Title: The associations between digit ratio (2D:4D and right - left 2D:4D), maximal oxygen consumption and ventilatory thresholds in professional male football players

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Abstract

Introduction: Digit ratio (2D:4D: the relative length of the 2nd and 4th digit) is thought to be a negative correlate of prenatal testosterone. The 2D:4D is related to oxygen metabolism, but the precise nature of this relationship is unclear. The purpose of the present study was to consider associations between digit ratios (right 2D:4D, left 2D:4D, right-left 2D:4D [Dr-l]) and $VO_{2max}$ and ventilatory thresholds (VT1 and VT2). Methods: One hundred and thirty-three Caucasian (n=133) professional football players competing in Cyprus participated in the study. Players underwent anthropometric measurements, and digit lengths were measured from hand scans. They also completed an incremental cardiopulmonary test to exhaustion on a treadmill. Results: There were negative correlations between digit ratios and $VO_{2max}$ (right 2D:4D, $r$ = -.65; left 2D:4D $r$ = -.37, both $p$<.0001; Dr-l $r$ = -.30, $p$ = .0005). There were no relationships between digit ratios and VT1. For VT2, there were negative relationships with digit ratios (right 2D:4D, $r$ = -.43, $p$<.0001; left 2D:4D, $r$ = -.21 and Dr-l, $r$ = -.21, both $p$ = .02).Digit ratios are negatively related to $VO_{2max}$ with large (right 2D:4D) and medium (left 2D:4D, Dr-l) effect sizes. For VT2, there were also negative correlations, which were medium (right 2D:4D) and small (left 2D:4D, Dr-l). Conclusion: Our findings may help clarify the relationships between digit ratios and high-intensity actions for extended periods, which are dependent on efficient oxygen metabolism.

Key Words: Prenatal testosterone, Aerobic fitness, Digit ratios, Soccer

## Introduction

The relative lengths of the 2nd and 4th digits (2D:4D) and the side difference in 2D:4D (Dr-l: right-left 2D:4D) are thought to be negative correlates of 1st-trimester testosterone and positive correlates of 1st-trimester oestrogen (Manning et al., 1998; Manning et al., 2002; Breedlove, 2010; Swift-Gallant et al., 2020). The 2D:4D and Dr-l show sexual dimorphism (males<females); the sex difference appears in the 1st trimester and shows little change in children, juveniles and adults (Malas et al., 2006; Trivers et al., 2006; Manning et al., 2022). In contrast to the links between digit ratios and prenatal sex steroids, there is little evidence of associations between 2D:4D and background levels of testosterone or oestrogen in adults (Hönekopp et al., 2007).

Manning and Taylor (2001)were the first to report that 2D:4D was negatively associated with performance among male participants from a range of sports, including >300 elite footballers competing in the English Leagues. In addition, meta-analyses have found negative relationships between 2D:4D and performance in a number of sports with mean right-hand effect sizes of r = -0.28 (Hönekopp and Schuster, 2010) and weak negative relationships with hand grip strength (Pasanen et al., 2022). With regard to endurance disciplines, Manning et al., (2007 )have reported strong correlations between 2D:4D and running speed in middle- and long-distance races($r^2$ values of approximately 25% for males and females). They suggested that 2D:4D may be a strong correlate of vascular health. However, reports of associations between 2D:4D, $VO_{2max}$ and ventilatory threshold (VT) employing objective lab-based measures of $VO_{2max}$ and VT have yielded mixed results from samples that were small and were recruited from a range of backgrounds in sports(Hill et al., 2012; Holzapfel et al., 2016; Lombardo et al., 2020).In this regard, it is important to examine the relationships between digit ratios (2D:4D and Dr-l) and oxygen metabolism in a larger sample of athletes who participate in the same sport. The latter includes $VO_{2max}$(maximal oxygen consumption;

Hill and Lupton, 1923) and ventilator thresholds [(VT1 the point during exercise at which pulmonary ventilation and carbon dioxide output begin to increase exponentially; Cerezuela-Espejo et al., 2018), and VT2 or RC (the point associated with hyperventilation at which lactate is rapidly increasing with intensity; Meyer et al., 2004).

Evidence for links between digit ratios and oxygen metabolism may be indicated by the types of sport linked to 2D:4D or Dr-l. Low values of digit ratios have been reported to be associated with high performance in a range of sports. For males, these include football (soccer; Manning and Taylor, 2001), rugby (Bennett et al., 2010), skiing (Manning, 2002), rowing(Longman et al., 2011), surfing (Kilduff et al., 2011), wrestling (Keshavarz et al., 2017), basketball (Klapprodt et al., 2018) and for females, rowing (Hull et al., 2015), skiing (Manning 2002), and Olympic athletes participating in power, endurance and technical sports (Eklund et al., 2020). Therefore, low digit ratios may be linked to both strength and endurance. However, a consideration of associations between 2D:4D and running speed suggests that the latter shows greater effect sizes than the former. In this regard, Manning et al., (2007) and Longman et al., (2015) have reported correlations between 2D:4D and running speed in long-distance races ranging in strength from $r = .40$ to $r = .60$ in males and $r = .20$ to $r = .30$ in females. In contrast, 2D:4D was indicated to be weakly related to sprinting speed, with correlations averaging about $r = .10$ (Hönekopp and Schuster, 2010; Manning and Hill, 2009). Physiological variables ($VO_{2max}$, velocity at maximal oxygen uptake, and changes in lactate levels), training load and fat mass are considered the main factors determining performance in long-distance races (Alvero-Cruz et al, 2020). The strong relationship between 2D:4D and speed in long-distance races suggests that 2D:4D may be a negative correlate of maximal aerobic performance, and in particular, it is likely to be predictive of maximal oxygen uptake ($VO_{2max}$) and/or Ventilatory Thresholds (VT1 and VT2).

However, attempts to quantify relationships between digit ratio and $VO_{2max}$ and VT1 and VT2 have met with mixed results. Hill et al., (2012) considered relationships between digit ratios and oxygen metabolism in 41 boys (mean age 13.9 [SD1.3] years). They found no significant relationships for right or left 2D:4D, but there were negative correlations of medium strength for Dr-l and $VO_{2max}$. In contrast, Holzapfel et al., (2016) reported no significant correlations between 2D:4D (Dr-l was not considered) and $VO_{2max}$ in a sample of 26 men and 28 women, but strong negative relationships were demonstrated for 2D:4D and VT. Furthermore, Lombardo and Otieno (2020) reported on digit ratio and aerobic fitness variables in 11 boys and 15 girls, aged between 11 and 19 years, who were the top five finishers in 10 or more races of 10 km. In their study, boys (but not girls) with lower right 2D:4D had significantly greater $VO_{2max}$. Girls (but not boys) with lower right 2D:4D had significantly greater VT. Thus, it appears that digit ratios are related to maximal aerobic performance, but the strength of the relationship and the relative importance of $VO_{2max}$ and VT need to be clarified. In general, sample sizes thus far were small, and participants varied in their participation in sports. Therefore, we consider relationships between digit ratios (2D:4D and Dr-l) and $VO_{2max}$, and VT1 and VT2 in a large sample of male professional football players.

## Materials and Methods

### *Participants*

An initial sample of 143 professional male football players (age: 25.21 ± 5.47 years, height:180.15 ± 6.12cm, weight: 76.40 ± 7.12 kg) participating in Division 1 and 2 in the Eastern Mediterranean was recruited. The sample included 133 Caucasian and 10 Black participants. Due to significant differences in the anthropometric characteristics and digit ratios between the Caucasian and Black players, our statistical analyses were mainly focused on the Caucasian players (n=133).

Testing was undertaken during the months of June and July before the pre-season period. Exclusion criteria included injuries within the last two months before the testing. Anthropometric measurements (age, stature, body weight, body fat and hand scans) were recorded before the physical tests. Players' characteristics are given in Table 1. The players completed an incremental cardiopulmonary test to exhaustion on a treadmill. All players were familiar with the testing protocol as this was part of their annual testing. They were instructed to avoid heavy physical activity the day prior to the testing. All participants completed an informed consent after being briefed about the procedures, and the technical director of the team approved all the testing protocols. The research complied with the relevant national regulations, was conducted in accordance with the Declaration of Helsinki, and was approved by the National Committee of Bioethics (EEBK EP 2022.01.290).

**Procedure**

*Anthropometric measurements*

Anthropometric measurements were conducted using a wall stadiometer (Leicester; Tanita, Tokyo, Japan) to determine the players' stature and a leg-to-leg bioelectrical impedance analyser (BC418MA; Tanita) to assess body composition (% body fat). The players were instructed to follow the standard guidelines prior to the bioelectrical impedance testing (Kyle et al., 2004).

*Hand scans*

Players were asked to place their hand on the surface of the photocopier (EPSON scanner, DS-50000) with the palm facing downwards and fingers as straight as possible according to the methodology described by previous investigators (Manning, 2002). They were instructed not to exert too much pressure but lightly place their fingers on the photocopier and wait until the scan was completed. The scan was evaluated by a single examiner, and in cases where it was not clear, it was repeated. The finger length was measured twice by the same investigator, blind to the oxygen data, and the 2D:4D ratio was calculated from each set of scans. Digit length was measured to an accuracy of 0.05 mm using Vernier callipers (Mitutoyo, D15, Japan).

*Incremental cardiopulmonary testing on a treadmill*

The players completed an incremental cardiopulmonary test to exhaustion (CPET) on a treadmill (HP Cosmos Quasar med, HP Cosmos Sports, and Medical GmbH, Nussdorf-Traunstein, Germany). Gas exchange measurements were collected with reusable masks, a turbine flow meter, and a two-way nonrebreathing valve (model 7940, Hans Rudolph, Kansas City, MO). Heart rate (COSMED wireless HR monitor, Rome, Italy), VO2, carbon dioxide (VCO2) production and expired minute volume (VE) were continuously monitored throughout the test, and a breath-by-breath analysis was performed on a computerised (Cosmed Quark CPET, Rome, Italy) system. Before each test, the air VO2 flowmeter and oxygen-carbon dioxide meters were calibrated with a three-litre air syringe and a gas of known oxygen (16.5%) based on the manufacturer's recommendations. Throughout the testing, laboratory conditions were kept constant, with the temperature being around 21-22 degrees (C) and the relative humidity around 50%.

During the test, the inclination was kept constant at 1%. The players started the test at a speed of 8km/hr, and the speed was increased every 3.15 minutes by 2km/hr until they reached volitional exhaustion or could no longer continue. The recovery speed was 5 km/h for 2-3 minutes. The $VO_{2max}$ was identified after filtering the results by indicating the highest value for an average of 10 seconds and was expressed relative to body mass (ml/kg/min). The ventilatory threshold (VT1) was identified through the V-slope method (the point at which the increase in the rate of elimination of carbon dioxide is greater than the increase in VO2) and was verified at the nadir of the VE/VO2 curve. The respiratory compensation point (VT2 or RC) was determined at the nadir of the VE/VCO2 curve (Beaver et al., 1986).

*Statistical Analysis*

Means and standard deviations (mean ± SD) were calculated for all the parameters. The homogeneity of variance was tested using the Brown-Forsythe test, and the normality assumption was verified using the Shapiro–Wilk test. Interclass correlation coefficients (ICC) (absolute agreement) between the first and second 2D:4D's of the right and left digits were calculated. Pearson-product moment correlation coefficients were used to determine the association between 2D:4D, $VO_{2max}$ and its associated ventilatory thresholds. Correlations were referred to as trivial (0–0.1), small (0.1–0.3), moderate (0.3– 0.5), large (0.5–0.7), very large (0.7–0.9), nearly perfect (>0.9) and perfect (1.0) (Hopkins et al., 2009). Three multiple regression analyses with independent variables age, right 2D:4D and left 2D:4D and dependent variables $VO_{2max}$ or VT1 or VT2 were performed. All statistical analyses were performed in IBM® SPSS® Statistics, version 26.0, for Windows (SPSS Inc., Chicago, IL, USA), and the statistical significance was set at $p < 0.05$.

## Results

Two values of digit ratios were calculated. Intra-class correlations coefficients ($r_I$, used for the assessment of the consistency of the measurements) were high and significant for right 2D:4D ($n = 142$, $r_I = .976$, $F = 82.79$, $p < .0001$), left 2D:4D ($n = 140$, $r_I = .960$, $F = 48.48$, $p < .0001$) and Dr-l ($n = 139$, $r_I = .954$, $F = 42.29$, $p < .0001$). The average of the two measurements was used to obtain the final values for right and left 2D:4D and Dr-l ratios.

Descriptive statistics for the total sample and the sample split by ethnicity are given in Table 1. In comparison to Caucasians, Black players had greater mass, BMI, % body fat, and VO2 at VT as well as lower right and left 2D:4D. Therefore, we removed the Black players from the sample and reported relationships for Caucasians (n = 133) only for the following analyses.

There were no significant relationships between digit ratios (right and left and Dr-l) and age or body size variables (*r*=0.04 between age and right 2D:4D, *r*= 0.06 between height and right 2D:4D, *r*=0.03 between weight and right 2D:4D, *r*=0.04 between age and left 2D:4D, *r*=0.06 between height and left 2D:4D, *r*=0.07 between weight and left 2D:4D, all $p$>.05).

The correlations between digit ratios (right and left and Dr-l), and $VO_{2max}$ and ventilatory thresholds VT1 and VT2 are given in Table 2. Correlations were strongest between digit ratios and $VO_{2max}$, effect sizes were greatest for right 2D:4D, and all correlations were negative. With regard to $VO_{2max}$, there was a large correlation with right 2D:4D (*r* = -0.65; Figure 1) and medium correlations with left 2D:4D (*r* = -0.37) and Dr-l (*r* = -0.30). There were no significant relationships between digit ratios and VT1 (*r* varying from -0.02 to -0.12). Considering VT2, right 2D:4D showed a moderate correlation (*r* = -0.43), and there were small correlation coefficients for left 2D:4D and Dr-l (both *r* = -0.21). $VO_{2max}$, V1 and V2 were interrelated with varying strengths (very large,$VO_{2max}$ and VT2, *r* = .73; large, VT1 and VT2, *r* = .59; moderate, $VO_{2max}$ and VT1, *r* = .34: all $p$<.0001).

In addition to the correlations (*r*) for the Caucasian participants, we also considered the total sample (i.e. Caucasian and Black players, *n* = 143) together with the total sample after ethnicity effects were removed (standardised regression coefficient, *b*)—the values of *b*are presented in parenthesis in Table 2. There was one notable change in *r* and *p* values, i.e. for the total sample of Caucasian plus Black players; right 2D:4D was now negatively and significantly related to VT1 (*r* = -.21, *p* = .01). There were no substantial differences in effect sizes and *p* values between the Caucasian sample and the total sample when ethnicity effects were removed.

Age may influence $VO_{2max}$, VT1 and VT2. Therefore, we performed three multiple regression analyses with independent variables age, right 2D:4D and left 2D:4D and dependent variables $VO_{2max}$ or VT1 or VT2. With regard to $VO_{2max}$, the overall relationship was $r = 0.67$ ($r^2 = 0.45$, age $b = -.08$, SE = .06, $p = .25$, right 2D:4D $b = -0.61$, SE = 9.77, $t = -8.35$, $p<.0001$, left 2D:4D $b = -0.13$, SE = 10.81, $t = -1.80$, $p = 0.08$). For VT1 the overall relationship was $r = 0.21$ ($r^2 = 0.04$). There was a small negative relationship for age but no relationships for digit ratios (age $b = -0.17$, SE = .06, $t = -1.98$, $p = 0.049$). Considering VT2, the overall relationship was $r = 0.48$ ($r^2 = 0.23$). There was a small negative relationship with age and a moderate negative association for right 2D:4D (age $b = -0.22$, SE = .06, $t = -2.82$, $p = 0.006$, right 2D:4D $b = -0.41$, SE = 10.40, $t = -4.84$, $p<.0001$). There was no relationship for left 2D:4D.

**Discussion**

Football is an intermittent sport with repeated high-intensity phases. As a result of improvements in training techniques, football players today are much more similar to endurance athletes than 50 years ago (Edwards et a., 2003). Therefore, comparisons between our results and those from endurance athletes are appropriate.

Our finding of a mean $VO_{2max}$ of 56.05±4.53 was close to large sample measures of elite football players (range, goalkeeper 50.42±4.2 to winger-sides back 60.53±5.02, median 58.25: Manari et al., 2016). In our total sample of 143 participants, there were 133 Caucasians and 10 Black football players. The latter differed from the former in their 2D:4D (Caucasian>Black) and in mass, BMI, % body fat, and VO2 at VT1. High 2D:4D in Caucasians and low 2D:4D in Black populations have been reported in a number of studies (Manning, 2002; Butovskaya et al., 2021). Such differences can obscure relationships.

Therefore, the less numerous group was removed, and subsequent analyses focused on Caucasians.

With regard to our Caucasian sample, we have found significant negative relationships between all three-digit ratio variables (right 2D:4D, left 2D:4D and Dr-l) and $VO_{2max}$. The large correlation between right 2D:4D and $VO_{2max}$ was the strongest of the three associations, such that right 2D:4D explained 42% of the variance in $VO_{2max}$. Associations for left 2D:4D and Dr-l with $VO_{2max}$ were medium in strength. There were no significant relationships between digit ratios and VT1. For VT2, all digit ratio correlations were negative and significant, with a moderate (and strongest) relationship for right 2D:4D and small correlations for left 2D:4D and Dr-l. Our study is one of the larger studies to consider relationships between digit ratios and $VO_{2max}$ and VTs in males. The sample was relatively homogeneous in that the participants were all male Caucasian professional football players competing in Leagues 1 and 2, Eastern Mediterranean. Moreover, they can be regarded as being relatively homogenous in terms of their exercise regime.

A similar study by Hill et al., (2012) indicated no association between 2D:4D (right or left) and $VO_{2max}$ but reported a significant negative correlation for Dr-l in young athletic teenage boys of Middle East origin (age: 13.9 ± 1.3 years) during an incremental treadmill test. We have replicated this latter association in our larger adult male sample. Hill et al., (2012) participants were drawn from a wide range of sports with different training regimes (soccer, squash, table tennis and athletics). This may have masked the relationship between right and left 2D:4D and $VO_{2max}$. Importantly, both our present sample and that of Hill et al., (2012) controlled for ethnicity by considering a single ethnic group.

Holzapfel et al., (2016)reported little or no relationship between 2D:4D (Dr-l was not considered) and $VO_{2max}$ in a sample of 26 men (13 sedentary and 13 distance runners).

However, they found large negative correlations between 2D:4D and VT. On the contrary, in our sample, there were no relationships between digit ratios and VT1. The distance runners in the Holzapfel et al., (2016)study had higher mean $VO_{2max}$ (62.6±11.2) than our sample of football players (55.91±4.51, Cohen's $d$ = .78). However, this was unlikely to account for the differences as there were large correlations between digit ratios and VT in both their sedentary and runner samples. Their sample was recruited from the student population of a South-Eastern US University, and the authors did not report any controls for ethnicity. Thus, the discrepancies between the Holzapfel et al., (2016) study and the Hill et al., (2012) and the present study may have arisen as the result of differences in sample size and controls for ethnicity. In this regard, the removal of ethnicity controls in our present study resulted in a significant relationship between right 2D:4D and VT1.

A similar study by Lombardo and Otieno (2020)reported correlations between right 2D:4D and $VO_{2peak}$, VT and Point of Equivalent Change (PEC) in 11 boys who were elite distance runners. All three variables were negatively related to right 2D:4D with two ($VO_{2peak}$ $r$ = -.62; PEC $r$ = -.66) showing significance at $p<.05$. However, significance was lost for both when adjusted for mass. The strength of the correlation with right 2D:4D was similar to that of our finding for right 2D:4D and $VO_{2max}$. These findings suffer from small sample sizes. However, we judge them to be not incompatible with our findings.

With regard to the value of 2D:4D to coaches and scouts. We suggest that 2D:4D may be of predictive value in sports that are performance-dependent on high values of $VO_{2max}$ (e.g. distance running, tennis, rowing & football). Values of 2D:4D appear to be more or less stable across puberty, thus, 2D:4D may yield predictive information in adolescents.

An explanation for the links between low 2D:4D and high values of $Vo_{2max}$ and VT2 may lie in the relationship between 2D:4D and prenatal testosterone. Manning (2002) has suggested that 2D:4D is a highly conserved trait that is linked to the early emergence of tetrapods from an aquatic to a terrestrial existence. Since this suggestion, there have been some 30 reports concerning 2D:4D sexual dimorphism in amphibians, reptiles, birds, artiodactyls, rodents, and primates (Lupu et al., 2023). The findings suggest a pattern of sex differences of moderate effect, with some species showing male 2D:4D lower than female 2D:4D and other species having the reverse pattern. However, not all species have significant sexual dimorphism in 2D:4D. For example, Lombardo & Thorpe (2008) did not report evidence of sexual dimorphism in 2D:4D green anolis lizards (*Anolis carolinensis*), and Lombardo et al., (2008) did not find evidence of sexual dimorphism in 2D:4D in four species of birds (house sparrows, tree swallows, budgerigars, chickens). When the four species were pooled, male 2D:4D was greater than female 2D:4D. With regard to more direct evidence that sex differences in 2D:4D are testosterone dependent, there are nine studies that included manipulations of testosterone or its receptor; six of these report a masculinisation effect, one a feminisation effect, and two a null effect (Manning and Fink, 2023). The emergence from an aquatic existence is associated with a suite of traits, including the ability to process gaseous oxygen (Manning, 2002; Manning and Fink, 2023). Low testosterone compromises mitochondrial function (Yan et al., 2017), and in human males, it is linked to cardiovascular disease (Harada, 2018). High 2D:4D is associated with elevated fibrinogen levels and early myocardial infarction (Manning et al., 2019). Thus, our expectation is that low 2D:4D is related to efficient oxygen metabolism.

Our study has a number of limitations. We have not considered non-Caucasian and female football players as it was not possible to recruit sufficient numbers. Moreover, we suggest that associations between 2D:4D and oxygen metabolism should be considered in a variety of sports. These could range from those that require a very high level of aerobic fitness (e.g.

professional cyclists participating in the Girod d'Italia, Tour de France and Vuelta de Espana) to those in which fitness is somewhat less important (e.g. table tennis).

## Conclusions

In conclusion, we have found significant negative correlations between digit ratios and $VO_{2max}$ in 133 professional male football players. They were large (right 2D:4D) and medium (left 2D:4D, Dr-l) in effect size. For VT2, there were also significant negative correlations, which were medium (right 2D:4D) and small (left 2D:4D, Dr-l) in effect size. There were no associations between digit ratios and VT1. All associations were controlled for ethnicity. We hope these findings help to clarify associations between digit ratios and oxygen metabolism in men. Further work is necessary to quantify these associations in women.

## Figure legends

**Figure 1**. VO2max and right 2D:4D ($r = -.65$, $R^2 = 0.425$)

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