Brunel University London

UEFA FINAL REPORT

Risks *versus* benefits:

should youth football players be encouraged to take creatine?

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INTRODUCTION

Owing to its well-established ergogenic potential (Cooper et al., 2012), creatine is a highly popular food supplement in sports. In English professional football, over a third of players use creatine (Waddington et al., 2005). The usage of creatine is, however, not limited to professional sports; a considerable number of children and adolescents use creatine, with male adolescent football players being amongst the heaviest users (Metzl et al., 2001).

The reason for the widespread use of creatine in football likely stems from the fact, that: i) it is not prohibited by the governing bodies of sport and ii) it is purported to be safe. The recognised potential of creatine to stimulate muscle strength and power, to increase muscle volume/fat-free mass and to stimulate recovery from exercise (Cooper et al., 2012) make it particularly attractive for use in football. However, recent findings cast doubt on the impact of creatine supplementation in field-based, football-specific situations (Williams et al., 2014). Further, whilst the current consensus is that creatine consumption is safe (Cooper et al., 2012, Buford et al., 2007), no study has so far evaluated the possible adverse effect of creatine on lung function. This is particularly striking considering that, in an animal model of asthma, creatine has been shown to exacerbate allergic-induced lung inflammation, remodelling and airway hyperresponsiveness (AHR) (Vieira et al., 2007, Ferreira et al., 2010).

Asthma is a chronic inflammatory lung disorder with strong allergic overtones that is characterised by reversible airway obstruction and AHR. In elite sports, asthma /AHR is the most common chronic medical condition, with a prevalence of ~8% (Fitch, 2012). However, both in professional (Ansley et al., 2012) and high-school football (Kukafka et al., 1998), asthma/AHR is largely misdiagnosed. This is worrying in that competitive and recreational

sporting activities have the potential to cause sudden fatal asthma exacerbations (Becker et al., 2004). In youth American football players, asthma has recently been identified as one of the most common cause of fatalities (Boden et al., 2013).

High-intensity exercise is known to cause injury and inflammation in the airways of athletes (Kippelen and Anderson, 2012). Further, repeated injury-repair of the airway epithelium is a key susceptibility factor for asthma/AHR development in elite athletes (Kippelen and Anderson, 2012). Whether the effects of high-intensity exercise *plus* creatine supplementation are additive and, therefore, their combination is particularly harmful to the airways of football players remains unknown.

Aim of the study

The aim of our study was to evaluate the risks – in terms of respiratory health – *versus* the benefits – in terms of field-based, football-specific performance – associated with creatine supplementation in youth football players.

More specifically, we aimed to establish whether:

- eight weeks of creatine supplementation in combination with football-specific training increases airway inflammation and airway responsiveness in Academy players,
- a standard course of creatine supplementation significantly improves 'traditional' indices of fitness performance *and* match performance [as assessed by global positioning system (GPS) tracking during competitive game play] in youth football players.

Contribution to European football and UEFA's mission

This project lies within the scope of one of the key missions of UEFA, namely to run a successful anti-doping programme aimed, amongst others, at safeguarding the players' health. Creatine is one of the most widely used ergogenic supplements in football, both at professional and recreational level. Although ingesting creatine as an oral supplement is regarded as safe, no experimental data are yet available on the possible negative effects of creatine on the respiratory health of football players.

With this project, we will establish whether creatine, when combined with high-intensity football training, initiates and/or exaggerates inflammatory-mediated lung disorders in youth, elite football players. We are also aiming to produce ballgame performance results (through GPS tracking during actual game play) that will inform team staff (incl. coaches, sport scientists, physicians) whether creatine makes a difference to the players' performance in real-life, competitive situations.

METHODS

Study participants

Nineteen under 18 (U18) and ten under 21 (U21), non-smoking, male players from Watford FC Academy agreed to take part to this project. Watford FC is a football club which plays in the English Championship. The Academy is the players' development part of the club, with its teams playing in the Football League Youth Alliance (South East Division). Each season, Watford FC Academy has approximately 200 boys of various ages (ranging from 6 to 21 years old). Watford is a unique and pioneering Academy which is dedicated to the holistic development of players. The club has a long and proud record of producing professional football players who have excelled at all levels of the game, right up to the international level.

Participants completed a medical questionnaire before the start of the study to check that none was currently injured or on medication (except for asthma). The only U18 player with a previous diagnosis of asthma was asked to keep his medication unchanged during the trial. Diet was not be standardised, but participants were asked not to change their dietary habits during the course of the study. None of the players used creatine supplements in the three months prior to the study.

The study was approved by the Research Ethics Committee from the School of Sport & Education, Brunel University London (#RE69-13) (approval letter attached). All participants gave informed, written consent before taking part (participant information sheet and consent form attached).

Study design

A double-blind, placebo-controlled, parallel-group trial of creatine supplementation was conducted over 8wk during the first half of the English competitive season (Oct-Dec 2014). The double-blinding procedure means that, neither the participants, not the players were aware which group they belonged to during the trial, *i.e.*, creatine or placebo ('control'). The double-blinding procedure is considered 'gold standard' for experimental trials, in that it avoids experimental bias (intentional or unconscious). Placebo-controlled means that, in addition to the group of subjects that received the creatine supplement, a separate control group received a sham 'placebo' supplement (maltodextrin) which was known to have no real effect. Parallel-group means that one group received the creatine supplement only, while the other group received maltodextrin. Stratified randomisation was used to ensure equal distribution between groups of: i) participants with allergic rhinitis or allergic asthma and ii) U18 and U21.

Players were tested at baseline and after 8wk of training plus supplementation. Due to logistical reasons (no full U21 team at Watford FC Academy this season and, hence, no possibility to collect GPS data during games in those players), the U18 players took part in the full study; for the U21 players, the focus was exclusively on the effect of creatine on lung health.

Lung health assessment

Airway inflammation. Fractional nitric oxide in exhaled breath (F_ENO) was measured at rest in all players as a non-invasive marker of airway inflammation. As *per* standard

recommendations [American Thoracic Society (ATS) & European Respiratory Society (ERS), 2005], at least two measurements agreeing within 10% of each other were recorded, and the mean of the two values were calculated. In agreement with international guidelines (ATS&ERS, 2005), players were asked to refrain from exercising, eating and drinking for an hour prior to the F_ENO measurements.

Lung function. Standard spirometry [*i.e.*, forced vital capacity (FVC) manoeuvers] was performed at rest in all players to determine baseline lung volume and expiratory flow rates. In line with the international recommendations (Miller et al., 2005), at least three technically acceptable manoeuvres were performed (up to a maximum of eight), with a minimum of two reproducible recordings [difference ≤150 ml for forced expiratory volume in 1 sec (FEV_1) and FVC]. The best FEV_1 and FVC readings out of the two reproducible manoeuvres were kept for analysis. Forced expiratory flow between 25 and 75% of FVC (FEF_{25-75}) was taken from the acceptable manoeuvre with the highest sum 'FEV_1+FVC'. The highest peak expiratory flow out of all acceptable attempts was kept for analysis.

Airway hyperresponsiveness (AHR). A standard 6-min eucapnic voluntary hyperpnoea (EVH) test with dry air was used to detect AHR. The test was performed in accordance with international recommendations (Anderson et al., 2001) on a commercially available system (EucapSys, SMTEC, Nyon, Swiss). The test involved 6 min of heavy breathing (to mimic the ventilatory demand of exercise) at a target ventilation rate of 30 times baseline FEV_1 [which equates to 85% of predicted maximum voluntary ventilation (MVV)] while inhaling a dry gas mixture containing 5% CO_2, 21% O_2, and balance nitrogen.

Standard drug withholding times were applied for the player on asthma medication, and all players abstained from caffeine and alcohol in the morning prior to the test, and from exercising for at least 4 hours.

Prior to, and at 2, 5, 10, 15 and 20 min of recovery FVC manoeuvres were performed. A minimum of three FVC manoeuvres were completed at baseline (see section on 'lung function') and at least two reproducible manoeuvres (up to four) were repeated at each recovery time point. The best FEV_1 out of the reproducible measurements were kept for analysis. The maximal fall in FEV_1 post-EVH (expressed as % of baseline) was calculated as an index of AHR. A test was considered positive for exercise-induced bronchoconstriction (EIB) if FEV_1 fell by ≥10% from baseline over two consecutive time points (Anderson and Kippelen, 2012).

Atopic status. Atopy was assessed by standard skin prick test (Bousquet et al., 2012), with the following allergens tested: house dust mite, timothy grass, silver birch, dog and cat hair (those being the most common aero-allergens British people are sensitized to). In line with current international recommendations (Bousquet et al., 2012), wheal diameters ≥3 mm were considered as positive.

Body composition

Body mass was measured using a calibrated electronic scale. Body composition was assessed *via* skinfold measurement. As recommended by the International Society for the Advancement of Kinanthropometry International Society for the Advancement of Kinanthropometry (ISAK), eight sites (*i.e.*, tricep, sub-scapular, bicep, iliac crest, supra-spinal, abdominal, front thigh and medial calf) were used. All measurements were taken by a

level1 ISAK qualified researcher (with an average technical error of measurement of 1.50 ± 0.31%).

Performance assessment

Lower body strength. The muscle strength measurements were performed on both the U18 and U21 players using an isokinetic dynamometer (EasyTech, Genu ISO, Florence, Italy). The protocol routinely performed by Watford FC Academy players was followed. Standardisation of the test was in line with the recommendations from the British Association of Sport and Exercise Sciences (Baltzopoulos et al., 2012). The players performed a standard 5-min warm-up on a stationary bike. The maximal isokinetic peak and average peak torque of the knee extensor and flexor muscles were recorded at an angular velocity of 90° *per* second over five repetitions. Both legs (starting with the right one) were tested and the same encouragements were provided to each player. Tests were performed early mornings (before the start of any training session) to limit prior muscle fatigue.

Repeated-sprint ability (RSA). The testing took place on an indoor football pitch (3GTurf) at Harefield Academy, and was preceded by a standardised warm-up. Air temperature was between 13 and 18°C, and relative humidity (RH) was between 61 and 80% pre-supplementation. Post-supplementation, these values were: 5 to 15°C for temperature, and 35 to 64% for RH. The exercise protocol used was the one described by Rampinini *et al.* (2009), wherein each U18 player completed initially a 40-m (20 m + 20 m) shuttle test (criterion score). Players then had at least 5 min of rest. The repeated sprint test consisted of six 40-m (20 m + 20 m) shuttle sprints, with 20 sec of passive recovery. If performance during the first sprint of the RSA was slower than the criterion score (time >2.5%), players were given an

additional 5 min of rest and were required to repeat the RSA with maximal effort after that period. No encouragements were provided. The time was recorded *via* photocells gates (Brower Timing System, Draper, Utah, USA). The shortest time in a single sprint (RSA_{best}), mean time (RSA_{mean}), and percent decrement (RSA_{dec}) were calculated.

Yo-Yo-Intermittent Recovery 2 test (Yo-Yo-IR2). The Yo-Yo-IR2 was conducted on the U18 players only. The testing took place in the indoor football pitch at Harefield Academy (air temperature 17°C and 50% RH pre-supplementation, and 6-13°C and 35-67% RH post-supplementation). Players were split into two groups, and the standard protocol devised by Bangsbo (1994) was followed. The Yo-Yo-IR2 consists of a repeated bout of 20-m shuttle runs, with 10-sec rest, at a progressive increased speed controlled by audio-bleeps from a tape recorder. The starting speed was 13 $km \cdot h^{-1}$ and the total distance covered by the players was recorded as index of a player's ability to perform intense intermittent exercise with a high rate of aerobic and anaerobic energy turnover (Krustrup et al., 2006).

Game play performance. GPS tracking was used to analyse full match performance of the U18 players. GPS game data was collected using a Catapult unit (Minimax, Catapult Innovations, Canberra, Australia) worn in a tight fitting vest (to reduce movement artefact) between the players' shoulder blades, with an operating sampling frequency of 10 Hz. One to five matches performed by each outfield player in the period leading to the supplementation period and in weeks 4 to 8 of the supplementation period were analysed. Only those matches in which athletes were playing for at least 60 min were analysed. Data were expressed in both absolute (m) and relative ($m \cdot min^{-1}$) terms to allow direct comparisons between individuals, and between pre- and post-supplementation without bias to individual variations in match

exposure [as done in previous research on youth football players (Harley et al., 2010)]. Post-match analysis enabled the quantification of total distance (m) and distances covered in higher velocity bands: high speed distance (19-22 $km \cdot h^{-1}$), and sprint distance (>22 $km \cdot h^{-1}$). Velocity bands were set by Watford FC Academy and were in line with published research (Osgnach et al., 2010). Work rate ($m \cdot min^{-1}$) in each velocity band was analysed, as done previously in youth football players (Harley et al., 2010). Overall match distance covered at high intensity (% distance >19 $km \cdot h^{-1}$) was expressed as a percentage of the total distance covered during a match and was used as primary index for high-intensity match performance.

Supplementation

After initial baseline control measurements, participants in each strata (*i.e.*, allergic and non-allergic; U18 and U21) were randomly assigned, either to the creatine supplementation group, or to the placebo group. A typical creatine monohydrate (CM) supplementation protocol was followed (Buford et al., 2007), whereby participants ingested 0.3 g CM/kg/d (MyProtein, Informed Sports Range, 100% pure) during a 1-wk loading phase. The placebo group received the same dosage of maltodextrin (MyProtein, Informed Sports Range, 100% pure). The supplement was mixed up with 90 g of the usual protein drink (Maxiraw Protein Complex; 60% whey protein concentrate, 20% soy protein isolate, 20% micellar casein) provided to players at Watford FC Academy, and ingested in four daily 150 ml intakes. This was followed by a 7-wk maintenance phase during which players ingested 5 g/day of creatine monohydrate, or of placebo, mixed up in 30 g of protein drink, and taken at the end of their daily training session or after games (at the weekend).

To ensure adherence, the supplements were prepared daily by the Head Academy Sport Scientist at Watford FC Academy and handed out directly to the players at the required times (expect on Sundays, when players were provided with shakers to take home).

Daily record of asthma symptoms, upper or lower respiratory tract infection symptoms, use of asthma reliever medication (salbutamol), and potential side-effects (*e.g.*, muscle cramps, musculo-skeletal injury, gastro-intestinal discomfort) were logged for all players.

Training programme

The researcher did not change the training schedule devised by the players' coaches. The 8-wk training programme included a mixture of football specific (*i.e.*, technical and tactical exercises), and strength and conditioning work (~1 session of 50 min *per* week). As part of the Football League Youth Alliance, the U18 team had regular match fixtures over the course of the trial.

Data analysis

Sample size. F_ENO was our primary outcome measure. To detect a 10 ppb difference in F_ENO between the creatine and the control group post-supplementation, with a statistical power of 80% and an alpha level of 0.05, a within-group standard deviation of 6 ppb, and a ratio of control to experimental participants of one, we calculated that a sample size of 14 players would be required (*i.e.*, seven players *per* group). Taking account of an estimated drop-out rate of 30%, a minimum of 20 players had to be recruited.

Statistics. Our primary study measures were: F_ENO, maximal fall in FEV_1 post-EVH, distance covered in the Yo-Yo-IR2, RSA_{best}, RSA_{mean}, RSA_{dec}, and GPS data. Secondary study outcomes included: sum of 8 skinfold, body mass, maximal isokinetic peak and average peak torque of the knee extensor and flexor muscles, baseline lung function parameters, V_E achieved during the EVH test.

Data were checked for normality using the Shapiro-Wilk test. Data showing a Gaussian distribution were analysed using unpaired t-tests (for between-group comparisons at the start of the trial) and repeated measures ANOVA (for between- and within-group comparisons during the trial). For non-Gaussian data (incl. V_E achieved during the EVH test, maximal FEV_1 fall post-EVH and F_ENO data) non-parametric tests were carried out (*i.e.*, Mann Whitney test for between-group comparisons and Wilcoxon test for within-group comparisons).

Data were analysed with SPSS 20 for Windows (SPSS, Chicago, IL). The level of significance was set at $P<0.05$. Unless otherwise stated, parametric data are presented as means ± SD, and non-parametric data as median (interquartile range).

RESULTS

Participants

Twenty nine players were initially recruited (incl. 19 U18 and 10 U21). Five players did not complete the supplementation (two went on loan, two did not regularly take the supplement, and one stopped attending the training sessions); they were therefore removed from the data analysis. An additional two players got injured in the first half of the supplementation; their data were also removed. During the trial, the dropout rate was therefore of 24%. Out of the 22 players who completed the full trial (15 U18 and 7 U21), ten were randomly allocated to the CM group (incl. the two U21 goal-keepers) and twelve to the placebo group.

At study entry, the participants had played on average for 10.6 ± 2.8 yr in football, and their weekly training volume was 11.9 ± 3.9 hours. Two U18 and four U21 were international players who represented their country at international fixtures.

Anthropometric data and body composition

At study entry, players were aged 17 ± 1 yr, had an average stature of 178.3 ± 5.2 cm and a body mass of 73.2 ± 5.4 kg. Eight weeks of CM or placebo supplementation did not significantly change the body mass of the players (Table 1). However, an overall 'time' effect was noticed for body composition; the sum of 8 skinfolds being significantly reduced post-supplementation in all players (P=0.008) (Table 1). The average reduction in the sum of 8 skinfolds post-supplementation was not significantly different between groups: 5.9 ± 5.1% in the creatine group *versus* 2.8 ± 7.6% in the placebo groups.

Table 1. Body composition in U18 and U21, male, football Academy players before and after 8 weeks of creatine monohydrate (CM) or placebo (PLA) supplementation

	CM (n=10)		PLA (n=12)	
	Pre-CM	Post-CM	Pre-PLA	Post-PLA
Body mass (kg)	74.9 ± 5.2	75.0 ± 4.6	71.8 ± 5.3	72.3 ± 5.3
Sum of 8 skinfolds (mm)	67.9 ± 13.3	63.7 ± 10.9**	60.5 ± 14.8	59.3 ± 16.9**

Note. Values are means ± SD. ** $P<0.01$, significantly different from pre-supplementation

Asthma and atopic status

At study entry, only one player (U18) had a prior medical diagnosis of asthma; this player was using salbutamol (as needed) and inhaled corticosteroids (daily).

Seventeen (77%) players were atopic (12 U18 and 5 U21), with grass being the most common allergen players were sensitized to: 13 (59%) players were positive to grass *versus* seven (32%) to house dust mite, seven (32%) to dog hair, six (27%) to cat hair, and two (9%) to silver birch.

F_ENO

The F_ENO values of two U18 players had to be removed from the statistical analysis since they had an upper or lower respiratory tract viral infection in the weeks preceding pre-supplementation data collection (one player – *i.e.*, the one with diagnosed asthma – had a chest infection, and the other player had tonsillitis). Upper and lower respiratory tract viral infections have previously been shown to impact on F_ENO readings (ATS&ERS, 2005,

Dressel et al., 2008). The F_ENO analysis was therefore carried out on 20 players (9 in the creatine group and 11 in the placebo group, which satisfies our initial sample size calculation).

Between-group comparisons at each time point (pre- and post-supplementation) did not reveal any significant difference in F_ENO values. However, within-group comparisons revealed a trend (P=0.086) for F_ENO to increase post-supplementation in the creatine group (Table 2).

Table 2. Fractional nitric oxide in exhaled air (F_ENO) in football Academy players before and after 8 weeks of creatine monohydrate (CM) or placebo (PLA) supplementation

	CM (n=11)		PLA (n=9)	
	Pre-CM	Post-CM	Pre-PLA	Post-PLA
Median F_ENO (ppb)	22	34[&]	21	20
Interquartile range	18-81	16-95	17-34	16-24

Note. [&] P=0.086 compared to pre-supplementation value.

Individual results highlighted that, at the start of the trial, 13 (65%) of the players had F_ENO value <25 ppb (healthy range), 15% had F_ENO values between 25 and 50 ppb [borderline range for airway eosinophilic inflammation (Dweik et al., 2011)], and 20% had F_ENO values >50 ppb [airway eosinophilic inflammation likely (Dweik et al., 2011)]. Post-supplementation, the percentage of players in each of these categories remained broadly unchanged, but two players from the creatine group 'moved up' to the 25 to 50 ppb range, whilst two players from the placebo group 'moved down' to the healthy category (Table 3).

Table 3. Distribution of fractional exhaled nitric oxide in exhaled air (F_ENO) in football Academy players before and after 8 weeks of creatine monohydrate (CM) or placebo (PLA) supplementation

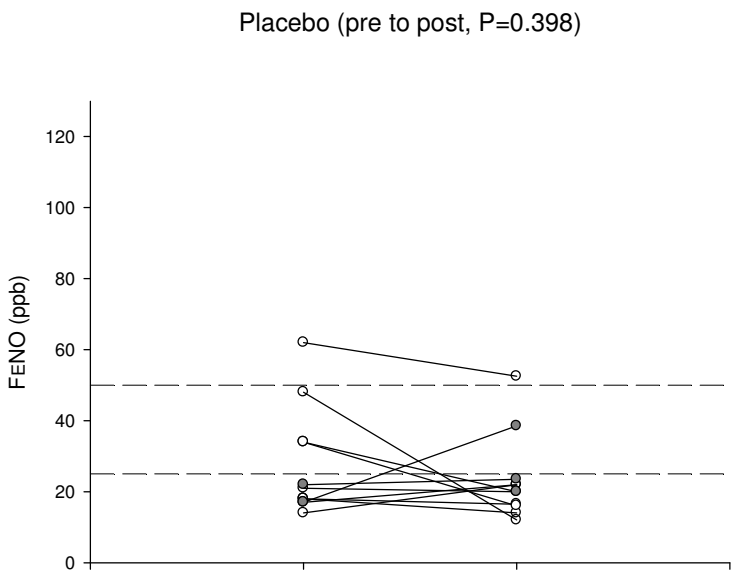
	CM (n=9)		PLA (n=11)	
	Pre-CM	Post-CM	Pre-PLA	Post-PLA
$F_ENO < 25$ ppb	6 (67)	4 (44)	7 (64)	9 (82)
$25 \leq F_ENO < 50$ ppb	0 (0)	2 (22)	3 (27)	1 (9)
$F_ENO > 50$ ppb	3 (33)	3 (33)	1 (9)	1 (9)

Note. Values are numbers (%)

Four (44%) players supplemented with creatine had an increase in F_ENO >10 ppb [*i.e.*, minimum significance threshold for intervention trials (Dweik et al., 2011)] from pre- to post-supplementation (Figure 1), while only one (9%) of the players under placebo had such an increase. The average relative increase in F_ENO for the sub-group of four players under creatine with a meaningful change in F_ENO post-supplementation was of 40% (range 14-80%). All four players were atopic.

Since animal studies suggest that creatine may be particularly damaging to the airways of allergen-sensitised mice (Vieira et al., 2007), further statistical analyses were carried out on the F_ENO data obtained in the 15 atopic players (n=7 in the creatine group and n=8 in the placebo group). Between-group comparisons revealed a trend (P=0.072) for F_ENO to be higher post-supplementation in the atopic players supplemented with creatine compared to the atopic players on placebo (Table 4). Within-group comparisons also showed a trend (P=0.063) for F_ENO values to increase post-supplementation in the atopic players supplemented with creatine, but not in those players on placebo (Table 4).

A.



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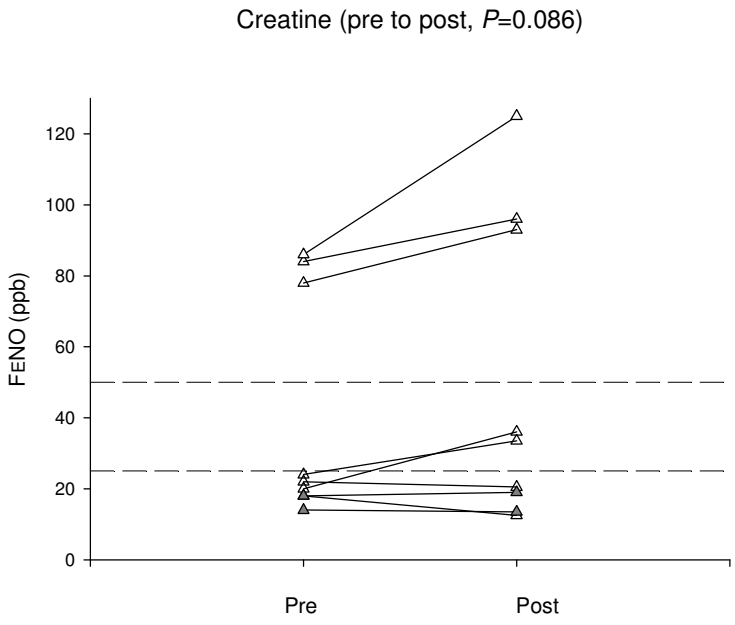

Figure 1. Individual data for fractional nitric oxide in exhaled air (F_ENO) in football Academy players before and after 8 weeks of creatine monohydrate (panel A) or placebo (panel B) supplementation. Open symbols are atopic players; closed symbols are non-atopic players. Broken lines represent clinically meaningful F_ENO thresholds: 25 and 50 ppb, borderline range for airway eosinophilic inflammation; > 50 ppb, airway eosinophilic inflammation likely (Dweik et al., 2011).

Table 4. Fractional nitric oxide in exhaled air (F_ENO) in atopic, football Academy players before and after 8 weeks of creatine monohydrate (CM) or placebo (PLA) supplementation

	CM (n=7)		PLA (n=8)	
	Pre-CM	Post-CM	Pre-PLA	Post-PLA
Median F_ENO (ppb)	24	36[&,£]	20	20
Interquartile range (ppb)	20-84	21-96	17-45	15-22

Note. [&] P=0.063 compared to pre-supplementation value; [&] P=0.072 compared to the placebo group post-supplementation.

Resting lung function

Resting spirometry data were available pre- and post-supplementation for all but two players; one was the U18 asthmatic player who had a chest infection in the week leading to the pre-supplementation data collection; the other one was an U21 player who broke a rib two weeks before the post-supplementation data collection and could not perform the forced expiratory manoeuvres. Therefore, the lung function analysis was conducted on a dataset of twenty (n=9 in the creatine group and n=11 in the placebo group).

Resting lung function values were within normal range for all the players, except one. One U18 had FEV_1, FVC and FEF_{25-75} values below the lower limit of normal, both pre- and post-supplementation: FEV_1 was ~2.66 L (*i.e.*, 72% of the predicted value) and FVC was ~3.39 L (*i.e.*, 80% of the predicted value) on both occasions. This player reported symptoms of asthma during strenuous exercise and had a family history of asthma, but no past medical diagnosis of asthma or EIB.

Statistical analysis revealed a small, but statistically significant (*P*=0.044) reduction in resting FEV_1 (% predicted) after 8 weeks, with no difference between groups (Table 5). This change was of ~1% (*i.e.*, 34 ± 80 ml in absolute term), which is not deemed clinically significant.

AHR

All players bar two (*i.e.*, the U18 player with asthma who had a chest infection the week prior to pre-supplementation data collection, and the U21 with a broken rib post-supplementation), completed the EVH test pre- and post-supplementation (n=20).

Overall, the players maintained a ventilation of 103 (90-116) $L \cdot min^{-1}$ over the 6 minutes, and ventilation did not significantly differ between pre- [104 (92-115) $L \cdot min^{-1}$] and post-supplementation [103 (86-116) $L \cdot min^{-1}$]. There was a trend for the creatine group to reach higher ventilation levels: 116 (94-137) $L \cdot min^{-1}$ *versus* 103 (87-106) $L \cdot min^{-1}$ in the placebo group pre-supplementation (*P*=0.067), and 116 (96-135) $L \cdot min^{-1}$ *versus* 102 (83-106) $L \cdot min^{-1}$ in the placebo group post-supplementation (*P*=0.080). However, this trend disappeared (*P*=0.381 for 'group' effect) once the data were expressed as % of predicted MVV [as calculated by baseline FEV_1 times 35 (Anderson et al., 2001)]: 71 ± 10% *versus* 67 ± 13% for the creatine and placebo groups, respectively, pre-supplementation, and 73 ± 11% *versus* 68 ± 14% for the creatine and placebo groups, respectively, post-supplementation.

Three (21%) of the 14 U18 players were unable to reach the minimum target ventilation of ≥60% of predicted MVV pre-supplementation, and two had the same problem post-supplementation (Figure 2). All the U21 players reached the minimum ventilatory threshold.

Table 5. Resting lung function data in football Academy players before and after 8 weeks of creatine monohydrate (CM) or placebo (PLA) supplementation

	CM (n=9)		PLA (n=11)	
	Pre-CM	Post-CM	Pre-PLA	Post-PLA
FEV_1 (L)	4.56 ± 0.79	4.52 ± 0.84	4.04 ± 0.75	4.01 ± 0.71
FEV_1 (% pred.)	104 ± 9	103 ± 10*	103 ± 16	101 ± 16*
FVC (L)	5.43 ± 1.03	5.43 ± 1.03	4.64 ± 0.83	4.65 ± 0.80
FVC (% pred.)	106 ± 8	105 ± 8	99 ± 12	99 ± 11
FEV_1/FVC (%)	84 ± 4	83 ± 5	89 ± 6	88 ± 6
PEF ($L \cdot sec^{-1}$)	9.73 ± 1.31	9.62 ± 1.34	9.07 ± 1.30	9.46 ± 1.50
FEF_{25-75} ($L \cdot sec^{-1}$)	4.66 ± 0.97	4.51 ± 1.10	4.64 ± 1.23	4.59 ± 1.40

Note. Values are means ± SD. FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; PEF, peak expiratory flow; MEF, mid expiratory flow between 25 and 75% of FVC; % pred., % of the predicted values (Quanjer et al., 2012). * $P<0.05$, significantly different from pre-supplementation

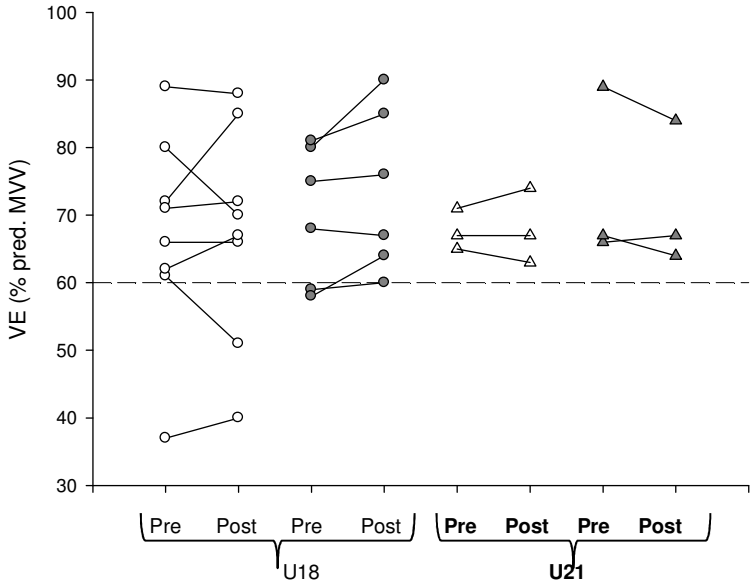

Figure 2. Individual data for ventilation [VE, expressed as percentage of predicted maximal voluntary ventilation (MVV)] achieved by the fourteen U18 (circles) and six U21 (triangles) football Academy players during 6 min of eucapnic voluntary hyperpnoea test of dry air before (pre) and after (post) 8 weeks of supplementation with creatine monohydrate (closed circles) or placebo (open circles)

Statistical analysis revealed significant within- and between-group differences for the maximal fall in FEV_1 post-EVH. Whilst the fall in FEV_1 post-EVH was not significantly different pre-supplementation between groups [6.3% (3.4-14.0%) in the creatine *versus* 6.8% (2.8-10.1%) in the placebo group, *P*=0.882], post-supplementation the creatine group showed a slightly, but significantly (*P*=0.038), larger fall in FEV_1 [6.8% (4.7-12.3%) *versus* 4.8% (3.6-5.3%) in the placebo group] (Figure 3). Further, over the supplementation period, the fall in FEV_1 post-EVH was slightly, but significantly (*P*=0.033) reduced in the placebo group (difference pre- to post-supplementation for FEV_1 fall: 2.0 ± 2.8%), while it remained unchanged in the creatine group (difference pre- to post-supplementation for FEV_1 fall: 0.7 ± 3.1%, *P*=0.594).

Using the recommended threshold (*i.e.*, a ≥10% fall in FEV_1 post-EVH over at least two consecutive time points) for a diagnosis of EIB (Anderson and Kippelen, 2012), three players (two in the creatine group) were positive for EIB pre-supplementation, and two players (both in the creatine group) were positive for EIB post-supplementation (Figure 3). None of the players with a positive response to EVH had a history of asthma and/or EIB, but they all had F_ENO values suggestive of eosinophilic airway inflammation (F_ENO range: 62-86 ppb). The player who was positive for EIB pre-supplementation, but negative post-supplementation, was the one who had an abnormally low lung function at rest at both time points (consistent with baseline airway obstruction). One player in the creatine group with a history of respiratory symptoms on exertion had a borderline response to EVH (non-sustained maximum FEV_1 fall of 11%) post-supplementation and markedly increased F_ENO at both time points (84 and 96 ppb pre- and post-supplementation, respectively). Together, the data from this player were therefore supportive of EIB.

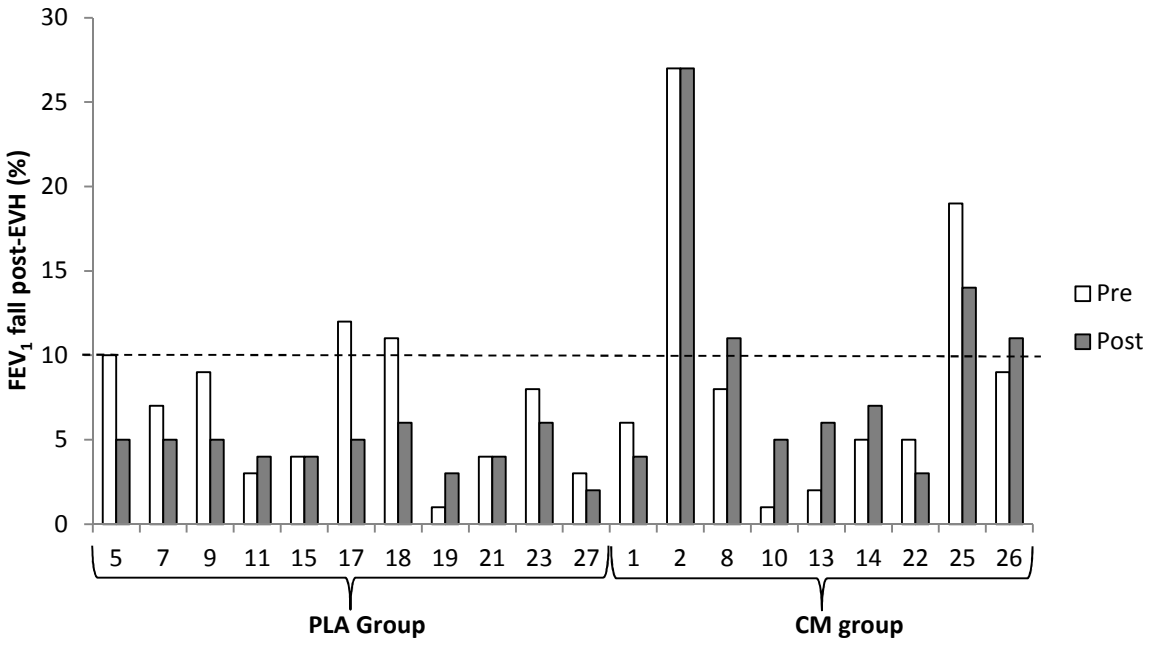

Figure 3. Pre- and post-supplementation individual values for the fall in forced expiratory volume in 1 sec (FEV_1) after 6 min of eucapnic voluntary hyperpnoea (EVH) of dry air in football Academy players supplemented for 8 weeks with creatine monohydrate (CM) or a placebo (PLA).

Altogether, four players (20%) – 2 U18 and 2 U21 – with no past history of asthma/EIB were diagnosed with asthma/EIB in the course of this study. The only (U18) player who had a prior medical diagnosis of asthma/EIB and who was using preventive and rescue medications at the time of the trial did not do the EVH test pre-supplementation (because of a chest infection). His post-supplementation test results were however clearly positive (with a 32% fall in FEV_1 post-EVH and a F_ENO of 54 ppb), suggestive of poorly controlled asthma under the current pharmacotherapy. Taken together, these data highlight that 25% of the studied population had objective evidence of asthma and/or EIB.

Lower body strength

All fifteen U18 players performed the isokinetic test pre- and post-supplementation. Due to lack of availability, only three out of seven U21 players performed the test on both occasions. Hence, results are presented for 18 players (n=9 in the creatine group and n=9 in the placebo group).

The maximal isokinetic peak and average peak torque of the knee extensor muscles for both, the dominant and non-dominant legs, did not significantly differ between groups (creatine *versus* placebo) and times (pre- *versus* post-supplementation) (Table 6). For the knee flexor muscles, a significant 'time' effect was noticed for maximal isokinetic torque for the dominant ($P<0.001$ and $P=0.022$ for peak and average torque, respectively) and non-dominant leg ($P<0.001$ and $P=0.032$ for peak and average torque, respectively), with post-supplementation values being significantly higher than pre-supplementation values in both groups (Table 6).

Field-based performance tests.

RSA

Thirteen out of the fifteen (87%) U18 players (n=6 in the creatine group and n=7 in the placebo group) performed the RSA on both occasions (the missing players were recovering from injury or illness on the day of testing).

RSA_{best}, RSA_{mean} and RSA_{dec} values did not significantly differ between 'groups' and 'times' (Table 7) and no 'interaction' effect was noticed, suggesting that creatine supplementation had no significant impact on repeated sprint performance in the U18 players.

Table 6. Maximal isokinetic peak and average peak torque of the knee extensor and flexor muscles of football Academy players before and after 8 weeks of creatine monohydrate (CM) or placebo (PLA) supplementation

	DL				NDL			
	CM (n=9)		PLA (n=9)		CM (n=9)		PLA (n=9)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Knee extensors								
Peak torque (N·m)	196 ± 34	202 ± 28	183 ± 24	185 ± 13	194 ± 26	191 ± 27	169 ± 32	187 ± 18
Average torque (N·m)	183 ± 30	189 ± 24	168 ± 18	173 ± 14	180 ± 23	184 ± 34	160 ± 32	176 ± 14
Knee flexors								
Peak torque (N·m)	151 ± 22	160 ± 28***	134 ± 18	147 ± 19***	139 ± 20	154 ± 24***	124 ± 17	143 ± 20***
Average torque (N·m)	140 ± 24	149 ± 29*	124 ± 20	139 ± 19*	130 ± 21	146 ± 27*	116 ± 21	136 ± 20*

Note. Values are means ± SD. DL, dominant leg; NDL, non-dominant leg. * $P<0.05$, *** $P<0.001$, significantly different from pre-supplementation.

Table 7. Repeated sprint test results in U18 Academy players before and after 8 weeks of creatine monohydrate (CM) or placebo (PLA) supplementation

	CM (n=6)		PLA (n=7)	
	Pre-CM	Post-CM	Pre-PLA	Post-PLA
RSA_{best} (s)	7.10 ± 0.26	7.13 ± 0.25	7.01 ± 0.21	6.81 ± 0.22
RSA_{mean} (s)	7.57 ± 0.18	7.52 ± 0.22	7.43 ± 0.30	7.26 ± 0.21
RSA_{per} (%)	6.6 ± 2.5	5.6 ± 2.0	6.0 ± 2.1	6.6 ± 1.1

Note. Values are means ± SD. RSA_{best}, shortest time in a single sprint during the repeated sprint ability (RSA) test; RSA_{mean}, mean time during the RSA test; RSA_{per}, percent decrement during the RSA

Yo-Yo-IR2

Fourteen out of the fifteen (93%) U18 players (n=7 in the creatine group and n=7 in the placebo group) performed the Yo-Yo-IR2 on both occasions (one player was returning to running following an injury only two weeks before the Yo-Yo-IR2 test pre-supplementation, and did therefore not perform the test).

Significant 'time' (P=0.005) and 'interaction' effects (P=0.044) [and close to significant 'group' effect (P=0.051)] were noticed for the distance covered in the Yo-Yo-IR2 by the players, with pairwise comparisons revealing that only the players under placebo significantly (P=0.010) improved their performance in the Yo-Yo-IR2 post-supplementation. Furthermore, the post-supplementation performance of the players on placebo was significantly better than the performance of the creatine group (P=0.017) (Table 6). The group difference in the % increase pre- to post-supplementation in distance covered during the Yo-Yo-IR2 test just

failed to reached significance (26 ± 19% in the placebo group *versus* 7 ± 18% in the creatine group, *P*=0.076).

Large inter-individual differences were noticed in the performance of the players during the Yo-Yo-IR2 test, mainly depending on their playing position (Table 8).

GPS data

GPS data were obtained pre- and post-supplementation in ten U18 players (n=4 in the creatine and n=6 in the placebo group). No GPS data were obtained for the two goalkeepers (both were randomly allocated to the creatine group) and data for three players were not available (two were returning from injury and had not played in any game in the month leading to pre-supplementation data collection, and GPS data were not available for one player).

Creatine supplementation had no significant effect on any of the GPS parameters measured during game play, with the statistical analysis revealing no ‘group’, ‘time’ or ‘interaction’ effects (Table 9).

Table 8. Individual data for distance covered in the Yo-Yo-IR2 test by U18 football Academy players supplemented for 8 weeks with creatine monohydrate (CM) or a placebo (PLA)

ID#	Position	Pre (m)	Post (m)	Change pre to post (%)
CM				
1	GK	480	400	-17
2	GK	480	560	17
8	CB	600	760	27
10	RB	880	840	-5
12	CM	640	640	0
13	CF	640	600	-6
14	CM	520	680	31
Mean		606	640	7
SD		139	142	18
Placebo				
5	CB	560	720	29
7	CB	600	880	47
11	CM	840	1160	38
15	CF	720	840	17
17	CF	480	680	42
18	LWB	840	800	-5
19	CF	960	1080	13
Mean		714	880[*]	26[&]
SD		175	179	19

Note. GK, goalkeeper; CB, centre back; RB, right fullback; CM, centre midfielder; CF, centre forward; LWB, left wing back. [*] $P<0.05$, significant between- and within-group difference; [&] $P=0.076$ compared to the creatine group.

Table 9. Game performance indexes (as assessed by GPS) in U18 Academy players before and after 8 weeks of creatine monohydrate (CM) or placebo (PLA) supplementation

	CM (n=4)		PLA (n=6)	
	Pre-CM	Post-CM	Pre-PLA	Post-PLA
Game distance (m)	9981 ± 334	10053 ± 327	9972 ± 979	10271 ± 819
HI speed distance (m)	278 ± 29	338 ± 72	379 ± 118	396 ± 141
Sprint distance (m)	163 ± 82	223 ± 98	369 ± 201	352 ± 182
Max velocity ($km \cdot h^{-1}$)	27.5 ± 1.7	27.4 ± 1.8	29.3 ± 0.9	29.0 ± 1.3
Game W/R ($m \cdot min^{-1}$)	107.2 ± 3.3	107.5 ± 3.7	108.3 ± 6.8	111.3 ± 7.8
HI speed W/R ($m \cdot min^{-1}$)	3.3 ± 0.6	3.6 ± 0.7	4.0 ± 1.1	4.3 ± 1.5
Sprint distance W/R ($m \cdot min^{-1}$)	1.9 ± 0.8	2.4 ± 1.0	3.9 ± 2.1	3.8 ± 1.9
% distance >19 $km \cdot h^{-1}$ of game distance	4.8 ± 0.7	5.6 ± 1.6	7.3 ± 2.8	7.2 ± 2.9

Note. Values are means ± SD. HI speed, 19-22 $km \cdot h^{-1}$; Sprint, >22 km/h; W/R, work rate; % distance >19 $km \cdot h^{-1}$, overall distance covered at 'high intensity' and expressed as % of the total time spent on the field

Visual inspection of individual data from the creatine group [to account for inter-individual differences in the response to creatine supplementation (Syrotuik and Bell, 2004)] shows that one of the four players on creatine (a centre forward) had a median post-supplementation value for % game distance >19 $km \cdot h^{-1}$ (*i.e.*, the most likely variable to be affected by creatine supplementation) that was outside the pre-supplementation 95% confidence interval (CI) (Figure 4). In the three other players supplemented with creatine [one centre back player

(Figure 4) and two centre midfielders (Figure 5)] post-supplementation % game distance >19 $km \cdot h^{-1}$ was within the 95% CI of pre-supplementation value, suggesting that creatine had no significant effect on high-intensity work during game play. Further, none of the players in the placebo group (two centre backs, one centre midfielder, two centre forwards and one left wing back) had a post-supplementation median value for % game distance >19 $km \cdot h^{-1}$ outside the 95% CI of pre-supplementation value (Figure 6).

Side effects

None of the players reported asthma exacerbations or developed upper or lower respiratory tract infection symptoms over the course of the supplementation.

No significant side-effects (e.g., muscle cramps, musculo-skeletal injury, gastro-intestinal discomfort) were reported by players during the trial.

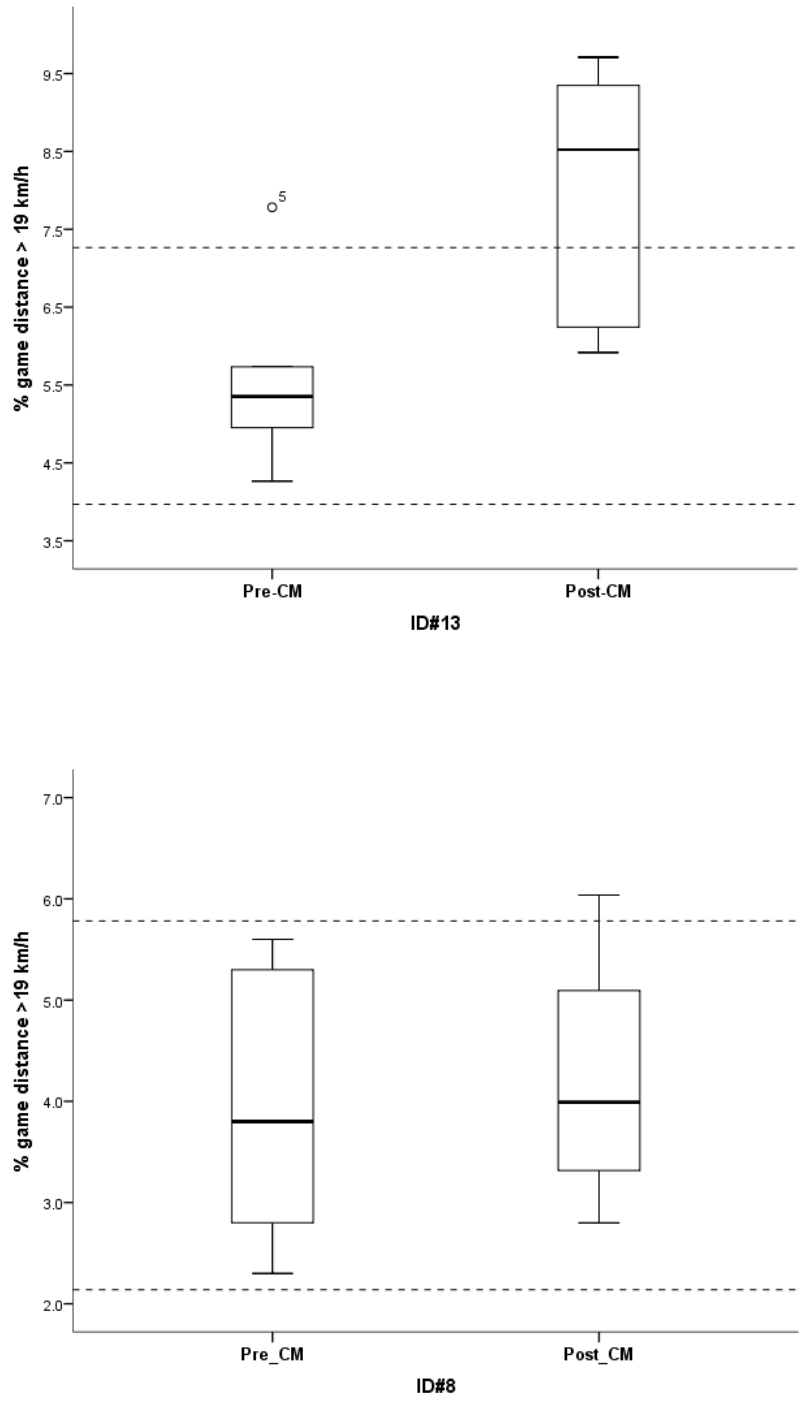

Figure 4. Box- and whisker-plots for overall distance *per* game covered at high-intensity (*i.e.*, speed >19 km·h^{-1} expressed as % of the total time spent on the field) in two U18 football Academy players before (pre) and after (post) 8 weeks of supplementation with creatine monohydrate (CM): ID#13 played as centre forward, while ID#8 played as centre back. Broken lines represent lower and upper bound for 95% confidence interval

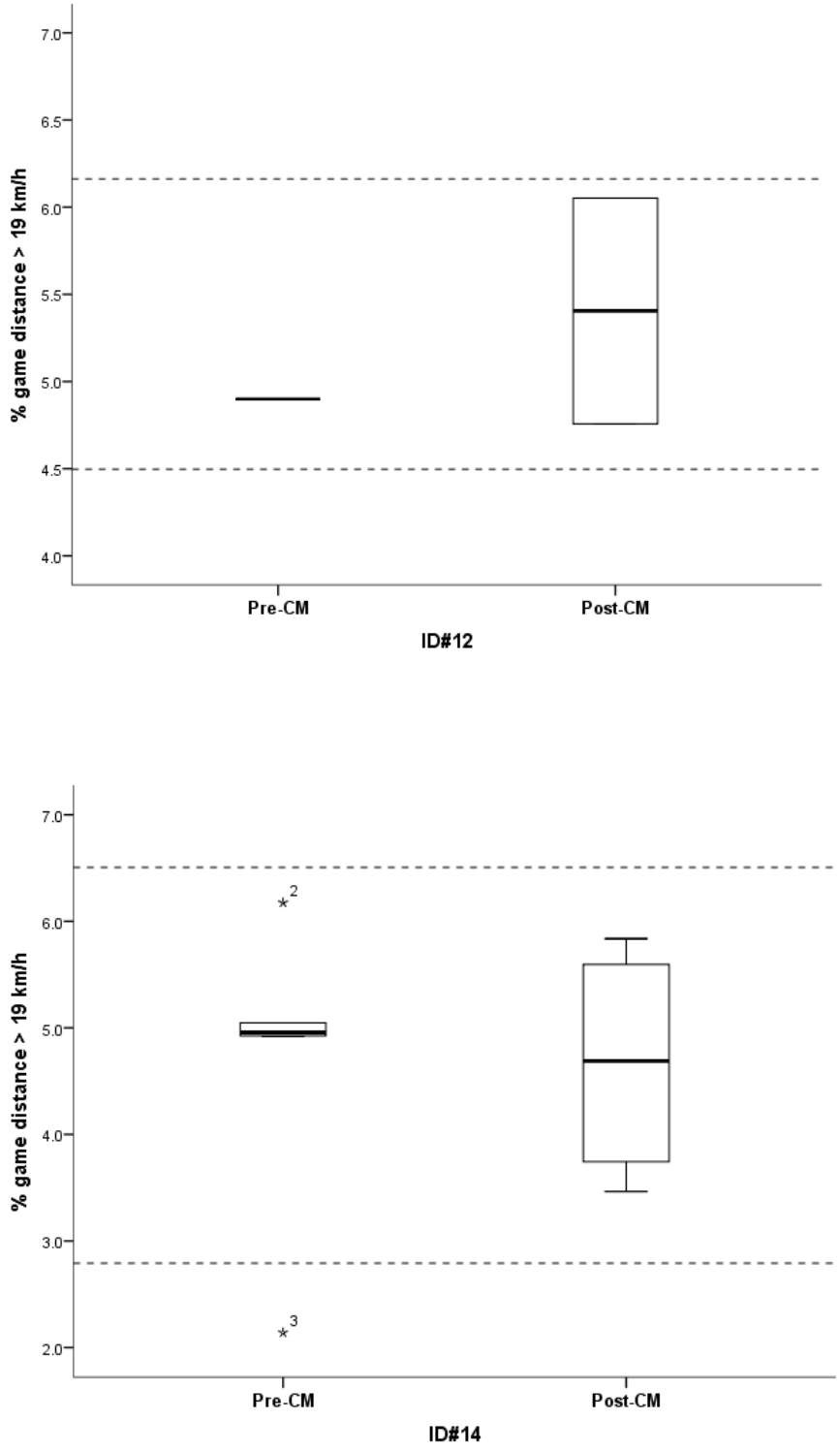

Figure 5. Box- and whisker-plots for overall distance *per* game covered at high-intensity (*i.e.*, speed > 19 km·h^{-1} expressed as % of the total time spent on the field) in two U18 centre midfielders before (pre) and after (post) 8 weeks of supplementation with creatine monohydrate (CM). Broken lines represent lower and upper bound for 95% confidence interval. Note that for player ID#12 GPS data were available for only one game pre-supplementation; hence the 95% CI was calculated combining the pre-supplementation data available from all the midfielders.

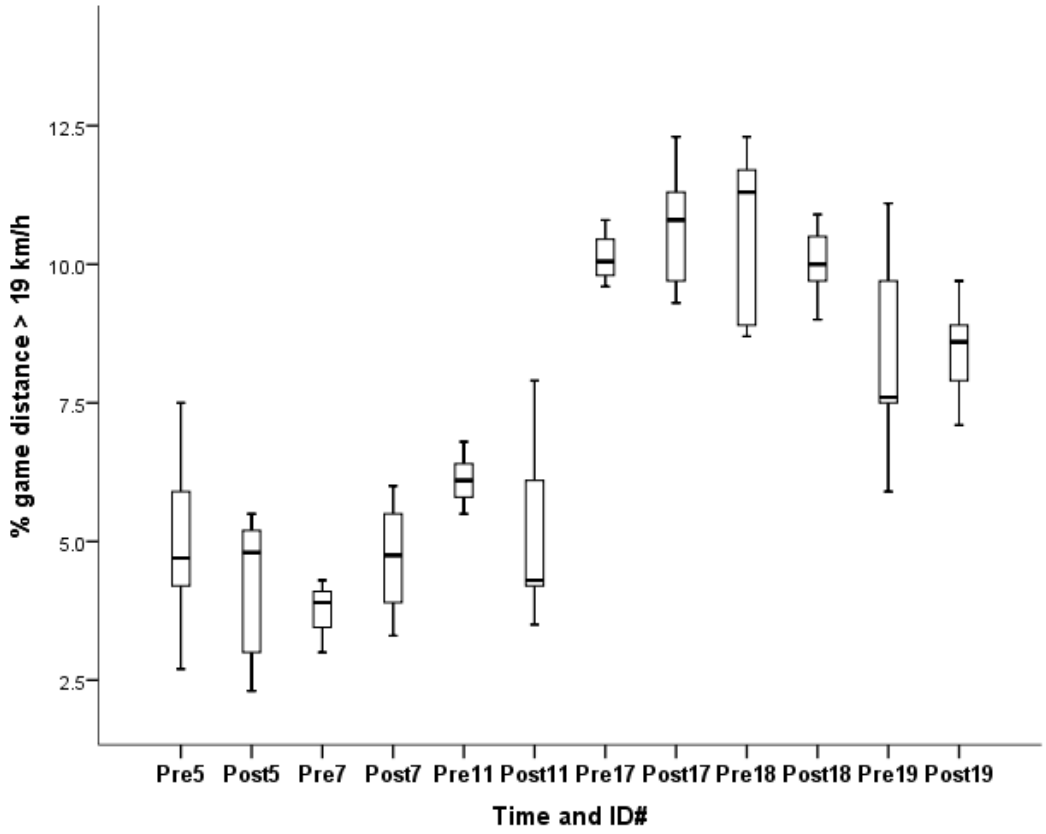

Figure 7. Box- and whisker-plots for overall distance *per* game covered at high-intensity (*i.e.*, speed >19 km·h^{-1} expressed as % of the total time spent on the field) in six U18 football Academy players before (pre) and after (post) 8 weeks of supplementation with a placebo (maltodextrin). Playing positions were as follows: ID#5 and ID#7, centre backs; ID#11, centre midfielder; ID#17 and ID#19, centre forwards; and ID#18, left wing back

DISCUSSION

Main findings

The primary aim of this study was to determine whether eight weeks of creatine supplementation in combination with football-specific training increased airway inflammation and airway responsiveness in youth, elite football players. A trend for increased F_ENO values was noticed post-supplementation in male, U18 and U21 Academy players taking creatine, but not in those under placebo; therefore, we cannot exclude the potential for creatine to increase airway inflammation in susceptible, youth, elite football players. While airway responsiveness remained essentially unchanged over the 8-wk supplementation period in the creatine group, the placebo group had a reduced airway response to dry air after 8 weeks; this suggests that creatine supplementation may interfere with the natural variability in airway responsiveness over the course of a football season.

Our secondary aim was to establish whether a standard course of creatine supplementation improved match performance of youth, elite football players alongside 'traditional' indices of fitness performance. Our results suggest that neither ballgame performance (as assessed by GPS data during competitive games), nor football-specific, field-based, fitness tests (*i.e.*, RSA and Yo-Yo-IR2) significantly increase after creatine supplementation in youth, highly-trained, male players.

Together, these results therefore cast doubt about both, the safety and the effectiveness of creatine as an ergogenic aid in youth football.

Creatine and lung health

To the best of our knowledge, this is the first study to evaluate the possible adverse effects of creatine supplementation on lung function in humans. Whilst the safety of creatine has been widely investigated in the past through serum and urinary clinical health markers of metabolic, hepatic, renal and muscular function (Kreider et al., 2003, Cancela et al., 2008) – the general consensus being that, at the recommended dosage, creatine is safe (Buford et al., 2007, Cooper et al., 2012) –, no such data are available as yet for lung function.

Our data showed a trend for airway inflammation – as measured by increased F_ENO – to increase after 8-wk of a standard course of creatine supplementation. These results are in line with previous animal-based studies (Ferreira et al., 2010, Vieira et al., 2007). In mice sensitized with intraperitoneal injections of ovalbumin (a model of asthma), 32 days of creatine supplementation led to increased airway inflammation, as measured by the infiltration of eosinophils and pro-inflammatory cytokines in the airways (Vieira et al., 2007). F_ENO is a well-established marker for eosinophilic airway inflammation in humans (Dweik et al., 2011), which is widely used in clinics and in research to indirectly – and non-invasively – detect lower airway inflammation. That 44% of the atopic players supplemented with creatine had an increase in F_ENO >10 ppb [*i.e.*, the minimum significance threshold for intervention trials (Dweik et al., 2011)] post-supplementation suggests that a sub-group of players could be particularly susceptible to the pro-inflammatory effect of creatine that is thought mediated by activation of airway epithelial cells (Ferreira et al., 2010).

Due to time frame of the funding, we were unable to tests players over the spring and summer seasons (*i.e.*, when the pollen count is high in the UK). It is however likely that, when intensive training takes place during the natural pollen season, some of the observed changes are exaggerated, particularly in those players with pollen sensitization.

In our study ~60% of the youth players were sensitized to grass pollen. This high prevalence of atopy is in line with the observation of widespread allergic diseases (particularly rhinitis) in elite sport (Alaranta et al., 2005). High pollen levels have previously been recorded, on and around training and competition venues (Katelaris et al., 2000). Further, natural exposure to airborne allergens during the pollen season has been shown to be associated with an increase in airway responsiveness (Prieto et al., 2002) and in F_ENO in non-asthmatic, nonathletic individuals with allergic rhinitis (Henriksen et al., 1999, Prieto et al., 2002, Ciebiada et al., 2012). In elite Finnish runners, Helenius et *al.* (1998b) showed that allergic sensitization is a major risk factor for asthma/EIB (with odds ratios of increased airway responsiveness and asthma increasing with the number of positive skin test reactions). Furthermore, the same authors (Helenius et al., 1998a) observed that some elite athletes develop mild EIB only during the pollen season. We therefore cannot exclude that the pro-inflammatory effect of creatine puts susceptible, atopic football players at increased risk for asthma during the natural pollen season.

In our study, we noticed a slight improvement in airway responsiveness to dry air in the placebo group post-supplementation (but not in the creatine group), suggesting that creatine may somehow alter the natural fluctuation in airway responsiveness occurring over the course of a competitive season in youth, elite players. It is not the first time that fluctuations in airway responsiveness have been observed in elite athletes. Seasonal variability in the airway response to exercise has previously been observed in elite runners (Helenius et al., 1998a). More recently, Bougault *et al.* (2011) also highlighted the transient nature of AHR in elite swimmers, with AHR being mainly present at times of intensive training (but not 'out of season'). Whether repetitive courses of creatine supplementation alter the natural variability

of airway responsiveness in elite athletes and increase the risk for asthma has yet to be established.

Asthma in football

Throughout this trial, we identified five players (25%) with asthma or EIB, with only one having a previous medical diagnosis. In our study, we used the recommended test (*i.e.*, EVH of dry air) for asthma/EIB detection in elite athletes (Anderson et al., 2001). The high potency of this test makes it superior to exercise (Dickinson et al., 2006) and allows detection of even mild EIB, as commonly observed in elite athletes (Helenius et al., 1998a). Our results are in line with previous data (based on the same methodology) that showed a prevalence of asthma of ~21% in elite (Olympic) UK athletes (Dickinson et al., 2005). Our data reinforce the idea that incorrect diagnoses (un-diagnosis and misdiagnosis) of asthma/EIB are commonplace in elite football (Ansley et al., 2012, Dickinson et al., 2011). Since high-intensity training is thought to trigger or exacerbate respiratory dysfunctions in susceptible athletes (Kippelen and Anderson, 2012, Anderson and Kippelen, 2008), in line with previous recommendations (Ansley et al., 2012, Holzer and Brukner, 2004, Dickinson et al., 2005, Dickinson et al., 2006) our data support the need for implementing systematic screening programs for asthma/EIB in professional football.

Creatine and football performance

This is the first study to investigate the effect of creatine supplementation on real-life performance (as assessed by GPS tracking during competitive games) in football players. Our results do not support an ergogenic effect of the supplement on match performance in youth,

male, elite players, in that we showed no improvement in match performance (incl. high-intensity running) in the creatine group post-supplementation. Even though our match performance results are to be interpreted with caution (our final small sample size being limited to four participants), we found no concomitant improvements in football-specific, fitness test results (*i.e.*, Yo-Yo-IR2 and RSA).

Both the Yo-Yo-IR2 and the RSA are routinely used tests to measure fitness performance in professional soccer players. The Yo-Yo-IR2 has originally been designed to replicate the physiological demands of football games (Bangsbo, 1994); its reliability and validity to evaluate a player's ability to perform intense intermittent exercise with a high rate of aerobic and anaerobic turnover is well established (Krustrup et al., 2006). As for the repeated sprint test, RSA performance has been shown to relate to maximal aerobic capacity (VO_2max) and to selected physiological responses to a standardized, high-intensity, intermittent running (Rampinini et al., 2009). The use of both these tests was therefore particularly relevant in the context of our work.

The absence of effect of creatine supplementation on our performance indexes is in line with a recent study that demonstrated no beneficial effects of a short (7 days) course of creatine supplementation (20 g of Cr per day) on simulated game play [as assessed by ball-sport endurance and speed test, comprising measures of aerobic (circuit time), speed (12 and 20m sprint) and explosive power (vertical jump) abilities over 90 min] in amateur, male soccer players (aged ~26 yr) (Williams et al., 2014). To date there is only one single study that showed the potential for creatine to act as an effective ergogenic aid for soccer players. Cox *et al.* (2002) noticed an improvement in some (not all) sprint and agility tasks stimulating soccer match play in elite, female soccer players after 6 days of creatine supplementation (20

g of Cr per day). Whether those positive effects are sustained past the loading phase and are found in male players remains uncertain.

Alongside the lack of effect of creatine supplementation on performance indexes, we noticed no differences in lower body strength and body composition post-supplementation between our creatine and placebo groups. Skinfold thickness was reduced post-supplementation in both groups, while body mass remained unchanged. This suggests that creatine did not induce muscle mass gain beyond that observed with training alone. This result is in line with findings obtained in NCAA Division IA collegiate (19 ± 1.02 yr), male American football players (Wilder et al., 2001); ten weeks of creatine supplementation (at either 3 g per day, or 20 g per day for 7 days, followed by 5 g per day for the remainder of the study) combined with an off-season resistance training and conditioning program did not improve fat-free mass or lower body strength above the effects of resistance training alone in that highly-trained, young, male population.

Physiologically, these results can be explained by the fact that creatine does not seem to have a direct anabolic effect on protein synthesis (Parise et al., 2001, Louis et al., 2003) and muscle hypertrophy (Dangott et al., 2000). A recent animal study showed that the benefits of creatine supplementation on muscle mass gain, beyond what is observed with training alone, is dependent on an higher workload of supplemented trained muscles in relation to non-supplemented trained muscles (Aguiar et al., 2011).

In our study, we retrospectively looked at the number of strength and conditioning training sessions and time spent in the gym by the U18 players over the course of the supplementation period. Data were not significantly different between groups [all players did between nine to ten strength and conditioning sessions over the 8-wk period, and the average time spent at the gym was of 414 ± 20 min in the creatine group *versus* 405 ± 21 min in the placebo group

(P>0.05)]. This result highlights that, during the competitive phase of a football season (*i.e.*, when the training focus is not on development of strength and conditioning), youth, male, elite players gain no benefits (in terms of body composition and muscle strength) from creatine supplementation.

Study limitations

Due to the many drop-outs (linked to injuries, players going on loans, or non-adherence to the supplementation protocol), we ended up with a somewhat limited sample size for some of our statistical analyses (especially in regards to match analysis).

For F_ENO values, between- and within-group comparisons were all close to (*P* values between 0.05 and 0.10), but did not quite reach statistical significance. Large inter-individual variability in F_ENO has been observed in the past; the distribution of F_ENO in an unselected population has been shown to be skewed to the right [with a median value of 16.0 ppb and a range of 2.4 to 199 ppb (Dweik et al., 2011)]. In our study, F_ENO ranged from 14 to 86 ppb pre-supplementation and from 12 to 125 ppb post-supplementation. Even when individuals with atopy or diagnosed asthma are excluded, the upper limit of 'normal' ranges for F_ENO have been shown to be significantly influenced by the age and height of the individuals (Olin et al., 2007). Whilst the age of our study population was homogeneous (range: 16-21 yr), height varied from 168.9 cm to 186.8 cm, which could have contributed to the large spread of F_ENO data and limited our ability to reach statistical significance with our intervention.

One of the inherent limitations of GPS game analysis in football is the high match-to-match variation in physical performance linked to un-controllable factors (*i.e.*, change in opposition, the weather, the score, and position in league/competition) (Hewitt et al., 2014). However, to

maintain ecological validity, this real-life approach was deemed most appropriate in the context of our work. While random allocation to experimental groups is good research practice (to avoid any possibility of selection bias), in our study, the randomisation led to an uneven split of players from various positions between the two groups (*e.g.*, both U18 goalkeepers were randomised to the creatine group and GPS data were not recorded in those players). The physiological demand of a football match is known to vary greatly with playing positions (Mohr et al., 2003, Gonçalves et al., 2014), mainly in line with the different tactical roles and physical capacities of the players (Bangsbo, 1994). In our study design, the use of an additional strata based on the different playing positions would have required a very large initial sample size, which was not available to us (we initially recruited all the U18 and U21 players from Watford FC Academy). The recruitment of players from another Academy would not have been suitable either, since too many potential confounding factors would then have been introduced (*e.g.*, different training regimes and diet).

Practical implications and recommendations to UEFA

Notwithstanding the above-mentioned limitations, several practical recommendations can be drawn from our work.

First, based on the fact that 25% of the youth, elite players recruited in this study tested positive for asthma/EIB (with only one player having a prior medical diagnosis), systematic screening for asthma/EIB should be implemented in football Academies. Alike the successful pre-participation cardiac screening implemented in elite sport in some European countries (Corrado et al., 2006), respiratory screening should become routine in elite football. Even if less mediatised than sudden cardiac deaths (since usually happening shortly after cessation of – not during –exercise, *i.e.*, when athletes are away from the 'spotlights'), asthma deaths

occur in sport (Becker et al., 2004), and youth, white males are at highest risk (Becker et al., 2004). Screening and education programs are likely to prevent some of those deaths; in the UK alone, a staggering 50% of asthma deaths could be avoided with better routine care (Royal College of Physicians, 2014). Starting the screening already at Academy level is important, in that it shall ensure early detection of respiratory dysfunctions in youth, elite players. As demonstrated by the successful asthma program run by the IOC medical commission between 2001 to 2009 (Fitch, 2012), objective, standardised and validated procedures should be used to detect and improve the management of asthma/EIB in athletes. Based on bronchodilator tests and/or bronchial provocation test results (Boulet and O'Byrne, 2015), health care professionals will be able to put youth athletes with asthma/EIB under appropriate pharmacotherapy. This approach will benefit the players, their club, and European football as a whole, in that it will ensure that players with asthma/EIB can reach their full physical potential and produce their best possible performance when playing competitively. Furthermore, it will minimize the risk for their sporting career to have long-term damaging effect on their respiratory health.

Since safeguarding the players' health is part of UEFA's mission statement, and considering the capacity of UEFA to generate impact, UEFA should consider implementing a European-wide education campaign on asthma/EIB and to promote the use of screening programs in professional football clubs and football Academies. Education of club doctors is also warranted to ensure the best level of care is provided to all players (not only to the elite athletes). Whilst asthma/EIB is nowadays a recognised issue in elite sport (Fitch et al., 2008, Fitch, 2012), evidence suggest that the condition may also be under-recognised (and hence, poorly managed) in recreational sport (Molphy et al., 2014, Kukafka et al., 1998). In line with previous work (Rundell et al., 2001, Holzer et al., 2002), we recently demonstrated that a

symptom-based approach for EIB diagnosis – as commonly used by physicians – is highly inappropriate in athletes (Simpson et al., 2015). Hence, club doctors need to be made aware of the latest recommendations for asthma/EIB detection and management in athletes (Boulet and O'Byrne, 2015) to provide the best level of care to the players.

As 21% of the U18 players tested in this study were not able to reach the minimum recommended ventilatory threshold of 60% pred. MVV during the EVH test (Anderson et al., 2001), follow-up research seems warranted. Future studies should establish whether EVH is the most appropriate bronchial provocation test to be used in a youth (and still maturing) players to detect asthma/EIB, and whether adjustments in the target ventilation are necessary.

Considering our negative findings on the effect of creatine supplementation on football performance – as assessed by sport-specific, field-based fitness tests and GPS match performance data – and the trend for an increased F_ENO post-creatine supplementation, the potential health risks of the nutritional supplement seem to outweigh its performance effects. Until further work has been done to investigate in depth (*via* multiple, direct and indirect markers of airway inflammation) the effects of creatine supplementation on lung health in the exercising human during the natural pollen season, caution should prevail, *i.e.*, youth, elite football players *should not* be encouraged to use creatine supplements.

CONCLUSION

In summary, we showed a trend for F_ENO to increase and for the natural course of airway responsiveness to be altered after a standard course of creatine supplementation in youth, male, elite football players. Based on those findings, we cannot exclude that, in susceptible players, creatine supplementation in combination with high-intensity, football-specific training may increase inflammation of the airways. Our findings also suggest that creatine supplementation does not promote gains in muscle mass or in muscular strength in youth, male, elite players beyond the effects observed with football training alone. Finally, 8wk of creatine supplementation did not lead to an improvement in fitness levels or match performance (as assessed by GPS tracking during competitive games) in youth, elite football players. Altogether, these results indicate that the health risks of the nutritional supplement outweigh its ergogenic effects; therefore, creatine may not be a worthwhile strategy for youth, male, elite football players and its use *should not* be promoted in football Academies.

REFERENCES

Aguiar, A. F., de Souza, R. W., Aguiar, D. H., Aguiar, R. C., Vechetti, I. J. & Dal-Pai-Silva, M. 2011. Creatine does not promote hypertrophy in skeletal muscle in supplemented compared with nonsupplemented rats subjected to a similar workload. *Nutr Res,* **31,** 652-7.

Alaranta, A., Alaranta, H., Heliövaara, M., Alha, P., Palmu, P. & Helenius, I. 2005. Allergic rhinitis and pharmacological management in elite athletes. *Med Sci Sports Exerc,* **37,** 707-11.

American Thoracic Society & European Respiratory Society. 2005. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med,* **171,** 912-30.

Anderson, S. D., Argyros, G. J., Magnussen, H. & Holzer, K. 2001. Provocation by eucapnic voluntary hyperpnoea to identify exercise induced bronchoconstriction. *Br J Sports Med,* **35,** 344-7.

Anderson, S. D. & Kippelen, P. 2008. Airway injury as a mechanism for exercise-induced bronchoconstriction in elite athletes. *J Allergy Clin Immunol,* **122,** 225-35.

Anderson, S. D. & Kippelen, P. 2012. Assessment and prevention of exercise-induced bronchoconstriction. *Br J Sports Med,* **46,** 391-6.

Ansley, L., Kippelen, P., Dickinson, J. & Hull, J. H. 2012. Misdiagnosis of exercise-induced bronchoconstriction in professional soccer players. *Allergy,* **67,** 390-5.

Baltzopoulos, B., King, M., Gleeson, N. & De Ste Croix, M. 2012. The BASES Expert Statement on Measurement of Muscle Strength with Isokinetic Dynamometry. *The Sport and Exercise Scientist.,* **Spring,** 12-13.

Bangsbo, J. 1994. Fitness training in football - A scientific approach. Bagsvaerd, Denmark, HO+Storm.

Becker, J. M., Rogers, J., Rossini, G., Mirchandani, H. & D'Alonzo, G. E. 2004. Asthma deaths during sports: report of a 7-year experience. *J Allergy Clin Immunol,* **113,** 264-7.

Boden, B. P., Breit, I., Beachler, J. A., Williams, A. & Mueller, F. O. 2013. Fatalities in high school and college football players. *Am J Sports Med,* **41,** 1108-16.

Bougault, V., Turmel, J. & Boulet, L. P. 2011. Airway hyperresponsiveness in elite swimmers: is it a transient phenomenon? *J Allergy Clin Immunol,* **127,** 892-8.

Boulet, L. P. & O'Byrne, P. M. 2015. Asthma and exercise-induced bronchoconstriction in athletes. *N Engl J Med,* **372,** 641-8.

Bousquet, J., Heinzerling, L., Bachert, C., Papadopoulos, N. G., Bousquet, P. J., Burney, P. G., Canonica, G. W., Carlsen, K. H., Cox, L., Haahtela, T., Lodrup Carlsen, K. C., Price, D., Samolinski, B., Simons, F. E., Wickman, M., Annesi-Maesano, I.*, et al.* 2012. Practical guide to skin prick tests in allergy to aeroallergens. *Allergy,* **67,** 18-24.

Buford, T. W., Kreider, R. B., Stout, J. R., Greenwood, M., Campbell, B., Spano, M., Ziegenfuss, T., Lopez, H., Landis, J. & Antonio, J. 2007. International Society of Sports Nutrition position stand: creatine supplementation and exercise. *J Int Soc Sports Nutr,* **4,** 6.

Cancela, P., Ohanian, C., Cuitiño, E. & Hackney, A. C. 2008. Creatine supplementation does not affect clinical health markers in football players. *Br J Sports Med,* **42,** 731-5.

Ciebiada, M., Cichocki, P., Kasztalska, K., Majewski, S., Gorska-Ciebiada, M. & Gorski, P. 2012. Orally exhaled nitric oxide in patients with seasonal allergic rhinitis during natural pollen season. *Am J Rhinol Allergy,* **26,** e32-6.

Cooper, R., Naclerio, F., Allgrove, J. & Jimenez, A. 2012. Creatine supplementation with specific view to exercise/sports performance: an update. *J Int Soc Sports Nutr,* **9,** 33.

Corrado, D., Basso, C., Pavei, A., Michieli, P., Schiavon, M. & Thiene, G. 2006. Trends in sudden cardiovascular death in young competitive athletes after implementation of a preparticipation screening program. *JAMA,* **296,** 1593-601.

Cox, G., Mujika, I., Tumilty, D. & Burke, L. 2002. Acute creatine supplementation and performance during a field test simulating match play in elite female soccer players. *Int J Sport Nutr Exerc Metab,* **12,** 33-46.

Dangott, B., Schultz, E. & Mozdziak, P. E. 2000. Dietary creatine monohydrate supplementation increases satellite cell mitotic activity during compensatory hypertrophy. *Int J Sports Med,* **21,** 13-6.

Dickinson, J., McConnell, A. & Whyte, G. 2011. Diagnosis of exercise-induced bronchoconstriction: eucapnic voluntary hyperpnoea challenges identify previously undiagnosed elite athletes with exercise-induced bronchoconstriction. *Br J Sports Med,* **45,** 1126-31.

Dickinson, J. W., Whyte, G. P., McConnell, A. K. & Harries, M. G. 2005. Impact of changes in the IOC-MC asthma criteria: a British perspective. *Thorax,* **60,** 629-32.

Dickinson, J. W., Whyte, G. P., McConnell, A. K. & Harries, M. G. 2006. Screening elite winter athletes for exercise induced asthma: a comparison of three challenge methods. *Br J Sports Med,* **40,** 179-82; discussion 179-82.

Dressel, H., de la Motte, D., Reichert, J., Ochmann, U., Petru, R., Angerer, P., Holz, O., Nowak, D. & Jörres, R. A. 2008. Exhaled nitric oxide: independent effects of atopy, smoking, respiratory tract infection, gender and height. *Respir Med,* **102,** 962-9.

Dweik, R. A., Boggs, P. B., Erzurum, S. C., Irvin, C. G., Leigh, M. W., Lundberg, J. O., Olin, A. C., Plummer, A. L., & Taylor, D. R. on behalf of the American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. 2011. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med,* **184,** 602-15.

Ferreira, S. C., Toledo, A. C., Hage, M., Santos, A. B., Medeiros, M. C., Martins, M. A., Carvalho, C. R., Dolhnikoff, M. & Vieira, R. P. 2010. Creatine activates airway epithelium in asthma. *Int J Sports Med,* **31,** 906-12.

Fitch, K. D. 2012. An overview of asthma and airway hyper-responsiveness in Olympic athletes. *Br J Sports Med,* **46,** 413-6.

Fitch, K. D., Sue-Chu, M., Anderson, S. D., Boulet, L. P., Hancox, R. J., McKenzie, D. C., Backer, V., Rundell, K. W., Alonso, J. M., Kippelen, P., Cummiskey, J. M., Garnier, A. & Ljungqvist, A. 2008. Asthma and the elite athlete: summary of the International Olympic Committee's consensus conference, Lausanne, Switzerland, January 22-24, 2008. *J Allergy Clin Immunol,* **122,** 254-60, 260.e1-7.

Gonçalves, B. V., Figueira, B. E., Maçãs, V. & Sampaio, J. 2014. Effect of player position on movement behaviour, physical and physiological performances during an 11-a-side football game. *J Sports Sci,* **32,** 191-9.

Harley, J. A., Barnes, C. A., Portas, M., Lovell, R., Barrett, S., Paul, D. & Weston, M. 2010. Motion analysis of match-play in elite U12 to U16 age-group soccer players. *J Sports Sci,* **28,** 1391-7.

Helenius, I. J., Tikkanen, H. O. & Haahtela, T. 1998a. Occurrence of exercise induced bronchospasm in elite runners: dependence on atopy and exposure to cold air and pollen. *Br J Sports Med,* **32,** 125-9.

Helenius, I. J., Tikkanen, H. O., Sarna, S. & Haahtela, T. 1998b. Asthma and increased bronchial responsiveness in elite athletes: atopy and sport event as risk factors. *J Allergy Clin Immunol,* **101,** 646-52.

Henriksen, A. H., Sue-Chu, M., Holmen, T. L., Langhammer, A. & Bjermer, L. 1999. Exhaled and nasal NO levels in allergic rhinitis: relation to sensitization, pollen season and bronchial hyperresponsiveness. *Eur Respir J,* **13,** 301-6.

Hewitt, A., Norton, K. & Lyons, K. 2014. Movement profiles of elite women soccer players during international matches and the effect of opposition's team ranking. *J Sports Sci,* **32,** 1874-1880.

Holzer, K., Anderson, S. D. & Douglass, J. 2002. Exercise in elite summer athletes: Challenges for diagnosis. *J Allergy Clin Immunol,* **110,** 374-80.

Holzer, K. & Brukner, P. 2004. Screening of athletes for exercise-induced bronchoconstriction. *Clin J Sport Med,* **14,** 134-8.

Katelaris, C. H., Carrozzi, F. M., Burke, T. V. & Byth, K. 2000. A springtime olympics demands special consideration for allergic athletes. *J Allergy Clin Immunol,* **106,** 260-6.

Kippelen, P. & Anderson, S. D. 2012. Airway injury during high-level exercise. *Br J Sports Med,* **46,** 385-90.

Kreider, R. B., Melton, C., Rasmussen, C. J., Greenwood, M., Lancaster, S., Cantler, E. C., Milnor, P. & Almada, A. L. 2003. Long-term creatine supplementation does not significantly affect clinical markers of health in athletes. *Mol Cell Biochem,* **244,** 95-104.

Krustrup, P., Mohr, M., Nybo, L., Jensen, J. M., Nielsen, J. J. & Bangsbo, J. 2006. The Yo-Yo IR2 test: physiological response, reliability, and application to elite soccer. *Med Sci Sports Exerc,* **38,** 1666-73.

Kukafka, D. S., Lang, D. M., Porter, S., Rogers, J., Ciccolella, D., Polansky, M. & D'Alonzo, G. E. 1998. Exercise-induced bronchospasm in high school athletes via a free running test: incidence and epidemiology. *Chest,* **114,** 1613-22.

Louis, M., Poortmans, J. R., Francaux, M., Berré, J., Boisseau, N., Brassine, E., Cuthbertson, D. J., Smith, K., Babraj, J. A., Waddell, T. & Rennie, M. J. 2003. No effect of creatine supplementation on human myofibrillar and sarcoplasmic protein synthesis after resistance exercise. *Am J Physiol Endocrinol Metab,* **285,** E1089-94.

Metzl, J. D., Small, E., Levine, S. R. & Gershel, J. C. 2001. Creatine use among young athletes. *Pediatrics,* **108,** 421-5.

Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., van der Grinten, C. P., Gustafsson, P., Jensen, R., Johnson, D. C., MacIntyre, N., McKay, R., Navajas, D., Pedersen, O. F.*, et al.* 2005. Standardisation of spirometry. *Eur Respir J,* **26,** 319-38.

Mohr, M., Krustrup, P. & Bangsbo, J. 2003. Match performance of high-standard soccer players with special reference to development of fatigue. *J Sports Sci,* **21,** 519-28.

Molphy, J., Dickinson, J., Hu, J., Chester, N. & Whyte, G. 2014. Prevalence of bronchoconstriction induced by eucapnic voluntary hyperpnoea in recreationally active individuals. *J Asthma,* **51,** 44-50.

Olin, A. C., Bake, B. & Torén, K. 2007. Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers. *Chest,* **131,** 1852-6.

Osgnach, C., Poser, S., Bernardini, R., Rinaldo, R. & di Prampero, P. E. 2010. Energy cost and metabolic power in elite soccer: a new match analysis approach. *Med Sci Sports Exerc,* **42,** 170-8.

Parise, G., Mihic, S., MacLennan, D., Yarasheski, K. E. & Tarnopolsky, M. A. 2001. Effects of acute creatine monohydrate supplementation on leucine kinetics and mixed-muscle protein synthesis. *J Appl Physiol (1985),* **91,** 1041-7.

Prieto, L., Uixera, S., Gutiérrez, V. & Bruno, L. 2002. Modifications of airway responsiveness to adenosine 5'-monophosphate and exhaled nitric oxide concentrations after the pollen season in subjects with pollen-induced rhinitis. *Chest,* **122,** 940-7.

Quanjer, P. H., Stanojevic, S., Cole, T. J., Baur, X., Hall, G. L., Culver, B. H., Enright, P. L., Hankinson, J. L., Ip, M. S., Zheng, J., Stocks, J. & Initiative, E. G. L. F. 2012. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J,* **40,** 1324-43.

Rampinini, E., Sassi, A., Morelli, A., Mazzoni, S., Fanchini, M. & Coutts, A. J. 2009. Repeated-sprint ability in professional and amateur soccer players. *Appl Physiol Nutr Metab,* **34,** 1048-54.

Royal College of Physicians, 2014. Why asthma still kills: the National Review of Asthma Deaths (NRAD). http://www.asthma.org.uk/nrad-report. [assessed 30/03/2015].

Rundell, K. W., Im, J., Mayers, L. B., Wilber, R. L., Szmedra, L. & Schmitz, H. R. 2001. Self-reported symptoms and exercise-induced asthma in the elite athlete. *Med Sci Sports Exerc,* **33,** 208-13.

Simpson, A. J., Romer, L. M. & Kippelen, P. 2015. Self-reported Symptoms after Induced and Inhibited Bronchoconstriction in Athletes. *Med Sci Sports Exerc*. [Epub ahead of print].

Syrotuik, D. G. & Bell, G. J. 2004. Acute creatine monohydrate supplementation: a descriptive physiological profile of responders vs. nonresponders. *J Strength Cond Res,* **18,** 610-7.

Vieira, R. P., Duarte, A. C., Claudino, R. C., Perini, A., Santos, A. B., Moriya, H. T., Arantes-Costa, F. M., Martins, M. A., Carvalho, C. R. & Dolhnikoff, M. 2007. Creatine supplementation exacerbates allergic lung inflammation and airway remodeling in mice. *Am J Respir Cell Mol Biol,* **37,** 660-7.

Waddington, I., Malcolm, D., Roderick, M. & Naik, R. 2005. Drug use in English professional football. *Br J Sports Med,* **39,** e18; discussion e18.

Wilder, N., Deivert, R. G., Hagerman, F. & Gilders, R. 2001. The Effects of Low-Dose Creatine Supplementation Versus Creatine Loading in Collegiate Football Players. *J Athl Train,* **36,** 124-129.

Williams, J., Abt, G. & Kilding, A. E. 2014. Effects of creatine monohydrate supplementation on simulated soccer performance. *Int J Sports Physiol Perform,* **9,** 503-10.

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Dr Pascale Kippelen
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21st July 2014

Dear Pascale

RE69-13 Creatine supplementation in youth football players

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application connected to the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University/School Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to grant ethics approval to the proposed study.

Any changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee for review.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your study.

Yours sincerely

Dr Richard J Godfrey
Chair of Research Ethics Committee
School Of Sport and Education

PARTICIPANT INFORMATION SHEET

Creatine supplementation in youth football players

We would like to invite you to take part in a research project. Before you decide whether to take part, we'd like to tell you more about the project and what it would involve for you.

What is the purpose of the study? Creatine is the most effective performance-enhancing nutritional supplement currently available to athletes in terms of increasing high-intensity exercise capacity and muscle mass during training. The use of creatine as a nutritional supplement is considered effective, safe, and ethical. With this study, we want to determine whether field-based, football-specific performance of youth football players is improved following a typical course of creatine supplementation. We also want to confirm that, at the recommended dosage, creatine has no negative effect on the health of youth players.

Why have been invited to participate? You have been invited to take part since you are part of an under 18 (U18) or under 21 (U21) academy football team.

Do I have to take part? No. Your participation is voluntary and you can withdraw at any time without penalty or giving any reasons.

What will happen to me if I take part? You will be asked to attend several visits at your club training ground during which we will evaluate your general fitness level, your football-specific performance, and your health. Each visit will take up to 3 h. You will also be asked to take a standard dosage of creatine or a placebo (an inactive substance) for 8 weeks.

What do I have to do?

Initial assessment (pre-supplementation):

- You will be asked to provide a urine sample (to check your kidney function).
- You will be asked to perform various breathing manoeuvres (to assess your respiratory health). These will involve full inhalation and full exhalation (at various speeds) through a mouthpiece, while connected to two different breathing apparatus (pictured below). You will be asked to perform each maneuver 3 to 8 times on each apparatus to get consistent readings.

Fig1. Spirometer

Fig2. Exhaled nitric oxide analyser

- You will be asked to perform a dry air challenge (to determine whether you have overly twitchy airways, which may cause asthma attacks during exercise). The test involves hyperventilating dry air at a high flow rate for 6 minutes. The rate will be similar to that associated with very hard exercise, but you will be sitting in a chair. The gas mixture that you will breathe comprises medical air and a small amount of carbon dioxide. The carbon dioxide is a gas that is exhaled naturally, and is added to the gas mixture to prevent you becoming light-headed during the hyperventilation. The hyperventilation of the dry air may induce wheezing and shortness of breath similar to an asthma attack. The dry air may also induce some salivation. After the hyperventilation challenge you will be allowed to recover spontaneously while your lung function is measured at 3, 5, 10, 15 and 20 minutes of recovery. If at any stage the wheeze or chest tightness becomes too uncomfortable, then the airway narrowing can be reversed quickly using bronchodilator.
- We will carry out a skin prick test. This involves putting drops of different substances on your forearm and then pricking the skin through the drop to test whether you have an allergic reaction to any of them.
- We will assess your body mass and body composition (% body fat) using a calibrated electronic scale and by measuring skinfold thickness at various sites of your body (using the same technique as used routinely in your club).
- You will be asked to perform two sets of maximal voluntary contractions (with appropriate recovery time in-between) on your club's isokinetic dynamometer. The protocol will consist of rapid repetitions of knee extension, whilst seated (and strapped) on the dynamometer's chair. The first set will be of 5 repetitions at 90 degrees *per* second. The second set will be of 15 repetitions at 180 degrees *per* second. This will allow us to determine your lower body strength and endurance.
- You will be asked to perform a repeated sprints test and an incremental treadmill test (with appropriate recovery time in-between). The protocols will not significantly differ to the ones you are familiar with, and will include the recording of your heart rate (with a chest belt) and of blood lactate (*via* a small pin-prick made in the earlobe with a sterilised disposable lancet). During the treadmill test, at regular intervals, we will ask you to wear a nose clip and to breathe through a

mouthpiece (to collect expired gas samples). We will ask you to run to exhaustion, so that we can determine your maximal level of oxygen consumption (VO_2max, an index of cardio-respiratory fitness).

Supplementation period:

- Once this initial assessment has been carried out, you will be randomly allocated to one of the following groups: creatine or placebo. Neither you, nor the researchers taking the measurements will know which group you are part of.
- During the first week of supplementation, you will be asked to ingest a dose of 0.3 g creatine/kg/day (or placebo). You will be requested to dissolve the powder provided in your usual protein drink and to take it at regular intervals (4 times a day).
- You will also be asked to record the food you eat and fluid you ingest over 3 non-consecutive days during the first week of supplementation.
- After a week, the dosage of the supplement will be reduced; over the following 7 weeks, you will be provided with just one intake per day of 5 g of creatine (or placebo), to take with your protein drink.
- You will be asked to record daily any side effects that you may get from the supplement.
- You will also need to let us know about any changes in any medication you take.
- GPS data collected on game days by your club in the period preceding the start of the supplementation will be analysed (so as to determine your 'baseline' field performance). All through the supplementation period, your training workload and performance during games will also be recorded *via* GPS tracking to identify changes.

Post-supplementation:

- You will be asked to repeat all the tests performed pre-supplementation, so that we can record changes in your performance and health parameters.

Pre-participation health check questionnaire. Health and safety within this investigation is of paramount importance. For this reason we need to be aware of your current health status before you begin any testing procedures. To identify whether you are able to participate in this investigation, we will ask you to fill in a standard pre-participation health check questionnaire.

Requirements and abstentions imposed upon the participants prior to the tests.

- There are certain standard restrictions on what you can eat or drink, what exercise you can perform, and what medications you can take ahead of the tests. You will be required to abstain from alcohol and caffeine from 20:00 h the evening before each study day (pre- and post-intervention), and no vigorous exercise will be permitted for 24 h before the fitness tests and the simulated game plays. You will also need to avoid exercising for at least 4 h before the breathing tests.
- For those of you on asthma treatment, you will need to withhold drug(s) for an appropriate period of time. Inhaled corticosteroids should not be taken on the day of the study. Inhaled short acting

beta2-agonist (blue reliever inhalers: Salbutamol, Terbutaline, Ventolin, Bricanyl, Salamol) need to be withhold for a minimum of 8 h; long acting beta2-agonist (Oxis, Foradil, Serevent, Seretide, Symbicort, Spiriva) for 48 h; anti-histamines, leukotriene antagonists (Singulair or Accolate) and non-steroidal anti-inflammatory drugs for 7 days; cromones (Intal or Tilade) for at least 48 h; ipratropium (Combivent) for at least 24 h and tiotropium (Spiriva) for at least 72 h. In case you are unable to withhold your medication(s) for the appropriate period of time, please get in touch with Dr Kippelen (contact details below) and she will be able to advise you on possible alternatives.

What are the possible benefits of taking part?

- This investigation will highlight whether the perceived performance gains associated with the ingestion of creatine are real.
- We will also check that your health does not deteriorate during the period of supplementation.
- For those of you with a physician-diagnosis of asthma/exercise-induced bronchoconstriction, performing the breathing tests is a way to assess that your treatment is adequate and that your condition is well controlled.

What are the possible disadvantages and risks of taking part?

- If you have a positive test result to the dry air challenge, you can expect to develop respiratory symptoms, such as shortness of breath and cough with mucus production. However these symptoms are only transient and, as such, should not affect you adversely. If at any stage the wheeze or chest tightness become too uncomfortable, then the airway narrowing will be reversed quickly using bronchodilator (i.e., reliever) inhalers. In the unlikely event of the tests inducing a severe asthma attack, the emergency services will be called immediately.
- Exercise testing comes with inherent discomfort and risk of injury. Discomforts include temporary fatigue and shortness of breath. This will normally subside a few minutes after the completion of the tests. There is also a low risk of injury (due to strained muscles) and of muscle soreness. The risks will be minimised by including warm-up and cool-down periods. Risk of sudden cardiac death associated with exercise is very rare, especially in young, highly-trained athletes like you.
- Allergy skin prick testing may induce local redness and itchiness (similar to a nettle sting). This will be reversed with an antihistamine cream. There is a very small risk (<0.02%) of anaphylactic reaction (i.e., severe allergic response) to skin prick testing. Before entering the study, we will ask you to report any history of severe allergic reaction.
- The earlobe pin-prick (for lactate measurement) will result in a slight transient ‘scratch’ sensation. There is the remote possibility of cross-contamination with the handling of blood samples and, as such, the researcher will wear disposable powder-free latex gloves during all experimental procedures. The Pre-Participation Health check Questionnaire requires you to state if you have Hepatitis, HIV or any blood clotting disorders.
- At the proposed dosage, creatine supplement is considered to be safe. However, to check for any possible side-effects, you will be asked to fill in a daily symptoms diary. A urine sample will also be

collected pre- and post-intervention to ensure your albumin levels remain within normal range (i.e., no sign of kidney problem).

What if something goes wrong? If you have any questions about the tests, please contact Dr Pascale Kippelen (contact details below). For complaints, you can contact the Chair of the School of Sport & Education Research Ethics Committee, Dr Richard Godfrey (richard.godfrey@brunel.ac.uk)

Will my taking part in this study be kept confidential? Your personal information will remain confidential, and we will not disclose any of your personal information without your permission. The data will be stored at the School of Sport & Education, Brunel University, for a maximum of 5 years.

What will happen to the results of the research study? The results may be presented at national/international scientific conferences and in research publications. Anonymity of your data will be preserved at all time. A report including the results from your tests will be sent to you by post or email upon completion of the study. If deemed necessary, and with your agreement, we will also pass on your personal performance and health tests results to your club coaching team and to your club Doctor and GP.

Who is organising and funding the research? Dr Pascale Kippelen, Senior Lecturer at Brunel University, is the principal investigator on this project, which is funded by UEFA.

What are the indemnity arrangements? Brunel University has an insurance policy (NHE-01CA29-0013) with public and products liabilities of £30m.

Who has reviewed the study? **To protect your interests, this study has been reviewed and given favourable opinion by the School of Sport & Education Research Ethics Committee.**

What if I have questions? If you have any questions about this research project, please contact:

Dr Pascale Kippelen; email : pascale.kippelen@brunel.ac.uk; phone : 01895 267649

Thanks for your time.

Creatine supplementation in youth football players

	YES	NO
The participant should complete the whole of this sheet ***Please tick the appropriate box***		
Have you read the Research Participant Information sheet?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Have you received satisfactory answers to all your questions?	☐	☐
Who have you spoken to? ……		
Do you understand that you will not be referred to by name in any report concerning the study?	☐	☐
Do you understand that you are free to withdraw from the study:		
• at any time?	☐	☐
• without having to give a reason for withdrawing?	☐	☐
• without affecting your future as player within your current football club?	☐	☐
I agree to my urine samples being gifted to be used for future studies.	☐	☐
I agree to my GP and Club Doctor to be informed about my health tests results.	☐	☐
I agree to my coaching team to be informed about my sport performance tests results.	☐	☐
Do you agree to take part in this study?	☐	☐

Signature of Research Participant:

Date:

Name in capitals:

Witness statement

I am satisfied that the above-named has given informed consent.

Witnessed by:

Date:
Name in capitals:

Researcher name:	**Signature:**
Supervisor name:	**Signature:**