64% of FT fibers after 90 min and 82% of ST and 78% of FT fibers after 120 min were completely or almost empty of glycogen compared with baseline (31% for ST and 35% for FT; Fig. 5C).

**Hematological responses.** Hematocrit increased from baseline both at 90 min ($P < 0.01$; ES = 0.65 (−1.17 to −0.13)) and at 120 min ($P < 0.01$; ES = 0.71 (−1.23 to −0.19)), without any effect of extra time, whereas hemoglobin concentration remained unaltered throughout the 120-min game (Table 3). RBC count increased at 90 min ($P < 0.01$; ES = 0.99 (−1.52 to −0.45)) and subsequently declined ($P < 0.01$; ES = 0.49 (−0.02 to 1.01); Table 3). WBC, monocyte, and granulocyte counts had increased at 90 min ($P < 0.01$; WBC: ES = 2.81 (−3.53 to −2.10); MON: ES = −2.36 (−3.01 to −1.70); GRAN: ES = 2.66 (−3.36 to −1.97)) and 120 min ($P < 0.01$; WBC: ES = 3.06 (−3.80 to −2.31); MON: ES = 2.32 (−2.97 to −1.66); GRAN: ES = 3.08 (−3.82 to −2.33)) compared with baseline, without any effect of extra time (Table 3). No changes were noted for lymphocytes (Table 3).

## DISCUSSION

The present study is the first to investigate how extended match time affects muscle metabolic and physiological responses of importance for performance and fatigue development in well-trained competitive male soccer players. Our results demonstrate that the extended game time exacerbated glycogen depletion of single fibers, elevated fat breakdown, lowered blood glucose, and induced hyperammonemia. These metabolic disturbances were associated with considerable deterioration of performance assessed as game distances covered, frequency of intense actions, and impaired postmatch muscle function, sprint ability, and jump performance.

We found that physical game performance was markedly lower during the extra 30 min compared with the first 90 min. Indeed, both total ground covered and high-intensity running expressed as distance per minute were 10%–12% lower in the extra-time period. Also, sprint distance and maximal running speed, as well as acceleration and deceleration counts, declined. Finally, all of these physical performance markers were lower in extra time compared with the end of normal time. Thus, tracking parameters representing endurance, high-intensity performance, and ballistic/explosive movements in competitive soccer (7,12,24) seemed to be impaired during extra time compared with normal time. This is supported by lower average locomotion speed and heart rates, also indicating that the general game intensity was compromised either by fatigue and/or by a changed pacing strategy. Decrement in performance toward the end of a normal soccer game is a consistent finding in tracking research (25) and has been suggested to be caused by physiologically mediated fatigue (26). A recent systematic review confirmed our findings of decreased work rate in the extra-time period versus normal time (15) and reported a decrement in the same magnitude range (5%–12%) as our study.

In support of our tracking data, a greater fatigue response was also observed after extra time compared with normal time in both RSA and CMJ performance (Fig. 3). Several studies have demonstrated that RSA is impaired after a 90-min game (4,5,7,9,27), whereas some (28–30), but not all (31,32), report an effect on CMJ performance. Moreover, it was observed that peak isometric force had also deteriorated by approximately 10% both in knee extensors and flexors after extra time, which previously has been shown after normal games (6). Our

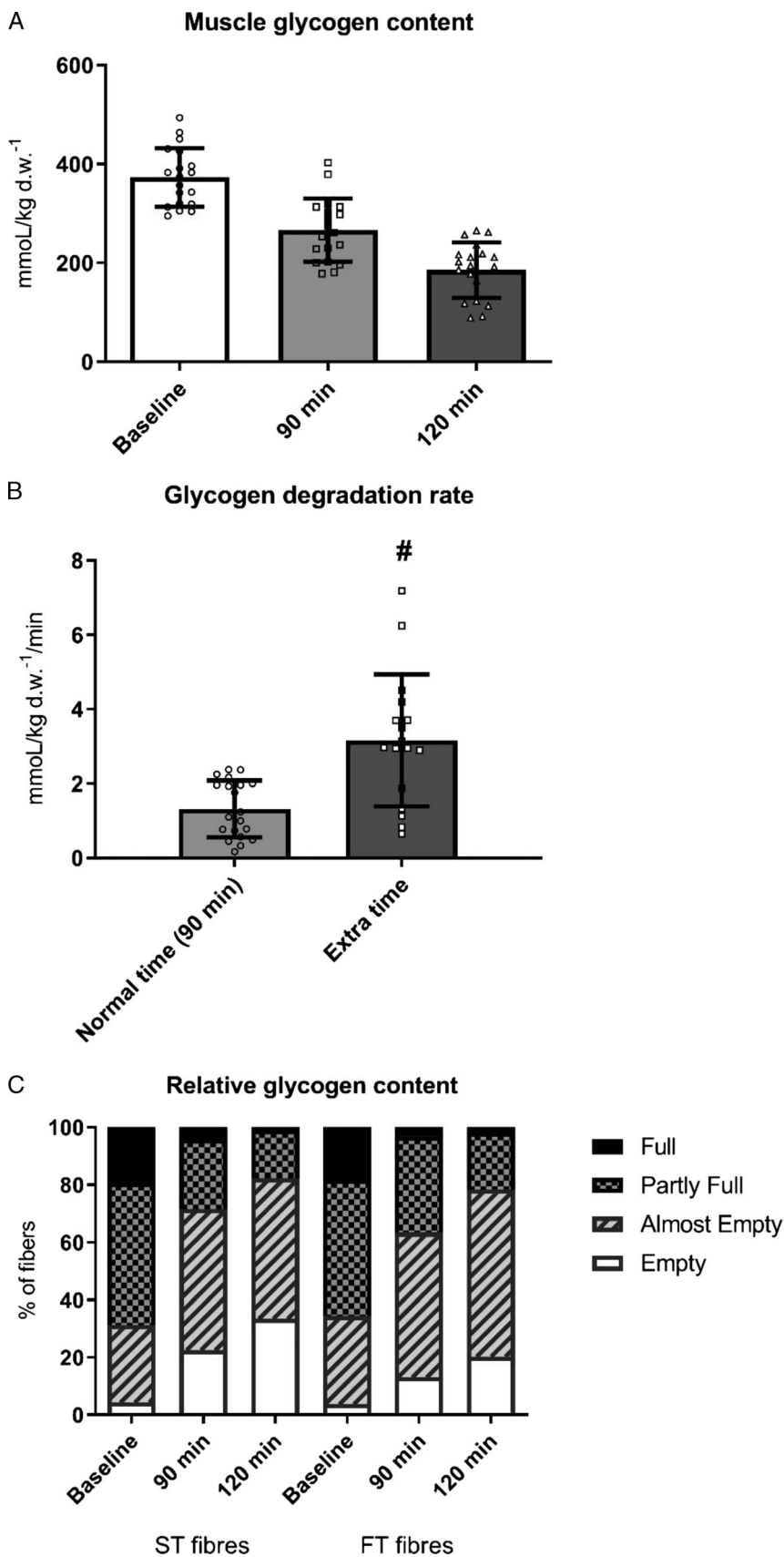**FIGURE 5—Muscle glycogen concentration at whole-muscle level (*n* = 19; A), muscle glycogen degradation rate (*n* = 19; B), and fiber-type–specific glycogen depletion pattern (*n* = 11; C) during the 120-min game protocol. * indicates significant difference compared with baseline; # indicates significant difference compared with 90 min.**

TABLE 3. WBC, RBC, hematocrit, and hemoglobin responses at baseline, after the match (90 min) and after extra time (120 min).

| | Baseline | 90 min | 120 min |
|---|---|---|---|
| WBC ($10^3 \cdot \mu L^{-1}$) | 6.0 ± 1.2 | 12.7 ± 3.1* | 14.1 ± 3.5* |
| Lymphocytes ($10^3 \cdot \mu L^{-1}$) | 2.4 ± 0.4 | 2.6 ± 0.7 | 2.6 ± 0.6 |
| Monocytes ($10^3 \cdot \mu L^{-1}$) | 0.26 ± 0.07 | 0.48 ± 0.11* | 0.55 ± 0.16* |
| Granulocytes ($10^3 \cdot \mu L^{-1}$) | 3.4 ± 1.0 | 9.8 ± 3.2* | 11.0 ± 3.3* |
| Hematocrit (%) | 43.7 ± 1.6 | 44.9 ± 2.0* | 45.0 ± 2.0* |
| Hemoglobin ($g \cdot dL^{-1}$) | 15.1 ± 0.7 | 15.3 ± 0.9 | 15.2 ± 0.9 |
| RBC ($10^6 \cdot \mu L^{-1}$) | 4.9 ± 0.2 | 5.1 ± 0.2* | 5.0 ± 0.2** |

*Significant difference with baseline.
**Significant difference with 90 min ($P < 0.05$).

findings discussed previously are supported by others reporting an exaggerated neuromuscular fatigue (14) response after extra time compared with normal time. Thus, the accumulated fatigue response observed at the end of a 90-min soccer game seems to progress substantially during an extra-time period in trained male soccer athletes.

After the initial 90-min game duration, skeletal muscle glycogen stores were reduced to $266 \pm 64$ mmol·kg$^{-1}$ dw, which is in line with other previous studies in soccer (4,5,9,11,33). More so, after the extra-time period, a further drop to $186 \pm 56$ mmol·kg$^{-1}$ dw was evident corresponding to an approximate 50% reduction compared with baseline values. There seems to be a critical glycogen threshold around 250 mmol·kg$^{-1}$ dw where high-intensity exercise performance is impaired by reductions in glycogen content (10,34). Accordingly, the muscle glycogen concentration was just above this potential threshold after normal time but declined markedly below this point during extra time, which may explain the exacerbated fatigue response observed. Surprisingly, the muscle glycogen degradation rate was considerably faster during extra time than normal time, which is also indicative of high glycolytic activity during the final stages. This was unexpected because the amount of high-intensity activity was lower in extra time, and a downregulation of glycolysis is to be expected at this point because of the degraded glycogen levels with a concomitant upregulated beta oxidation (9). However, importantly, this may be biased by our experimental design with the repeated sprint test performed before the extra-time period, which have contributed to the reductions in muscle glycogen during the extra-time period (35). Despite this, the glycogen utilization during the repeated sprint test is expected to be minor compared with the total load on the players during the complete 120-min game with several sprinting and high-intensity actions performed, meaning that the end-exercise glycogen concentrations are likely only slightly underestimated by the addition of the repeated sprints. Collectively, very low muscle glycogen concentrations are reached after the extra-time period in soccer, which may evoke the greater fatigue response.

The mediocre muscle glycogen stores measured in the muscle homogenate were further supported by the fiber-type–specific analysis demonstrating that 76% of all muscle fibers were low on glycogen after 120 min, a pattern evident in both ST and FT fibers. At the single-fiber level, a greater glycogen deficiency was observed after extra time versus normal time, which backs up the findings at whole-muscle level. The discovery of a large number of individual fibers being nominal on glycogen after prolonged high-intensity intermittent exercise is in line with previous data after a 90-min soccer game (5,9), simulated soccer (33), and elite ice-hockey match play (34). Accordingly, although the glycogen stores at whole-muscle level (measured in a muscle homogenate sample) are nondepleted, several individual fibers reach levels that may be critically low to sustain normal function. Depleted glycogen in a large fraction of myocytes or subcellular compartments has not yet been solidly coupled to a single fatiguing mechanism but has been suggested to disturb several acts in the E–C coupling, such as the Na$^+$-K$^+$ ATPases, Ca$^{2+}$ ATPases, and myosin ATPases (10). The potentiated fatigue response during extra time in a soccer game may therefore be associated with depleted muscle glycogen, which, in an integrated manner, may compromise key steps in the E–C coupling. Previous studies have shown statistical associations between degraded muscle glycogen and the decline in RSA after a team-sport game (5,9,34), which we failed to show in the present study, which may relate to the heterogensous distribution of glycogen in the myocytes.

A common finding during prolonged exercise is an accelerated reliance on fat to fuel ATP resynthesis (36), and in parallel, upregulation of beta oxidation has been shown when muscle glycogen becomes low (37). In our study, plasma glycerol rose by nearly 20% during the final 30 min, supporting a greater reliance on fat in the energy yield. During prolonged intense exercise, such as a soccer game, plasma insulin declines and catecholamines are being released into the bloodstream (10), as has also been shown during the later stages of a soccer game (9,37), which stimulates glucagon production and the release of glucose from the hepatic glycogen stores (38). Because the exercise intensity is lowered in the extra-time period (Table 2), the frequency of rest and low-intensity exercise periods is increased, which may elevate blood flow in the adipose tissue and therefore also the release of free fatty acids. A consistent finding in previous studies is that hypoglycemia does not occur during a 90-min soccer game (4,5,9,33), which again was confirmed by the 90-min measurement in this study. However, after 120 min, a ~15% decrease was observed compared with the 90-min time point, suggesting moderately lowered blood glucose levels, which may activate fatiguing mechanisms of central origin (39). A method to evaluate coordination skill performance during a game is to analyze technical performance or event data (40). In this study, the fraction of successful passes declined by >5% during extra time compared with normal time. This is supported by observations in a systematic review by Field et al. (15) demonstrating a reduction in technical performance events such as shot speed, number of passes, and number of dribbles during extra time. The same review provided evidence (15) that carbohydrate supplementation during extra time may partly counteract these effects, supporting the proposition that hypoglycemia, low glycogen, or muscle fatigue may also be affecting these variables in the extra-time period in soccer.

Interestingly, plasma ammonia increased by ~60% at 90 min, which confirms what others have observed in a male

soccer game (9), but was augmented even further (twofold) at 120 min compared with baseline, which represented an additional 90% increase as an effect of extra time alone. Ammonia is produced in the exercising muscles during energetically demanding work as result of purine nucleotide degradation, potentially in combination with amino acid catabolism (41). Ammonia has been substantiated to rise markedly during soccer-specific high-intensity intermittent exercise (9) and has been observed to cross the blood–brain barrier and accumulate in the cerebrospinal fluid during prolonged exercise (39). Because the brain has no effective uric cycle, ammonia may therefore disturb the neurotransmitter homeostasis in the brain and cause cerebral functions to deteriorate (42), which also may affect technical and physical performance. Thus, the extensive muscle and perhaps also liver glycogen depletion and blood glucose usage may provoke both mild-to-moderate hypoglycemia and hyperammonemia, which can be speculated to cause fatigue mediated centrally.

The total sweat rate was $2.04 \pm 0.57$ L during normal time, which is within the range usually observed in other studies at moderately high temperature (30), but rose to nearly 3.5 L during extra time, resulting in a 26% higher sweat rate in extra time compared with normal time. Despite the fact that no difference was observed in hematocrit levels between the 90- and 120-min time points, dehydration may become a component contributing to deteriorated performance in the extra-time period, especially because a high number of elite soccer players are hypohydrated in normal conditions (43).

A limitation in the study may be that the player knew beforehand that the game was going into extra time, which may have caused them to pace accordingly. However, the tracking data demonstrate a work rate very similar to real competitive games (37). Moreover, the degree of fatigue and the physiological response at 90 min are nearly identical to the literature (3–8). Thus, based on these observations, the 90-min game load is likely to be comparable to a real competitive game.

## CONCLUSIONS

In conclusion, our study demonstrates that the degree of fatigue after an extra-time period in competitive male soccer is exaggerated compared with a normal game, and this may be induced by low glycogen concentrations in a large portion of muscle fibers. Other factors facilitated by lowered blood glucose, hyperammonemia, and/or dehydration may contribute to the deterioration in performance, which points to an integrated and multifaceted fatigue scenario.

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