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**Title:** Motion analysis of match-play in elite U12-U16 year old soccer players.

**Running title:** Youth soccer match-play

**Keywords:** time-motion analysis; elite youth soccer; GPS; maturation.

## **Abstract**

The aim of this study was to quantify the motion demands of match-play in elite U12-U16 year old soccer players. 112 players from two professional soccer clubs at five age-group levels (U12-U16) were monitored during competitive matches ( $n = 14$ ) using 5 Hz non-differential Global Positioning System (NdGPS). Velocity thresholds were normalised for each age-group using the mean squad times for a flying 10 m sprint test as a reference point. Match performance was reported as total distance, high-intensity distance, very high-intensity distance and sprint distance. Data were reported both in absolute (m) and relative ( $\text{m} \cdot \text{min}^{-1}$ ) terms due to a rolling substitute policy. U15 ( $1.35 \pm 0.09$  s) and U16 ( $1.31 \pm 0.06$  s) players were significantly quicker than the U12 ( $1.58 \pm 0.10$  s), U13 ( $1.52 \pm 0.07$  s) and U14 ( $1.51 \pm 0.08$  s) players in the flying 10m sprint test ( $P < 0.001$ ). The U16 age-group covered significantly more absolute total distance (U16 > U12, U13, U14), high-intensity distance (U16 > U12, U13, U14, U15), very high-intensity distance (U16 > U12, U13) and sprint distance (U16 > U12, U13) than their younger counterparts ( $P < 0.05$ ). When the data are considered relative to match exposure, few differences are apparent. Training prescription for youth soccer players should consider the specific demands of competitive match-play at each age-group.

## Introduction

In recent years, the development of complex semi-automated video analysis systems (e.g. ProZone®, Amisco Pro®) have enabled the efficient and detailed tracking of both players and referees during elite soccer match-play (Di Salvo, Gregson, Atkinson, Tordoff & Drust, 2009; Weston, Castagna, Impellizzeri, Rampinini, & Abt, 2007), using multiple cameras which are a permanent fixture at club stadia. However, competitive matches at elite youth team level usually take place at training ground facilities, where such technology does not exist. To date, there are few studies that consider the match-play demands of elite youth soccer across a range of age-groups.

The match conditions for youth soccer at the elite-level vary, with pitch size and game period length being age dependent. Previous studies that have reported the motion demands of elite youth soccer players have assigned player movement into arbitrary speed categories (e.g. walking, jogging, running) using video analysis and observational coding (Capranica, Tessitore, Guidetti, & Figura, 2001; Strøyer, Hansen, & Klausen, 2004). One of the limitations of such methods is determining the exact point at which a player crosses movement category thresholds, leading to questions over both the inter- and intra-rater reliability of such systems. Furthermore, video-based observational coding has been shown to underestimate total distance covered and high-intensity running when compared to 5 Hz NdGPS and semi-automated video-analysis systems ( $P < 0.001$ ; Randers et al., 2010). Castagna, D'Ottavio & Abt (2003) used fixed velocity thresholds for speed zones (e.g. high-intensity run,  $3.64 - 5.0 \text{ m} \cdot \text{s}^{-1}$ ; maximal speed run,  $>5.0 \text{ m} \cdot \text{s}^{-1}$ ), therefore allowing direct comparisons to be made between individuals regarding the absolute work performed in each speed zone. However, the time constraints inherent in the method

of using triangulation to assess the movement of one player at a time resulted in a relatively small sample size from one age-group (age:  $11.8 \pm 0.6$  years;  $n = 11$ ), thus limiting the inferences that can be drawn regarding player development across a range of age-groups. Recent developments in non-differential Global Positioning System (NdGPS) technology offer the potential to overcome the logistical issues and restrictions of other time-motion analysis methods, and can provide a means of quantifying athlete-motion without space-limitations or the need for fixed equipment.

The match-play demands of elite senior players have previously been described in detail (Mohr, Krstrup & Bangsbo, 2003; Bangsbo, Nørregaard, & Thorsø, 1991; Reilly & Thomas, 1976), with recent studies reporting the distance covered by players in a series of defined speed thresholds (running,  $4\text{-}5.5 \text{ m} \cdot \text{s}^{-1}$ ; high-speed running,  $5.5\text{-}7 \text{ m} \cdot \text{s}^{-1}$ ; sprinting,  $>7 \text{ m} \cdot \text{s}^{-1}$ ; Bradley et al., 2009). Due to the innate differences in performance capabilities between elite-level junior and senior players, it would be inappropriate to use the speed thresholds commonly applied to elite senior players to an elite youth player. As sprint performance has been reported to be positively related to the age of elite youth players (Mujika, Spencer, Santisteban, Goiriena & Bishop, 2009), one approach to normalise thresholds for youth players could be to use age-specific ranges of measured sprint velocities, considered relative to those displayed by senior players. Understanding the match-play demands of elite youth soccer could have practical implications for training prescription, talent identification and the quantification of player training loads. In particular, understanding the demands of match-play across a range of age-groups may provide an insight into the development profile of players at the different

levels. Therefore, the aim of the present study was to assess the motion demands of elite youth soccer players, aged between U12-U16 years.

## **Methods**

### *Participants*

Parental and subject written consent was given for one hundred and twelve (112) youth elite-level soccer players to participate in the study. All were male, aged between 11-16 years, representing two professional English clubs. Participants were categorised in five different age-groups, classed as under 12 (U12;  $n = 22$ ), under 13 (U13;  $n = 20$ ), under 14 (U14;  $n = 25$ ), under 15 (U15;  $n = 21$ ) and under 16 (U16;  $n = 24$ ). All participants generally undertook 4.5 hours of soccer training during each week plus one competitive match at weekends. U15 and U16 age-groups also undertook 1.5 hours of strength training and conditioning per week as part of their usual training programme. All procedures were approved by the Institutional Review Board and the University's Faculty Research-Ethics Committee.

### *Match configuration*

All games were played in accordance with the rules outlined by the English Football Association, and were refereed by qualified officials. All age-groups played with 11 players, and adopted a 'rolling substitute' policy, whereby each individual player can interchange with any substitutes an unlimited number of times during the match. U12 and U13 age-groups played on a  $\frac{3}{4}$  size soccer pitch (77 m x 60 m); U14, U15 and U16 groups played on a full sized pitch (99 m x 65 m). Period configuration varied between groups, with U12, U13, U14 and U15s playing 3 x 25 minute

periods, or 2 x 25 minute plus 2 x 12.5 minute periods. The U16 age-group played 2 x 40 minute periods.

### *Experimental design*

Data were collected from each age-group for a total of 14 competitive matches (U12,  $n = 2$ ; U13,  $n = 3$ ; U14,  $n = 4$ ; U15,  $n = 3$ ; U16,  $n = 2$ ), with all individual match observations being included in the final data set, and each individual player ( $n = 112$ ) being included for only one match.

Speed zones were normalised using the mean flying 10 m sprint times for each age-group, allowing comparisons to be drawn between age-groups based on average speed characteristics. Following a thorough warm-up, each participant completed five 20 m sprints, with timing gates placed at 10 m and 20 m, allowing a 'flying' 10 m sprint time to be obtained for each individual using the fastest recorded time. As sprint distances in soccer rarely exceed 20 m (Carling, Bloomfield, Nelsen, & Reilly, 2008; Stølen, Chamari, Castagna, & Wisløff, 2005; Di Salvo et al., 2007) a 20 m sprint with 10 m flying recorded time was deemed appropriate in this study for the assessment of peak velocity ( $V_{\text{peak}}$ ), which has previously been shown to produce highly reliable results with elite-level soccer players (Barnes, 2006). Individual  $V_{\text{peak}}$  ( $V_{\text{peakInd}}$ ) scores were used to calculate mean  $V_{\text{peak}}$  for each age-group ( $V_{\text{peakGrp}}$ ), which were compared relative to the mean measured  $V_{\text{peak}}$  for a sample of elite level senior players ( $V_{\text{peakSnr}}$ ;  $n = 13$ ; mean  $\pm$  s: age  $21.2 \pm 0.8$  years; height  $179.4 \pm 4.4$  cm; mass  $74.7 \pm 5.6$  kg). The [ $V_{\text{peakSnr}} \div V_{\text{peakGrp}}$ ] ratio was then applied to the commonly used thresholds for senior players (Th-S; Bradley et al., [2009]; Table 1) to produce age-group specific speed zones, according to the formula:

$$(V_{\text{peakSnr}} \div V_{\text{peakGrp}}) \times \text{Th-S}$$

Before each game, all starting outfield players were fitted with a NdGPS unit (MinimaxX, Catapult Innovations, Canberra, Australia) which operated at a sampling frequency of 5 Hz. Units were worn between the shoulder-blades in custom-made, tight-fitting vests to reduce movement artifact. Previous studies have reported that the reliability of NdGPS is compromised during high-intensity activity using a sample frequency of 1 Hz (Coutts & Duffield, 2008). More recently, it has been reported that 5 Hz NdGPS seemed more accurate than 1 Hz when measuring distance and velocity for movement patterns at higher velocities, whilst there was still some discrepancy when compared to the criterion measure (Duffield, Reid, Baker & Spratford, 2009). We previously found that 5 Hz NdGPS displayed good levels of accuracy (%error <1%) and reliability (CV <5%) when compared to trundle-wheel measured total distance for soccer-specific courses derived from semi-automated video analysis data (ProZone ®) for position-specific bouts of activity (Portas, Harley, Barnes & Rush, 2010).

Post-game analysis enabled the quantification of total distance covered (sum of zones 1-6), and distance covered at high-intensity (zone 4, 5 and 6), very high-intensity (zone 5 and 6) and at sprinting pace (zone 6) in line with previous studies (Bradley et al., 2009; Table 1). Data were presented in both absolute (m) and relative ( $\text{m} \cdot \text{min}^{-1}$ ) terms to allow direct comparisons to be made between groups without bias to individual variations in match exposure. Data were excluded from the analysis for injured players who had to withdraw from the game. For all data, mean  $\pm s$  satellite coverage (located satellites) was  $7.1 \pm 2.3$ ; mean horizontal dilution of precision (HDOP) was < 2.3.



### *Statistical analyses*

Statistical analyses were conducted using SPSS Version 16.0 (SPSS Inc, Chicago, Ill). Before using parametric statistical procedures, the assumptions of normality and sphericity were verified. Differences in flying 10 m sprint time (s), match exposure (min), absolute (m) and relative ( $\text{m} \cdot \text{min}^{-1}$ ) distances covered were analysed using a one-way analysis of variance (ANOVA), with Tukey post hoc test applied to explore exact differences between age-groups. Effect size (Cohen's  $d$  [95% Confidence Interval]) was reported for each significant variable to assess the magnitude of the observed difference. The relationship between match exposure (min) and work-load (m) was analysed by simple linear regression, from which the Pearson Correlation was calculated, and coefficients of determination ( $r^2$ ) reported for each relationship. Statistical significance was set at  $P < 0.05$ .

## **Results**

### *Flying 10 m sprint time*

The U15 ( $1.35 \pm 0.09$  s) and U16 ( $1.31 \pm 0.06$  s) age-groups displayed faster flying 10 m sprint times than the U12 ( $1.58 \pm 0.10$  s), U13 ( $1.52 \pm 0.07$  s) and U14 ( $1.51 \pm 0.08$  s) age-groups, respectively ( $P < 0.001$ ). Sprint times between the U12, U13 and U14 age-groups, and between the U15 and U16 age-groups were not different ( $P > 0.05$ ).

### *Match exposure*

The U16 age-group displayed higher levels of match exposure (U16:  $71.0 \pm 26.4$  mins) than the U15 group (U15:  $50.8 \pm 11.7$  mins;  $P = 0.034$ ). Differences in match exposure between other age-groups (U12:  $57.2 \pm 10.2$  mins; U13:  $60.5 \pm 16.5$  mins;

U14:  $54.9 \pm 21.0$  mins) were non-significant ( $P>0.05$ ). Individual player match exposure ranged from 13 – 97 minutes. There were significant positive correlations between match exposure and absolute total distance ( $r^2=0.739$ ;  $P<0.001$ ), high-intensity distance ( $r^2=0.542$ ;  $P<0.001$ ), very high-intensity distance ( $r^2=0.378$ ;  $P<0.001$ ), and sprint distance ( $r^2=0.236$ ;  $P<0.001$ ).

#### *Total distance*

Absolute total distance (m) was significantly higher at U16 level ( $7672 \pm 2578$  m) than at U12 ( $5967 \pm 1277$  m;  $P=0.045$ ;  $d=0.8$  [0.0:1.7]), U13 ( $5813 \pm 1160$  m;  $P=0.017$ ;  $d=0.9$  [0.2:1.7]) and U14 ( $5715 \pm 2060$  m;  $P=0.004$ ;  $d=0.8$  [0.3:1.4]) levels (Fig. 1A). Relative total distance ( $\text{m} \cdot \text{min}^{-1}$ ) was higher at U15 level ( $118.7 \pm 12.2$   $\text{m} \cdot \text{min}^{-1}$ ) than at U12 ( $103.7 \pm 5.8$   $\text{m} \cdot \text{min}^{-1}$ ;  $P=0.026$ ;  $d=1.6$  [0.2:2.9]) and U13 ( $98.8 \pm 23.5$   $\text{m} \cdot \text{min}^{-1}$ ;  $P=0.001$ ;  $d=1.1$  [0.5:1.7]) levels. Relative total distance was also higher at U16 level ( $115.2 \pm 15.8$   $\text{m} \cdot \text{min}^{-1}$ ) than at U13 ( $98.8 \pm 23.5$   $\text{m} \cdot \text{min}^{-1}$ ;  $P=0.014$ ;  $d=0.8$  [0.2:1.5]) level (Fig. 1B).

#### *High-intensity distance*

High-intensity distance (m) was higher at U16 level ( $2481 \pm 1044$  m) than at U12 ( $1713 \pm 371$  m;  $P=0.006$ ;  $d=1.0$  [0.3:1.7]), U13 ( $1756 \pm 520$  m;  $P=0.008$ ;  $d=0.9$  [0.2:1.5]), U14 ( $1841 \pm 628$  m;  $P=0.013$ ;  $d=0.7$  [0.2:1.3]) and U15 ( $1755 \pm 591$  m;  $P=0.013$ ;  $d=0.9$  [0.2:1.5]) levels (Fig. 1C). When the data are considered in relative ( $\text{m} \cdot \text{min}^{-1}$ ) terms, no differences were observed in high-intensity distance ( $P>0.05$ ) between age-groups (Fig. 1D). High-intensity distance accounted for (mean [range]) 30.4% [17.1-42.6%] of total match distance for all age-groups, and for 9.2% [5.0-14.0%] of total match exposure.

### *Very high-intensity distance*

Very high-intensity distance (m) was higher at U16 level ( $951 \pm 479$  m) than at U12 ( $662 \pm 180$  m;  $P=0.045$ ;  $d=0.8$  [0.2:1.6]) and U13 ( $644 \pm 259$  m;  $P=0.022$ ;  $d=0.8$  [0.1:1.5]; Fig. 1E) levels. Relative very high-intensity distance ( $\text{m} \cdot \text{min}^{-1}$ ) was higher at U14 level ( $14.3 \pm 3.8$   $\text{m} \cdot \text{min}^{-1}$ ) than at U13 ( $11.1 \pm 4.7$   $\text{m} \cdot \text{min}^{-1}$ ;  $P=0.026$ ;  $d=0.8$  [0.1:1.4]; Fig. 1F) level. Very high-intensity distance accounted for 11.9% [4.5-22.7%] of total match distance for all age-groups, and for 3.1% [1.0-5.0%] of total match exposure.

### *Sprint distance*

Sprint distance (m) was higher at U16 level ( $302 \pm 184$  m) than at U12 ( $174 \pm 64$  m;  $P=0.033$ ;  $d=0.9$  [0.1:1.8]) and U13 ( $167 \pm 96$  m;  $P=0.016$ ;  $d=0.9$  [0.2:1.7]) levels (Fig. 1G). Relative sprint distance ( $\text{m} \cdot \text{min}^{-1}$ ) was higher at U14 level ( $4.7 \pm 2.4$   $\text{m} \cdot \text{min}^{-1}$ ) than at U13 ( $2.9 \pm 1.7$   $\text{m} \cdot \text{min}^{-1}$ ;  $P=0.006$ ;  $d=0.9$  [0.3:1.5]) level (Fig. 1H). Sprinting accounted for 3.6% [0.3-8.8%] of total match distance for each age-group, and for 1.01% [0.0-2.0%] of total match exposure.

## **Discussion**

The purpose of this study was to assess the motion demands of elite youth soccer players across different age-groups during competitive match-play. This is the first study to consider match-performances in speed zones calculated relative to group derived speed characteristics, and to report these distances in both absolute (m) and relative ( $\text{m} \cdot \text{min}^{-1}$ ) terms. The main findings of the study were that the U16 age-group displayed higher absolute total distance (U16 > U12, U13, U14), high-intensity distance (U16 > U12, U13, U14, U15), very high-intensity distance (U16 >

U12, U13) and sprint distance (U16 > U12, U13) than their younger counterparts. However, when the data are considered relative to match exposure, few differences in match work-rate are found between groups (total distance: U15 > U12, U13; U16 > U13; very high-intensity distance: U14 > U13; sprint distance: U14 > U13). The older age-groups (U15, U16) were also quicker than their younger counterparts (U12, U13, U14) when tested using a flying 10 m sprint protocol ( $P < 0.001$ ).

The findings of this study suggest that work-rate profiles of elite youth soccer players are similar between the age-levels of U12-U16, when the thresholds used to define movement categories are corrected relative to age-specific velocity characteristics. Castagna et al. (2003) reported the activity profile of U12 soccer players using set velocity thresholds for various movement categories, reporting that 9% of total match time was spent at high-intensity, using a threshold to define high-intensity work of movement above  $13 \text{ km} \cdot \text{h}^{-1}$  ( $3.61 \text{ m} \cdot \text{s}^{-1}$ ; Castagna et al., 2003). In comparison, the U12 group in the present study spent 10.8% of time at high-intensity, the threshold in our study being slightly lower at  $3.04 \text{ m} \cdot \text{s}^{-1}$  ( $10.94 \text{ km} \cdot \text{h}^{-1}$ ), based on age-specific velocity characteristics. In addition, mean sprint distance during match-play was reported as  $114 \pm 73 \text{ m}$  (34-250 m) by Castagna et al. (2003) which is lower than that reported for the U12 group in this study ( $174 \pm 64 \text{ m}$  [85-262 m]), despite a higher sprinting threshold being applied in the current study ( $5.32 \text{ m} \cdot \text{s}^{-1}$ ) for the U12 group, compared with  $5 \text{ m} \cdot \text{s}^{-1}$  used by Castagna et al. (2003). The mean match-exposure of the U12 age-group in the present study ( $57.2 \pm 10.2$  mins) was similar to the total match time reported by Castagna et al. (60 mins). Therefore, the higher sprint distances reported in this study may be in part influenced by the higher total playing time in the present study (75 mins), match-to-match variability between studies, differences in the assigned velocity categories for

sprints, and also by methodological differences between studies (NdGPS vs. camera based triangulation). Furthermore, the analysis of sprinting using NdGPS must be made with caution due to the reported accuracy of NdGPS during high-intensity activity (Duffield et al., 2009).

In the present study, speed zones were normalised for each age-group using the mean measured sprint performance for each group, relative to that measured for the senior group. Semi-automated video analysis systems categorise workloads into absolute speed-zones which are generally standardised for all players. Whilst the use of absolute speed-zones may provide useful information regarding the performance capacity of players, they do not account for the underlying individual physiological capacity that distinguishes different levels of play. Abt & Lovell (2009) suggested a method for individualising high-intensity speed thresholds based on the measured second ventilatory threshold in elite soccer players. However the use of such treadmill-based tests requiring maximal effort to volitional exhaustion may be unsuitable for youth soccer players for both economical and logistical (time-constraints, treadmill familiarisation) reasons. The method presented in this study may be used to make between-group comparisons based on the capabilities of different age-groups, or different levels of play. Limitations to this method, however, are that individual variations in speed capacity within age-groups are not accounted for. In addition, a degree of error may be incurred by individualising group thresholds based on  $V_{peak}$ , as this may also be influenced by other factors including anaerobic capacity and running mechanics.

A significant increase in measured peak speed was observed between the ages of U14 and U15 in the present study ( $P < 0.05$ ), with no differences between U12-U14 level, or between U15 to U16 level ( $P > 0.05$ ). These findings are in

agreement with Mujika et al. (2009), who reported an improved sprint performance between the ages of U14 and U15 ( $P<0.05$ ), with no further improvement being observed after U15 level (Mujika et al., 2009). It has been suggested that such improvements are likely related to the influence of maturation on maximal-effort exercise, and in particular differences in height and weight across age-groups (Mujika et al., 2009). Stratton, Reilly, Williams, & Richardson (2004) suggest that the average age at the onset of puberty is 13.5 years (U14) for boys, and that age at peak height velocity in sub-elite players occurs from 13.8 – 14.2 years (U14 – U15). It could therefore be implied that the physical performance characteristics measured in the present study (peak speed) were influenced by maturation status. However, as match-performance data were considered relative to these group-derived peak speed characteristics, no differences were observed in match performances between the ages of U14 and U15. In addition, the significantly higher absolute high-intensity distance at U16 level compared with the younger age-groups may be due to a high match exposure ( $71.0 \pm 26.4$  mins), as high-intensity distance showed a strong ( $r^2=0.542$ ;  $p<0.001$ ) correlation with match exposure. Alternatively, such differences may be attributed to an increased oxygen uptake capacity in mature children (Armstrong & Welsman, 1994), which has previously been reported to increase soccer performance (Helgerud, Engen, Wisløff & Hoff, 2001).

Future research into the effect of maturation levels on physical performance during competitive match-play in elite youth soccer players would be recommended to further investigate the findings of the present study. Such data could be used to identify players with the ability to play at a particular level, and to prepare players for the demands of successive playing levels through the modification of training loads according to the specific demands of match-play.

## Conclusions

This study highlights the importance of assessing match activities at youth team level in relative ( $\text{m} \cdot \text{min}^{-1}$ ) as well as absolute (m) terms due to variations in individual match exposure and the rolling substitute policy. Due to variations in performance characteristics across age-groups at youth team level, measures of match performance should be considered relative to age-group performance characteristics. In particular, when categorising player motion into speed zones, thresholds should be 'normalised' relative to individual or group derived speed capabilities, one method being the assessment of flying 10 m sprint time.

The relative match-play demands of elite youth soccer players appear to be consistent across the ages of U12-U16. However, players become significantly faster during this time, in particular between the ages of U14 and U15. Prescription of training drills for youth soccer players should consider the specific demands of competitive match-play at each age-level, with maturation levels being considered as an indicator of performance capacity.

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Table 1. Speed zone thresholds ( $\text{m} \cdot \text{s}^{-1}$ ) by age-group calculated from 10 m flying time

|               | 10 m                          | Speed Zone ( $< \text{m} \cdot \text{s}^{-1}$ ) |             |             |             |             |  |
|---------------|-------------------------------|---|-------------|-------------|-------------|-------------|--|
|               | flying time<br>(mean $\pm$ s) | 1   | 2           | 3           | 4           | 5           | 6<br>( $\geq \text{m} \cdot \text{s}^{-1}$ ) |
| <i>Senior</i> | <i>1.20</i>                   | <i>0.50</i>                                     | <i>2.00</i> | <i>4.00</i> | <i>5.50</i> | <i>7.00</i> | <i>7.00</i>                                  |
| U16           | 1.31 $\pm$ 0.06 *             | 0.46  | 1.83        | 3.66        | 5.04        | 6.41        | 6.41   |
| U15           | 1.35 $\pm$ 0.09 *             | 0.44  | 1.78        | 3.56        | 4.89        | 6.22        | 6.22   |
| U14           | 1.51 $\pm$ 0.08               | 0.40  | 1.59        | 3.18        | 4.37        | 5.56        | 5.56   |
| U13           | 1.52 $\pm$ 0.07               | 0.39  | 1.58        | 3.16        | 4.34        | 5.53        | 5.53   |
| U12           | 1.58 $\pm$ 0.10               | 0.38  | 1.52        | 3.04        | 4.18        | 5.32        | 5.32   |

\* significantly different to U12, U13 and U14 ( $P < 0.001$ ). Senior thresholds (Th-S) are shown in *italics*. Speed zones represent: (1, standing; 2, walking; 3, jogging; 4, running; 5, high speed running; 6, sprinting).

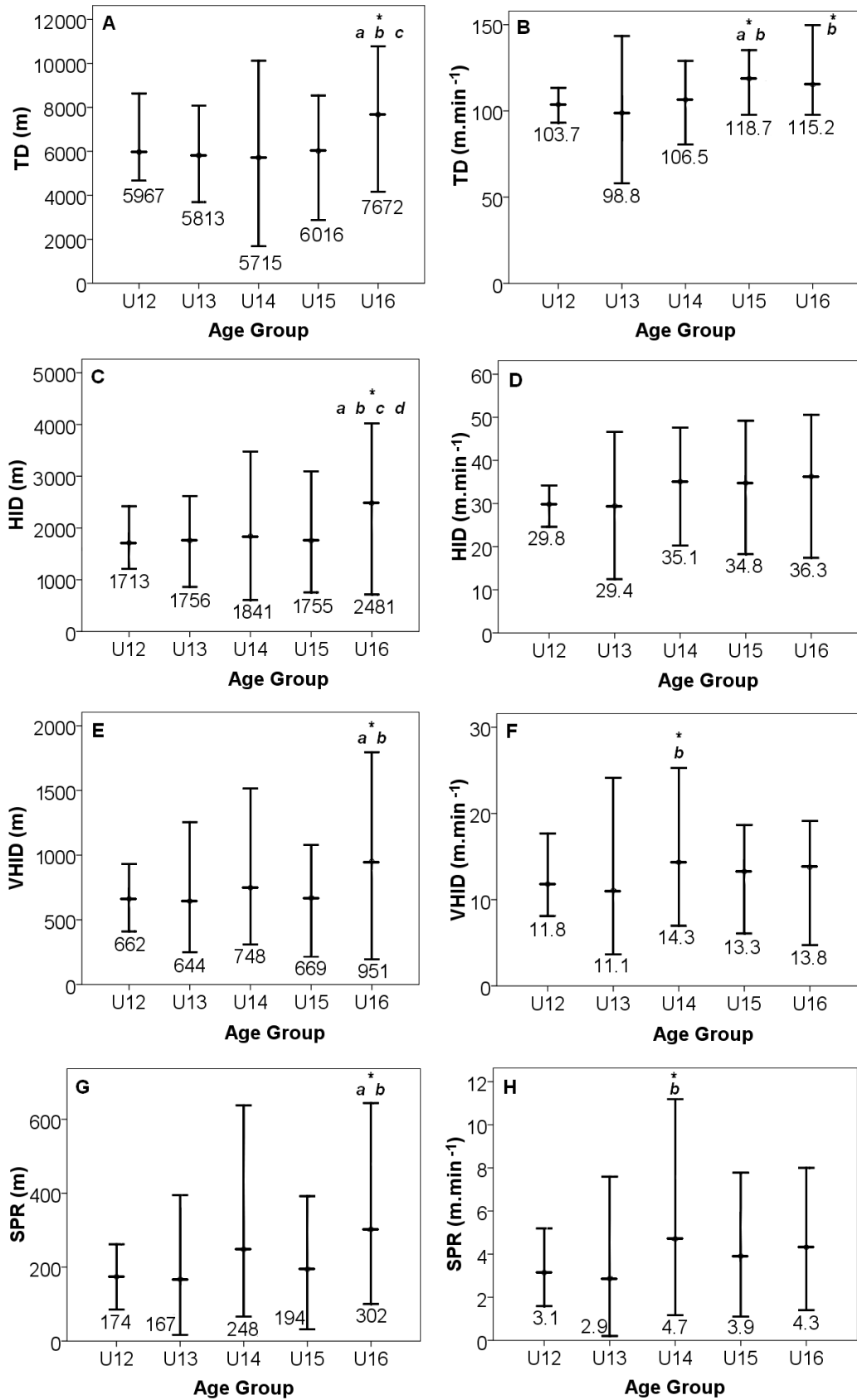


Figure 1. Absolute (m) and relative (m · min<sup>-1</sup>) total distance (TD), high-intensity distance (HID), very high-intensity distance (VHID) and sprint distance (SPR) for all

age-groups. Mean and range of data is illustrated, along with the mean data label. \* = significantly greater than (*a*, U12; *b*, U13; *c*, U14; *d*, U15;  $P < 0.05$ ).