Is Digit Ratio (2D:4D) a Biomarker for Lactate?

Evidence from a Cardiopulmonary Test on Professional Male Footballers

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Abstract

Background: Lactate accumulation is associated with vigorous exercise, cardiovascular disease and a number of cancers. Digit ratio (2D:4D) has also been linked to oxygen metabolism, myocardial infarction and various cancers. Such similarities suggest the possibility that 2D:4D is a biomarker of lactate. Here, we consider the relationship between 2D:4D and lactate during an incremental cardiopulmonary exercise test.

Method: The participants were male professional football players. The treadmill test began at a speed of 8km/h when the first lactate measurement was taken. The speed was increased by 2km/h every 3.15 minutes, with measurements at 10,12,14 and 16 km/h.

Results: There were 72 Caucasian and 7 Black participants, results are reported for the most numerous group. Lactate levels increased with running speed and were not correlated with age, body size or body composition. Median splits of digit ratios (right, left and right-left 2D:4D [Dr-l]) were calculated. In comparison to the Low ratio group, the High ratio group showed higher lactate levels across speeds. Effect sizes varied from very large to huge (right 2D:4D), large (left 2D:4D) and medium (Dr-l). At the individual level, positive correlations between digit ratios and lactate at the five different speeds varied from large (right 2D:4D), medium (left 2D:4D) and small (Dr-l).

Conclusion: There were large positive associations between right 2D:4D and lactate at all running speeds. We discuss our findings in relation to oxygen metabolism and suggest that 2D:4D may be a biomarker for lactate in the wider context of the latter's importance in health and disease.

Keywords: Ventilatory thresholds; Digit ratio; soccer

Introduction

Traditionally, lactate has been considered to be a metabolic waste product generated from glycolysis during vigorous exercise [1]. This view has been radically revised. Lactate is now considered to be an energy source that is released from and used within working muscles. As such, it is an important substrate for mitochondria in the muscles, heart and brain during exercise and it is the main gluconeogenic precursor in the liver [2]. In addition to its importance in sports performance, lactate has clinical importance as it is now apparent that levels of lactate are altered in many diseases. For example, lactate is a prognostic indicator of cardiovascular diseases such as myocardial infarction (MI) [3,4] and many cancers such as breast and prostate cancer [5]. The association between elevated lactate levels and vigorous exercise, cardiovascular diseases and cancer prognosis has marked similarities to the pattern of links between digit ratio (2D:4D) and oxygen metabolism and disease. Thus, 2D:4D is negatively related to the following: running speed in distance races [6], maximal oxygen capacity in incremental cardiopulmonary tests [7], age at MI [8], and breast cancer risk [9]. The common factor in these patterns of lactate and 2D:4D expression is likely to be hypoxia, i.e. a condition in which an area of the body is deprived of adequate oxygen supply at the tissue level. Therefore, the purpose of this study was to examine the relationship between 2D:4D and lactate in an incremental cardiopulmonary test. In order to clarify the expected relationships we first consider the background to both lactate and 2D:4D expression in health and disease.

The accumulation of lactate in arterial blood has long been considered as a consequence of an oxygen deficit at the onset of vigorous exercise and as such, it is eliminated during recovery with increased use of oxygen [10]. This model is now revised as lactate released during exercise is thought to be used as an important energy source [11]. Prolonged sub-maximal exercise releases lactate from the muscles and this is then taken up by the liver and converted to glucose. Therefore, a balance is established in blood lactate level. However, when VO_{2max} is approached, there is an exponential increase in lactate caused, in part, by constriction in the hepatic blood flow and a reduction in the conversion of lactate to glucose in the liver [12]. Thus, rapid accumulation of lactate occurs during vigorous exercise, particularly leg exercise, in healthy individuals. However, elevated levels of lactate are also important as prognostic factors in diseases such as MI and atherosclerosis [4,3]. With regard to the former, MI is caused by myocardial ischaemia as a result of coronary artery occlusion. This leads to a decrease in oxygen transport to myocardial cells and an increase in the production of lactate in cardiac myocytes. Therefore, elevated blood lactate levels predict poor outcomes in MI patients [13, 14]. With regard to atherosclerosis, this is a complex inflammatory process which has effects on vascular cells, i.e. endothelial and smooth muscle cells, in addition to monocytes, macrophages and lymphocytes. In common with MI it has been reported that carotid atherosclerosis is associated with elevated levels of lactate [15]. Associations between lactate, exercise and heart disease are intuitively appealing. However, perhaps more surprising are the links between elevated lactate levels and cancer. The link arises because the tumour microenvironment tends to be hypoxic. Thus, cancer cells take up high amounts of glucose with a marked increase in the production of lactate. This has been known since 1927 and is referred to as the "Warburg effect"[16, 17]. The accumulation of lactate in solid tumours is an important and early event in the development of malignancies. For example, lactate tumour concentrations have been shown to be inversely related to survival of patients and positively related to the incidence of metastases for a number of cancers, including cervical[18], and breast [19] cancers. However, it is to be noted that some cancers show lower levels of lactate compared to normal tissue e.g. thyroid cancer [19].

Digit ratio (2D:4D: the ratio between the 2nd and 4th digit) is sexually dimorphic (males<females) and is widely regarded to be a correlate of 1st trimester sex steroid exposure [20]. More specifically, 2D:4D is thought to be negatively related to prenatal testosterone and positively related to prenatal oestrogen [21, 22 but see 23]. There are links between lactate and testosterone. Exercise-induced testosterone secretion has been reported to be increased in rat testicular cells when cultured with lactate [24-26]. Performance in distance running is negatively related to 2D:4D in both men and women with effect sizes stronger in the former [6, 27, 28]. Oxygen metabolism in conditions of exercise-induced hypoxia has been reported to be strongly negatively related to 2D:4D[7, 29, 30]. High 2D:4D is also associated with MI and atherosclerosis in men. With regard to the former, high 2D:4D has been reported to be associated with early MI in men in five studies [8, 31-34] with one report showing a non-significant relationship [35]. Comparisons between MI patients and age-matched controls have reported higher 2D:4D in five studies [32-36]with two studies reporting no difference[8, 37]. Atherosclerotic plaque development may also be associated with high 2D:4D. A report of autopsy findings of young men found that high grade atherosclerosis was associated with high 2D:4D [38]. With regard to cancer, a review of 25 studies has shown high 2D:4D was linked to cancer in eleven studies, low 2D:4D in eight studies and five reported no association [39]. Examples of the former include breast cancer [9, 40] and of the latter prostate cancer [41, 42].

Therefore, there is evidence that lactate and 2D:4D show similar patterns in their links to metabolism and disease. That is, they are both correlates of the outcomes of vigorous exercise, cardiovascular disease and a number of cancers. We suggest that 2D:4D is a biomarker for between-individual variation in lactate production. The common link here may be hypoxic conditions at the tissue level which occur as a result of vigorous exercise, heart disease (such as MI) and rapid cell division in growing tumours. In order to test our hypothesised link, we consider lactate accumulation during an incremental cardiopulmonary test with a sample of professional footballers. Our prediction is that 2D:4D will be positively related to lactate levels at each point in the test. In general, effect sizes in 2D:4D studies are strongest for right 2D:4D e.g. [20]. Therefore, the positive relationship should be seen for right and left 2D:4D and for Dr-l, with effect sizes greatest for the former.

Methods

Details of the recruitment of participants and of the cardiopulmonary test are given in Parpa et al (2024) [7]. Briefly, the sample consisted of professional male football players participating in Division 1 and 2 in the Eastern Mediterranean. Testing was undertaken before the pre-season period. Exclusion criteria included injuries within the last two months before the testing. The players completed an incremental cardiopulmonary test to exhaustion on a treadmill. 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).

Anthropometric measurements were conducted using a wall stadiometer to determine stature (Leicester; Tanita, Tokyo, Japan) and a leg-to-leg bioelectrical impedance analyzer (BC418MA; Tanita) to assess body composition (% body fat). The participant's hands were scanned. The players placed their hand on the surface of an EPSON scanner (DS-50000) with the palm facing downwards and fingers as straight as possible. They were instructed to lightly place their fingers on the scanner and wait until the scan was completed. In cases where the scan was not clear, it was repeated. Digit length was measured twice by the same investigator [20], blind to the lactate 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 calipers (Mitutoyo, D15, Japan).

The players started the test at a speed of 8km/h (first lactate measurement was conducted at that time). Then the speed increased every 3.15 minutes by 2km/h. Therefore, measurements were taken at 10,12,14,16 and 18 km/h. Some players did not enter the 18km/h. With regard to the lactate values, the Nova blood lactate plus analyser was used for the blood lactate measurements. Lactate plus single-use test strip was touched to a drop of blood taken via finger prick (0.7 microliters) to initiate the test. The blood lactate values were available on-screen in 13 seconds. Measurements of blood samples were obtained every 3 minutes after completing each stage of the test.

Statistical analysis

Means and standard deviations (mean ± SD) were calculated for all the parameters. The normality assumption was verified using the Shapiro–Wilk test. Intra-class correlation coefficients (ICC) (absolute agreement) between the first and second 2D:4D's of the right and left digits were calculated. Median splits (Low and High) were calculated for right and left 2D:4D and right-left 2D:4D (Dr-l). Differences in mean (Cohen's *d*) were described as very small (.01), small (.20), medium (.50), large (.80), very large (1.20), huge (2.0) [43, 44]. Three two-factor ANOVA's were performed with dependent variable lactate levels and predictor variables Speed [8, 10, 12, 14, 16 Km/hr] and digit ratio [Low/High]). Pearson-product moment correlation coefficients were used to calculate associations. Correlations were described 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) [45]. All statistical analyses were performed in IBM® SPSS® Statistics, version 26.0, for Windows (SPSS Inc., Chicago, IL), and the statistical significance was set at $p < .05$.

Results

Repeatability of 2D:4D

Right 2D:4D could be measured from 79 participants and left 2D:4D from 76 participants. There was high repeatability between 2D:4D calculated from the first and second digit measurements. The intra-class correlation coefficients (ICC's) were: right 2D:4D, ICC = .975, F () = 77.68; left 2D:4D, ICC = .972, F () = 70.09, both p<.0001. Therefore, we calculated mean 2D:4D from first and second measurements and used these in all subsequent analyses.

Ethnicity

There were 72 Caucasian and 7 Black participants. There were significant ethnic differences (Caucasian>Black players) in mean (± SD) 2D:4D for right (n = 72, Caucasian .960±.036, Black .926±.025, t = 2.48, p = .02) and left 2D:4D (n = 76, Caucasian .955±.031, Black .926±.023, t = 2.18, p = .03). Therefore, the less numerous ethnic group was removed and all analyses were conducted on the Caucasian players.

Descriptive Statistics

The means, (SD's, and minimum and maximum values)for age, height, weight, BMI, % body fat and lactate values are given in Table 1. Lactate levels (mmol/L) are given for speeds at 8Km/hr, 10Km/hr, 12Km/hr, 12Km/hr, 14Km/hr, 16Km/hr, 18Km/hr. All players (n = 72) remained on the treadmill for speeds 8Km/hr through to 16Km/hr. For 18Km/hr there were *n* = 40 participants. Therefore, we focussed our analyses on lactate levels measured at speeds 8Km/hr to 16Km/hr.

Relationships with Lactate levels

Lactate levels increased with increasing running speeds (8Km/hr = 1.533 to 16Km/hr = 10.467: ANOVA, F[4, 355] = 312.856, p<.0001).

There were no significant correlations between age, body size (height, mass, BMI), body composition (% body fat) and the lactate levels at treadmill speeds of 8Km/hr to 16Km/hr (values of *r* varied from .001 to .20, all p>.05: Table 2).

Digit ratios and Lactate

Mean digit variables (SD plus minimum and maximum) were as follows: right 2D:4D .960±.036, .891 to 1.09, n = 72; left 2D:4D .955±.031, .869 to 1.021, n = 70; Dr-l .004±.037, -.102 to .109, n = 70; mean digit lengths ([2D+4D]/2) right hand 74.895±3.63mm, 64.125mm to 84.438mm; left hand74.378±3.745mm, 64.213mm to 85.338mm.

With regard to mean digit lengths per hand, there were no significant correlations with lactate levels at any running speed: right hand correlations with lactate varied from 16Km/hr *r* = -.008, p = .95 to 8Km/hr *r* = -.092, *p* = .44; left hand 14Km/hr *r* = .001, *p* = .99 to 16Km/hr *r* = .043, *p* = .73.

In order to consider the relationships between digit ratios (right 2D:4D, left 2D:4D and right-left 2D:4D [Dr-l]) and lactate levels across speeds 8KM/hr to 16Km/hr we first calculated median splits for each ratio (right 2D:4D, Low 1 = .891 to .956, n = 36, High 2 = .957 to 1.09, n = 36; left 2D:4D, Low 1 = .869 to .951, n = 35, High 2 = .952 to 1.02, n = 35; Dr-l, Low 1 = -.102 to .001, n = 35, High 2 = .002 to .109, n = 35). Mean lactate values for low and high digit ratio groups at speeds from 8KM/hr to 16Km/hr are given in Table 3.

Three two-factor ANOVA's were performed with dependent variable lactate levels and predictor variables Speed [8, 10, 12, 14, 16 Km/hr] and digit ratio [Low/High]). For right 2D:4D, there were significant main effects for Speed, F (4,350) = 698.199, p<.0001 and digit ratio, F(1,350) = 319.699, p<.0001, with a significant Speed*digit ratio interaction, F(4,50) = 30.638, p<.0001. Post hoc comparisons showed that at each speed the high right digit ratio group had significantly higher levels of lactate than that of the low right digit ratio group (Fisher's PLSD for lactate levels, Low-High mean difference -2.184, critical difference .240, p<.0001). The effect sizes (Cohen's *d*) for the Low-High lactate differences per speed varied from very large (12Km/hr, *d* = 1.74) to huge (16Km/hr, *d* = 2.29). For left 2D:4D, there were significant main effects for Speed, F (4,340) = 378.697, p<.0001 and digit ratio, F(1,340) = 68.597, p<.0001, with a significant Speed*digit ratio interaction, F(4,340) = 5.745, p = .0002. As with right 2D:4D, post hoc comparisons showed that at each speed the High left digit ratio group had significantly higher levels of lactate than that of the Low left digit ratio group (Fisher's PLSD for lactate levels, Low-High mean difference -1.373, critical difference .326, p<.0001). In contrast to right 2D:4D, the effect sizes for the Low-High lactate differences per speed were large and varied from *d* = .92 (14Km/hr) to *d* = 1.06 (12Km/hr and 16Km/hr). For Dr-l, there were significant main effects for Speed, F (4,340) = 324.131, p<.0001 and digit ratio, F(1,340) = 19.775, p<.0001, with a significant Speed*digit ratio interaction, F(4,340) = 2.404, p = .0495. Post hoc comparisons showed that at each speed the High Dr-l group had significantly higher levels of lactate than that of the Low Dr-l group (Fisher's PLSD for lactate levels, low-high mean difference -.797, critical difference .352, p<.0001). Effect sizes for the lactate differences in Low-High Dr-l groups were medium and varied from *d* = .42 (10Km/hr) to *d* = .61 (16Km/hr).

Focussing on individual differences in digit ratios and lactate, we considered product-moment correlations (*r*) between digit ratios (right 2D:4D; left 2D:4D and Dr-l) and blood lactate levels at running speeds of 8 Km/hr to 16Km/hr. Considering right 2D:4D,effects were large, varying from *r* = .616 for 12Km/hr to *r* = .78 for 16Km/hr, all p<.0001 (Figure 2). For left 2D:4D, effects were medium, varying from *r* = .416, *p* = .0003 for 14Km/hr to *r* = .567, *p*<.0001 for 10Km/hr. The effects for Dr-l were small. There was one non-significant correlation for 12Km/hr *r* = .217, *p* = .07, with the remaining associations varying from *r* = .246 *p* = .04 for 10Km/hr to *r* = .381, *p* = .001 for 14Km/hr (see Table 4).

Discussion

We have found that blood lactate levels increased with increasing treadmill speed such that at 8Km/hr the concentration was 1.533±.406 mmol/L and at 16Km/hr it was 10.467±2.786 mmol/L. Our focus here is not with the means but in predicting the between-individual differences in lactate. There were no significant correlations between lactate levels and age, height, mass, BMI, % body fat or mean digit lengths at any of the treadmill speeds. In contrast, a median split of ratios (Low and High) showed relative lactate levels to be Low<High at all treadmill speeds. Effect sizes (*d*) for right 2D:4D at each speed varied from very large to huge, left 2D:4D large and medium for Dr-l. Considering correlations between digit ratios and lactate at each speed, we found effect sizes (*r*) to vary from large for right 2D:4D, medium for left 2D:4D and small for Dr-l. These findings are supportive of our suggestion that digit ratio, particularly right 2D:4D, is a biomarker of lactate levels during vigorous exercise.

The positive association between 2D:4D and lactate could also be found more widely in health and disease. Aside from heart disease and cancers this might be the case for Covid-19 and related viruses. Hospitalized patients with Covid-19 show progressive hypoxia among the most seriously affected and duration of oxygen therapy has been reported to be positively related to 2D:4D [46]. Severe cases of Covid-19 do show elevated levels of lactate but more importantly they also show markedly high levels of lactate dehydrogenase (LDH), an enzyme that catalyses the inter-conversion of pyruvate and lactate [47]. This suggests that associations between Covid-19, 2D:4D, lactate and LDH should be investigated. Outcomes in emergency medicine might also be fruitfully investigated for links with 2D:4D and lactate.

Relative hyperlactatemia is associated with increased mortality in emergency department patients [48]. In the intensive care setting, lactate is commonly viewed as being associated with tissue hypoxia and sepsis [10].

Our study has limitations. The data relate to Caucasian males and should be extended to females and other ethnicities with larger samples. However, our findings are consistent with associations between digit ratios and oxygen metabolism measured by such variables as VO_{2max} and ventilator thresholds.

We conclude that digit ratios are correlates of lactate levels during vigorous exercise. Of the three ratios, right 2D:4D seems to be most strongly correlated with large effect sizes. Digit ratios and lactate show similarities in the pattern of their expression. In addition to vigorous exercise this pattern includes links with heart disease, viruses and cancers. We suggest that digit ratios are biomarkers for lactate levels and as such may be useful as predictors of between-individual differences in lactate production in health and disease.

References

[1]. B.S. Ferguson, M.J. Rogatzki, M.L. Goodwin, D.A. Kane, Z. Rightmire, L.B. Gladden. Lactate metabolism: historical context, prior misinterpretations, and current understanding. Eur. J. Appl. Physiol. 118 (4) (2018) 691-728.

[2]. J.D. Rabinowitz, S. Enerbäck. Lactate: the ugly duckling of energy metabolism. Nat. Metab. 2 (7) (2020) 566–571.

[3]. R.P. Vermeulen, M. Hoekstra, M.W. Nijsten, I.C. van der Horst, L.J. van Pelt, G.A. Jessurun, T. Jaarsma, F. Zijlstra, A.F. van den Heuvel. Clinical correlates of arterial lactate levels in patients with ST-segment elevation myocardial infarction at admission: a descriptive study. Crit. Care. 14 (5) (2010) R164.

[4]. J. Ouyang, H. Wang, J. Huang. The role of lactate in cardiovascular diseases. Cell Commun. Signal. 21 (1) (2023) 317.

[5]. M. Cai, J. Wan, K. Cai, H. Song, Y. Wang, W. Sun, J. Hu. Understanding the Contribution of Lactate Metabolism in Cancer Progress: A Perspective from Isomers. Cancers (Basel). 15 (1) (2022) 87.

[6]. J.T. Manning, L. Morris, N. Caswell. Endurance running and digit ratio (2D: 4D): Implications for fetal testosterone effects on running speed and vascular health. Am. J. Hum. Biol.19 (3) (2007) 416–421.

[7]. K. Parpa, J.T. Manning, M. Kobus, L. Mason, M. Michaelides. The associations between digit ratio (2D:4D and right – left 2D:4D), maximal oxygen consumption and ventilatory thresholds in professional male football players. Am. J. Hum. Biol. (2024) e24047.

[8]. J.T. Manning, P.E. Bundred, A. Kasielska-Trojan, T. Smith-Straney, L. Mason. Digit ratio (2D:4D), myocardial infarction and fibrinogen in men. Early Hum. Dev. 133 (2019) 18-22.

[9]. D. Muller, L. Baglietto, J. Manning, C. McLean, J. Hopper, D. English,. . . G. Severi, Second to fourth digit ratio (2D: 4D), breast cancer risk factors, and breast cancer risk: a prospective cohort study. Br. J. Cancer. 107 (9) (2012) 1631-1636.

[10]. U.W. Lepsen, R.R. Plovsing, K. Tjelle, N.B. Foss, C.S. Meyhoff, C.K. Ryrsø, M.G.R. Berg, N.H. Secher. The role of lactate in sepsis and COVID-19: Perspective fromcontracting skeletal muscle metabolism. Exp. Physiol. 107 (7) (2022) 665–673.

[11]. G. Van Hall, M. Strømstad, P. Rasmussen, O. Jans, M. Zaar, C. Gam, B. Quistorff, N.H. Secher, H.B. Nielsen. Blood lactate is an important energy source for the human brain. J. Cereb. Blood Flow Metab. 29 (6) (2009) 1121–1129.

[12]. H.B. Nielsen, M.A. Febbraio, P. Ott, P. Krustrup, N.H. Secher. Hepatic lactate uptake versus leg lactate output during exercise in humans. J. Appl. Physiol. 103 (4) (2007) 1227–1233.

[13]. Z. Mavrić, L. Zaputović, D. Zagar, A. Matana, D Smokvina. Usefulness of blood lactate as a predictor of shock development in acute myocardial infarction. Am. J. Cardiol. 67 (7) (1991) 565-8.

[14]. G. Gjesdal, O.Ö. Braun, J.G. Smith, F. Scherstén, P. Tydén. Blood lactate is a predictor of short-term mortality in patients with myocardial infarction complicated by heart failure but without cardiogenic shock. BMC Cardiovasc. Disord. 18 (1) (2018) 8.

[15]. G.P. Shantha, B. Wasserman, B.C. Astor, J. Coresh, F. Brancati, A.R. Sharrett, J.H. Young. Association of blood lactate with carotid atherosclerosis: the atherosclerosis risk in Communities (ARIC) Carotid MRI Study. Atherosclerosis. 228 (1) (2013) 249–55.

[16]. O. Warburg, F. Wind, E. Negelein, The metabolism of tumors in the body. J. Gen. Physiol. 8 (6) (1927) 519–53.

[17]. R. Pérez-Tomas, I. Pérez-Guillen Lactate in the Tumor Microenvironment: An Essential Molecule in Cancer Progression and Treatment. Cancers. 12 (11) (2020) 3244.

[18]. S. Walenta, M. Wetterling, M. Lehrke, G. Schwickert, K. Sundfør, E.K. Rofstad, W. Mueller-Klieser. High lactate levels predict likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers. Cancer Res. 60 (4) (2000) 916–21.

[19]. C. Wang, Z. Qu, L. Chen, Y. Pan, Y. Tang, G. Hu, R. Gao, R. Niu, Q. Liu, X. Gao, Y. Fang. Characterization of Lactate Metabolism Score in Breast and Thyroid Cancers to Assist Immunotherapy via Large-Scale Transcriptomic Data Analysis. Front. Pharmacol. 13 (2022) 928419.

[20]. J.T. Manning, D. Scutt, J. Wilson, D.I. Lewis-Jones. The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. Hum. Reprod. 13 (11) (1998) 3000-3004.

[21]. A. Swift-Gallant, B.A. Johnson, V. Di Rita, S.M. Breedlove, Through a glass, darkly: human digit ratios reflect prenatal androgens, imperfectly. Horm. Behav. 120 (2020) 104686.

[22]. J.T. Manning, B. Fink. Digit ratio (2D:4D) and its relationship to foetal and maternal sex steroids: A mini-review. Early Hum. Dev. 183 (2023) 105799.

[23]. C.M. McCormick, J.M. Care, Facing off with the phalangeal phenomenon and editorial policies: a commentary on Swift-Gallant, Johnson, Di Rita and Breedlove (2020). Horm. Behav. 120 (2020) 104710

[24]. L. Shin-Shan. Lactate and the effects of exercise on testosterone secretion: Evidence for the involvement of a cAMP-mediated. J. Chem. Inf. Model. 53 (1997) 1689–1699.

[25]. H. Lin, S.W. Wang, R.Y. Wang, P.S. Wang Stimulatory effect of lactate on testosterone production by rat Leydig cells. J. Cell. Biochem. 83 (1) (2001)147–154.

[26]. J.P. White, S. Gao, M.J. Puppa, S. Sato, S.L. Welle, J.A. Carson. Testosterone regulation of Akt/mTORC1/FoxO3a Signaling in Skeletal Muscle. Mol. Cell. Endocrinol. 365 (2) (2013) 174–186.

[27]. D. Longman, J.C. Wells, J.T. Stock. Can persistence hunting signal male quality? A test considering digit ratio in endurance athletes. PLoS One, 10 (4) (2015) e0121560.

[28]. E. Eklund, L. Ekström, J.O. Thörngren, M. Ericsson, B. Berglund, A.L. Hirschberg. Digit ratio (2D: 4D) and physical performance in female Olympic athletes. Frontiers in Endocrinology 11 (2020) 292.

[29]. R. Hill, B. Simpson, G. Millet, J. Manning, L. Kilduff. Right–left digit ratio (2D: 4D) and maximal oxygen uptake. J. Sports Sci. 30 (2) (2012) 129–134.

[30]. M.P. Lombardo, S. Otieno. The associations between digit ratio, aerobic fitness, physical skills, and overall physical fitness of elite youth distance runners. Am. J. Hum. Biol. 33 (1) (2021) e23448.

[31]. J.T. Manning, P.E. Bundred. The ratio of 2nd to 4th digit length and age at first myocardial infarction in men: a link with testosterone? Br. J. Cardiol. 8 (12) (2001) 720–723.

[32]. H. Zhenghao, L. Hong, D. Jie, F.L. Martin, Correlations between digit ratio and foetal origins of adult diseases in a Chinese population: a focus on coronary heart disease and breast cancer, Handbook of Anthropometry, Springer, (2012) 853–865.

[33]. H. Lu, Z. Huo, K. Zhang, N. Wei, Z. Shi, L. Peng, ... H. Jiao, Relations between digit ratio and coronary heart disease, Acta Anat. Sin. 39 (2008) 765–768.

[34]. H. Lu, Z. Ma, J. Zhao, Z. Huo, Second to fourth digit ratio (2D:4D) and coronary heart disease, Early Hum. Dev. 91 (2015) 417–420.

[35]. X.L. Wu, D.Y. Yang, W.H. Chai, M.L. Jin, X.C. Zhou, L. Peng, Y.S. Zhao, The ratio of second to fourth digit length (2D:4D) and coronary artery disease in a Han Chinese population, Int. J. Med. Sci. 10 (2013) 1584–1588.

[36]. I. Kyriakidis, P. Papaioannidou, V. Pantelidou, V. Kalles, K. Gemitzis, Digit ratios and relation to myocardial infarction in Greek men and women, Gend. Med. 7 (2010) 628–636.

[37]. F. Aksu, H. Topacoglu, C. Arman, S. Tetik, Neck circumference and 2:4 digit ratio in patients with acute myocardial infarction, Turkiye Klinikleri J. Cardiovasc. Sci. 21 (2009) 147–152.

[38]. O. Ozdogmus, Y.O. Cakmak, M. Coskun, U. Verimli, S. Cavdar, I. Uzun, The high 2D:4D finger length ratio effects on atherosclerotic plaque development, Atherosclerosis. 209 (2010) 195–196.

[39]. C.A.D. Fonseca, D.R.B. Martelli, C.M.F. Maia, V.O. Dias, A.A. Carvalho, H.M. Júnior. Digital biomarker 2D:4D as a predictor of cancer: A systematic review. Early Hum. Dev. 164 (2022)105521.

[40]. J.T. Manning, S.J. Leinster. re: The ratio of 2nd to 4th digit length and age at presentation of breast cancer: a link with prenatal oestrogen? Breast. 10 (4) (2021) 355-357.

[41]. A.A. Rahman, A. Lophatananon, S. Stewart-Brown, D. Harriss, J. Anderson, T. Parker, . . . K. Muir. Hand pattern indicates prostate cancer risk. Br. J. Cancer. 104 (1) (2011) 175-177.

[42]. D.C. Muller, G.G. Giles, J.T. Manning, J.L. Hopper, D.R. English, G. Severi. Second to fourth digit ratio (2D:4D) and prostate cancer risk in the Melbourne Collaborative Cohort Study. Br. J. Cancer. 105 (3) (2011) 438-40.

[43]. J. Cohen (1998). *Statistical Power Analysis for the Behavioral Sciences*. Routledge.

[44]. S. Sawilowski. New effect size rules of thumb. Journal of Modern Applied Statistical Methods. 8 (2) (2009) 467-474.

[45]. W. Hopkins, S. Marshall, A. Batterham, J. Hanin. Progressive statistics for studies in sports medicine and exercise science. Med. Sci. Sports Exerc.41(1) (2009) 3–12.

[46]. A. Kasielska-Trojan, M. Jabłkowski, J. Białkowska-Warzecha, O. Kwaśniewska, B. Antoszewski. Understanding Covid-19: Digit ratios and duration of oxygen therapy in hospitalized Covid-19 patients. Early Hum. Dev. 189 (2004) 105940.

[47]. B. Fialek, M. Pruc, J. Smereka, R. Jas, M. Rahnama-Hezavah, A. Denegri, A. Szarpak, M,J. Jaguszewski, F.W. Peacock, L. Szarpak. Diagnostic value of lactate dehydrogenase in COVID-19: A systematic review and meta-analysis. Cardiol. J. 29 (5) (2022) 751-758.

[48]. R. Bou Chebl, S. Jamali, N. Mikati, R. Al Assaad, K. Abdel Daem, N. Kattouf, R. Safa, M. Makki, H. Tamim, G. Abou Dagher. Relative Hyperlactatemia in the Emergency Department. Front Med (Lausanne). 7 (2020) 561.

Table 1

Descriptive statistics for the sample. All participants (*n* = 72) completed the treadmill test for speeds 8Km/hr to 16Km/hr, 40 players remained on the treadmill for 18Km/hr. Units of measurement for lactate (mmol/L).

trait	*n*	mean	SD	minimum	maximum
Age	72	25.431	5.272	17	36
Height	72	179.82	6.49	169	197
Weight	72	76.69	6.82	58.9	91.3
BMI	72	23.67	1.298	19.5	26.7
% body fat	72	11.237	2.47	4.98	17.8
Lactate 8Km/hr	72	1.533	.406	1.0	2.5
Lactate 10Km/hr	72	2.365	.845	1.1	4.2
Lactate 12Km/hr	72	3.939	1.16	1.6	6.1
Lactate 14Km/hr	72	6.465	2.236	2.6	11.6
Lactate 16Km/hr	72	10.467	2.786	4.4	14.6
Lactate 18Km/hr	40	11.718	1.593	9.1	14.7

Table 2

Product moment correlations (*r*) between age, body size (height, mass, BMI), body composition (%body fat) and lactate levels at five different treadmill speeds in 72 professional male footballers.

	Lactate levels (mmol/L) at five treadmill speeds				
Trait	8Km/hr	10Km/hr	12Km/hr	12Km/hr	16Km/hr
Age	.20	.03	.04	-.02	.08
Height	.04	-.01	.02	-.01	.06
Mass	-.01	.004	.02	-.05	.048
BMI	-.07	.025	.001	-.07	-.005
%body fat	.008	.083	.05	.007	.007

Table 3

Mean lactate levels (SD and SE mmol/L) of 72 professional football players at five speeds during a treadmill test. The means are given for median splits of 2D:4D (right, left and Dr-l).

	8 Km/hr	10 Km/hr	12 Km/hr	14 Km/hr	16 Km/hr
Low right 2D:4D n = 36	1.233 SD .269 SE .045	1.733 SD .542 SE .090	3.175 SD .77 SE .128	4.794 SD 1.051 SE .175	8.372 SD 2.26 SE .377
High right 2D:4D n = 36	1.833 SD .276 SE .046	2.997 SD .578 SE .096	4.703 SD .974 SE .162	8.136 SD 1.815 SE .303	12.561 SD 1.268 SE .211
Low left 2D:4D n = 35	1.354 SD .375 SE .063	1.917 SD .696 SE .118	3.429 SD 1.041 SE .176	5.571 SD 2.107 SE .356	9.14 SD 2.78 SE .47
High left 2D:4D n = 35	1.717 SD.35 SE .059	2.846 SD .719 SE .121	4.5 SD .988 SE .167	7.429 SD 1.938 SE .328	11.783 SD 2.188 SE .37
Low Dr-l n = 35	1.42 SD .393 SE .066	2.206 SD.896 SE .151	3.714 SD 1.159 SE .196	5.877 SD 1.95 SE .33	9.634 SD 2.915 SD .493
High Dr-l n = 35	1.651 SD .386 SE .065	2.557 SD .761 SE .129	4.214 SD 1.086 SE .183	7.123 SD 2.317 SE .392	11.289 SD 2.491 SE .421

Table 4

Product moment correlations (*r*) between digit ratios (right 2D:4D; left 2D:4D; Dr-l) and blood lactate levels(mmol/L) at five running speeds. Sample sizes: right 2D:4D *n* = 72, left 2D:4D and Dr-l *n* = 70.

	correlations (*r*) between digit ratios and blood lactate levels (mmol/L) at five running speeds				
Trait	8Km/hr	10Km/hr	12Km/hr	12Km/hr	16Km/hr
Right 2D:4D	.703 *p*<.0001	.709 *p*<.0001	.616 *p*<.0001	.711 *p*<.0001	.780 *p*<.0001
Left 2D:4D	.501 *p*<.0001	.567 *p*<.0001	.506 *p*<.0001	.416 *p* = .0003	.525 *p*<.0001
Dr-l	.288 *p* = .02	.246 *p* = .04	.217 *p* = .07	.381 *p* = .001	.327 *p* = .006

Figure 1

The relationship between mean (SE error bars) levels of lactate and running speed in football players with high or low right 2D:4D. The sample of 72 football players was median split into 36 players with right 2D:4D ranging from .891 to .956 (Low 2D:4D) and 36 football players with right 2D:4D ranging from .957 to 1.09 (High 2D:4D). The effect sizes (*d*) of differences in lactate levels between the groups varied from very large (12Km/hr, *d* = 1.74) to huge (16 Km/hr, *d* = 2.29).

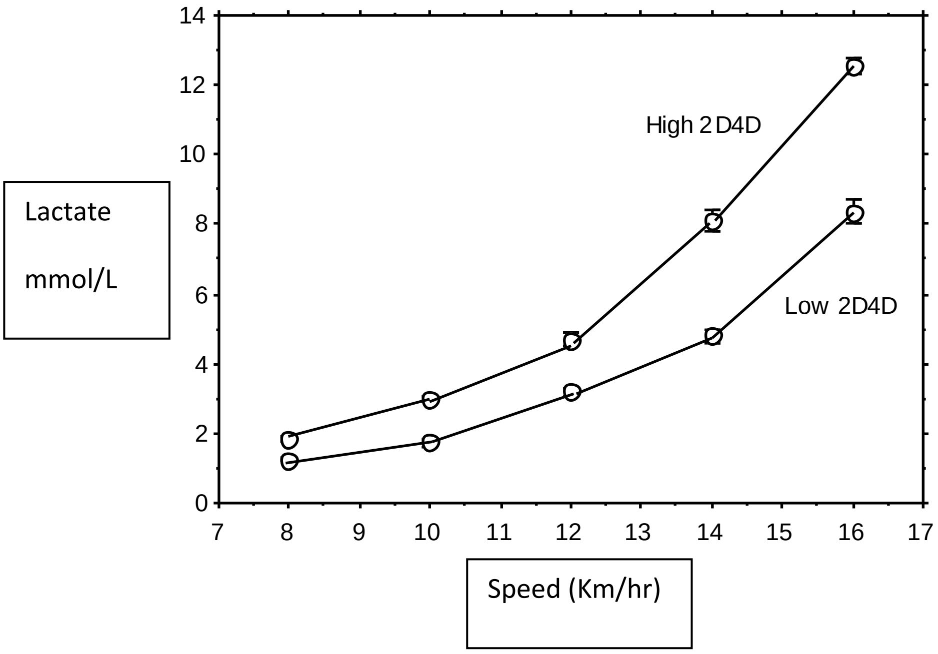

Figure 2

The relationship between right 2D:4D and lactate blood levels at 16Km/hr during a cardiopulmonary test in 72 professional male footballers. The equation for the line is: ***y* = 60.151*x* – 47.289, r_2 = .61.**