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Relationship Between a Proxy of Prenatal Testosterone (2D:4D) and Determinants of Endurance Running Performance

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By

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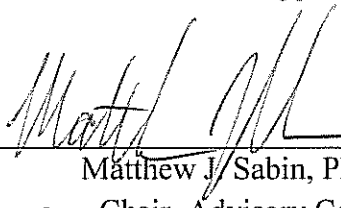

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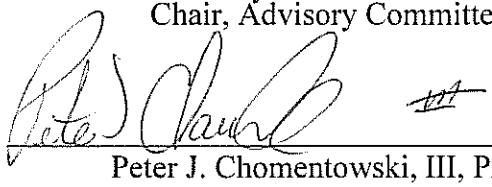

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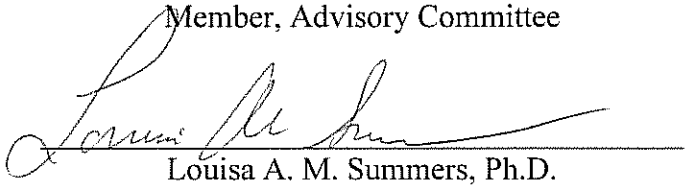

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RELATIONSHIP BETWEEN A PROXY OF PRENATAL TESTOSTERONE (2D:4D) AND DETERMINANTS OF ENDURANCE RUNNING PERFORMANCE

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ABSTRACT

Low ratios of the length of the second finger to the length of the fourth finger in the right (right 2D:4D) and the left hand (left 2D:4D) have been linked to high prenatal testosterone concentrations. Low R-L 2D:4D (subtracting left 2D:4D from right 2D:4D) has been associated with high androgen sensitivity as indicated by low numbers of cytosine-adenine-guanine (CAG) triplet repeats on exon-1 of the androgen receptor gene. Endurance training has led to higher increases in maximal oxygen uptake capacity ($\dot{V}O_{2max}$) in men with relatively low numbers of CAG triplet repeats, suggesting a relationship between R-L 2D:4D and $\dot{V}O_{2max}$. Moreover, Low right and left 2D:4D are associated with superior performances in sports such as fencing, rugby, soccer, basketball, and sumo wrestling. The strongest associations, however, have been found between right 2D:4D and endurance running performance ($r^2 = 0.25$). An inverse relationship between R-L 2D:4D and $\dot{V}O_{2max}$, running velocity at $\dot{V}O_{2max}$, and peak lactate concentration in pubertal boys has been reported.

The purpose of the present investigation was to examine the relationships between measures of digit ratios and performance variables ($\dot{V}O_{2max}$, maximal respiratory exchange ratio (RER_{max}), absolute running economy (RE_{abs}), relative running economy (RE_{rel}), and total time on the treadmill (ToT)) in post-pubertal sedentary populations and in trained endurance runners. The relationship between digit ratios and endurance running performance in the form of personal records (PRs) over different race distances in the trained runners was also examined in order to explore which performance variable moderates the relationship between prenatal testosterone and/or testosterone sensitivity and endurance running performance. A significant negative relationship between left 2D:4D and $\dot{V}O_{2max}$ ($r = 0.49$) was found only in the female sedentary group after removing the effects of weight using first order partial correlation analyses. In both the male and female trained runners, measures of digit ratio and $\dot{V}O_{2max}$ showed a trend to be positively related. RE_{abs} related negatively to digit ratios in the male and female sedentary groups, while RE_{rel} was negatively related to digit ratios in only the male sedentary group. All other relationships between digit ratios and performance variables were highly inconsistent across groups and often within groups. We found fairly consistent and moderately strong positive relationships between digit ratios and PRs which do not seem to be moderated by $\dot{V}O_{2max}$. However, The associations between 2D:4D and endurance running performance seemed be mediated by RER_{max} in male endurance runners, indicating that the capacity to buffer and/or clear lactic acid moderates the relationship between prenatal testosterone stimulation and endurance running capabilities. We recommend the investigation of the relationships among lactate threshold, endurance running performance and 2D:4D.

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INTRODUCTION

Prenatal Testosterone and Sexual Dimorphism

A mounting body of evidence suggests that prenatal testosterone exerts a permanent organizational effect on sexually dimorphic traits and characteristics. It determines to what degree a person possesses male or female characteristics. Pre-natal testosterone has organizational effects on a person's anatomy and physiology, masculinizing behavior, appearance, and physical ability (Cohen-Bendahan, Buitelaar, Van Goozen, Orlebeke, & Cohen-Kettenis, 2005; Hönekopp, Manning, & Müller, 2006). Sex differences upon birth seem to be caused by differences in testosterone concentrations and not estrogen concentrations as estrogen levels between male and female fetuses were found to be equal (McIntyre, 2006). Evidence exists that the degree of masculinization is a correlate of prenatal androgen levels as the absence of fetal testosterone leads to female phenotypical development (Zitzmann & Nieschlag, 2003). Research conducted with non-human animals has shown that the artificial elevation of prenatal testosterone levels leads to the masculinization of a variety of characteristics. Ethical concerns prohibit such experimentation with human fetuses; therefore investigators are limited to correlational studies to explore the relationships between early androgen stimulation and proxies thereof and adult characteristics in humans (Breedlove, 2010).

2D:4D and Sex Steroid Stimulation

The ratio of the length of the second digit to the length of the fourth digit (2D:4D) on the human hand is a sexually dimorphic trait, whereas men have lower 2D:4D than women (Hönekopp & Watson, 2010). The amount of testosterone the fetus produces between the 12^{th} and 24^{th} week, as measured by analysis of amniotic fluid (amniocentesis), negatively correlates with 2D:4D (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004). This means that greater testosterone production by the gonads of the fetus leads to a shorter index finger relative to the ring finger of the same hand (Lutchmaya et al., 2004). Besides amniocentesis other methods of establishing the link between *in utero* testosterone and digit ratios have been employed. These include the comparison of 2D:4D between females with congenital adrenal hyperplasia (CAH) and healthy controls (e.g. Buck, Williams, Hughes, & Acerini, 2003), the comparison of 2D:4D in dizygotic twins of the same and opposite sex (e.g. Anders, Vernon, & Wilbur, 2005), the comparison of 2D:4D between women with polycystic ovary syndrome and healthy controls

(e.g. Cattrall, Vollenhoven, & Weston, 2005), and the comparison of 2D:4D between men with Klinefelter's syndrome (KS) and healthy controls (Manning, Kilduff, & Trivers, 2013).

Typically, the index finger is slightly shorter than the ring finger, which causes the value of 2D:4D to be below 1.0 in most individuals. The sex differences in 2D:4D are more pronounced in the right hand (Hönekopp & Watson, 2010) and it seems that the fourth or ring finger is equipped with more androgen and estrogen receptors than the second or index finger especially in the right hand (Zheng & Cohn, 2011). Estrogen stimulates metaphyseal tissue to calcify while testosterone promotes bone growth (Weise et al., 2001). Evidence also suggests that testosterone sensitivity governed by the androgen receptor gene influences the development of sexually dimorphic traits (Breedlove, 2010). The ability of androgen receptor genes to transcribe the testosterone stimulus depends on their number of cytosine-adenine-guanine (CAG) triplet repeats (Chamberlain, Driver, & Miesfeld, 1994; Kazemi-Esfarjani, Trifiro, & Pinski, 1995). The higher the number of CAG triplet repeats the more inhibited the transcription process is (La Spada, Wilson, Lubahn, Harding, & Fishbeck, 1991). CAG triplet repeat length has been shown to positively correlate with 2D:4D and with difference between right and left 2D:4D (R-L 2D:4D) (Manning, Bundred, Newton, & Flanagan, 2003). Breedlove (2010) thus concludes that 2D:4D reflects total androgen stimulation which is proportionate to the concentration of and sensitivity to androgens. 2D:4D has been commonly used as a putative measure of *in utero* testosterone concentration and as an indicator of testosterone sensitivity.

Athletic Performance and 2D:4D

It has been shown that athletic performances, which are influenced by the sexually dimorphic traits such as muscular strength, muscular endurance, speed, and cardiovascular endurance, have a negative correlation with 2D:4D and R-L 2D:4D and thus a positive correlation with prenatal testosterone levels (Bennett, Manning, Cook, & Kilduff, 2010; Manning, 2002a; Manning, Morris, & Caswell, 2007; Tamiya, Lee, & Ohtake, 2011). Researchers found that a longer ring finger relative to the index finger in either hand predicts better performances in fencing (Voracek, Reimer, Ertl, & Dressler, 2006), skiing (Manning, 2002b), soccer (Manning & Taylor, 2001), field-based fitness tests (Hönekopp et al., 2006), sumo wrestling (Tamiya, Lee, & Ohtake, 2011), basketball (Tester & Campell, 2007), 50m dash (Manning & Hill, 2009), a hand-grip strength test (Fink, Thanzami, Seydel, & Manning, 2006), and 2,000m ergometer rowing (Longman, Stock, & Wells, 2011). The effect size of the relationship between 2D:4D of the right hand and athletic prowess (r = -0.26) is highly significant (Hönekopp & Schuster, 2010). The relationship between left 2D:4D and athletic prowess shows a similar and also significant effect size (r = -0.24). It seems that neither hand is a better predictor of athletic performance. The correlation between 2D:4D and endurance running performance measured in finishing position or time ranged from r = 0.30 to r = 0.51. The average

variance in athletic performance accounted for by 2D:4D ranges from 1% to 16% (Hönekopp & Schuster, 2010). The variance in endurance running performance explained by 2D:4D is 25% (Manning et al., 2007). Therefore, it seems that prenatal testosterone exerts greater effects on the function of the cardiovascular system than on other physiological variables, as endurance running is an activity that requires greater aerobic efficiency than many of the aforementioned activities (Manning et al., 2007).

Adult Testosterone Levels and Cardiovascular Health Components

While prenatal testosterone exposure seems to have beneficial effects on cardiovascular fitness, i.e. it makes one a better endurance runner, the effects of postnatal testosterone stimulation on cardiovascular health should also be discussed as digit ratio relates to adult testosterone sensitivity (Manning et al., 2003). Therefore, digit ratio may reflect not only prenatal testosterone stimulation but also, to some degree, adult testosterone stimulation. The effects of exercise on serum testosterone concentrations and the influence of serum testosterone levels on physiological variables in adults have been studied. Strength training and endurance training led to significant increases in serum testosterone, peaking approximately 20 minutes after commencement of training (Jensen et al., 1991; Vogel, Books, Ketchum, Zauner, & Murray, 1985). Elevations in serum testosterone levels have been shown to stimulate erythropoiesis, elevations in serum hemoglobin concentrations, Type I muscle fiber growth, growth of the myocardium, reductions in serum low-density lipoprotein levels, and elevations in serum high-density lipoprotein levels (Hartgens & Kuipers, 2004; Rebuffe-Scrive et al., 1991; Vermeulen et al., 1999; Zitzmann & Nieschlag, 2007). Testosterone supplementation has also caused decreases in diastolic and systolic blood pressure as well as resting heart rates (Zitzmann & Nieschlag, 2007). However, it has been shown that very high endurance training volumes led to chronic depressions in serum testosterone levels (Häkkinen, Pakarinen, Alén, Kauhanen, & Komi, 1988; MacConnie et al., 1986). The physiology of endurance athletes is characterized by most if not all of the benefits provided by elevated serum testosterone levels. It seems counterintuitive that endurance athletes would have depressed resting serum testosterone levels. Endurance athletes may thus be a very useful population when studying the effects of prenatal testosterone levels on human physiology as their depressed resting serum testosterone levels should cause less of the inter-individual variation in physiological parameters and thus making variation due to prenatal testosterone stimulation more easily detectable. Moreover, the elevation in serum testosterone associated with the repeated testosterone stimulus provided by endurance training could be reflected in 2D:4D as Kilduff, Cook, Bennett, Crewther, Bracken, and Manning (2012) found a significant negative correlation between R-L 2D:4D and free salivary testosterone in rugby players following a physical challenge. Therefore, testosterone sensitivity, with R-L 2D:4D as a postnatal proxy, and elevations

in serum testosterone associated with exercise could be the mediating variable between testosterone stimuli and physiological performance variables.

Evidence also suggests that cardiovascular risk factors and certain diseases are less prevalent in individuals with low 2D:4D (Abbott, Dumesic, & Franks, 2002; Fink, Manning, & Neave, 2006; Manning & Bundred, 2001; Manning, Taylor, & Bundred, 2003; Rebuffe-Scrive, Marin, & Bjorntrop, 1991; Singh, 1994; Vermeulen, Goemaere, & Kaufman, 1999). These findings seem to point towards beneficial effects of prenatal testosterone on cardiovascular health and fitness components (English, Mandour, Steeds, Diver, Jones, & Channer, 2000). The validation of 2D:4D as a biomarker of *in utero* androgen concentrations as well as sensitivity is important to provide for opportunities of quick and inexpensive studies and examinations of the genetic predisposition for health risk factors because direct measures of *in utero* androgen concentrations are not practical when studying their relationship to cardiovascular risk factors in adulthood.

Right and Left 2D:4D and Maximal Oxygen Uptake

The physiological measure that is widely accepted as the best measure of cardiovascular fitness is the maximal oxygen uptake or $\dot{V}O_{2max}$ (ACSM, 2010. Bassett & Howley, 1999. Mitchel & Blomqvist, 1971). $\dot{V}O_{2max}$ is the maximum amount of oxygen an individual can consume for energy production during vigorous exercise (Mitchel & Blomqvist, 1971). $\dot{V}O_{2max}$ can reflect poor cardiovascular fitness and inactivity, established as primary risk factors for cardiovascular heart disease (Blair & Kohl, 1989). Hill, Simpson, Manning, and Kilduff (2012) explored the relationship between digit ratios and $\dot{V}O_{2max}$, running velocity at $\dot{V}O_{2max}$ (v- $\dot{V}O_{2max}$), and peak lactate concentration (LA_{max}) in young athletic teenage boys (age: 13.9 ± 1.3 years) during an incremental treadmill test. All of these variables have been shown to be sexually dimorphic, whereas men display consistently higher values than women (Bouchard et al., 1998; Daniels & Daniels, 1991; Esbjörnsson-Liljedahl, Sundberg, Norman, & Jansson, 1999). Therefore, a significant negative relationship between 2D:4D and these variables was expected. However, Hill and colleagues (2012) did not find a significant relationship between right or left hand 2D:4D and $\dot{V}O_{2max}$, v-$\dot{V}O_{2max}$, or LA_{max}. These findings are somewhat surprising as Manning and colleagues (2007) found that right and left hand 2D:4D correlated significantly with endurance running performance. As lower right and left hand 2D:4D is associated with higher prenatal testosterone concentrations, these findings do not support strong favorable organizational effects conducive to cardiovascular health and fitness of prenatal testosterone on the human physiology. The lack of a significant correlation between 2D:4D and $\dot{V}O_{2max}$ in Hill's and colleagues (2012) study could be explained by the inclusion of a variety of sports, including soccer, squash, table tennis, and athletics (track and field), which the participants played. These sports require different amounts of running and movement which elicit varying acute cardiovascular responses

and therefore varying chronic adaptations of the cardiovascular and neuromuscular systems. For example, table tennis might not elicit heart rates as high as other athletic activities (e.g. track & field, soccer, etc.) and it certainly does not involve a comparable amount of locomotion in the sagittal plane (running). Therefore, table tennis players with low 2D:4D might have a low $\dot{V}O_{2max}$ compared to track & field runners with greater 2D:4D. The pre-pubertal and pubertal age of Hill's at al. (2012) sample presents further possible confounders because of the heterogeneity in age at the onset of puberty, the continuous elevation of testosterone levels, and the acceleration of growth and maturation during puberty (Mantzoros, Flier, & Rogol, 1997).

Attempts of synthesizing Manning's et al. (2007) and Hill's et al. (2012) reports to explain the influence of in-utero testosterone concentrations on determinants of endurance running performance bears only limited value because of significant methodological differences between those two studies. The participants in the study of Hill and colleagues (2012) were Middle-Eastern boys with a mean age of 13.9 ± 1.3 years who played a variety of sports and whose digit length was measured from photocopies of their hands. Manning and colleagues' (2007) sample consisted of trained Caucasian male and female distance runners with mean ages ranging from 24.04 ± 8.82 to 33.58 ± 9.25 years whose digit lengths were measured directly with steel vernier calipers. Further research is needed controlling for age to explore a possible influence of fetal testosterone stimulation on $\dot{V}O_{2max}$ related training effects.

R–L 2D:4D and Maximal Oxygen Uptake

Interestingly, in the same study by Hill and colleagues (2012), significant negative correlations were found between R-L 2D:4D and $\dot{V}O_{2max}$ (b = -0.33), v- $\dot{V}O_{2max}$ (b = -0.47), and LA_{max} (b = -0.50). As mentioned, low R-L 2D:4D, more strongly than right or left 2D:4D, has been associated with low numbers of cytosine-adenine-guanine triplet repeats, which in turn are associated with high testosterone sensitivity (Manning et al., 2007). Men with relatively few cytosine-adenine-guanine triplet repeats in exon 1 of the androgen receptor gene have reacted with larger increases in $\dot{V}O_{2max}$ than those with relatively large numbers of cytosine-adenine-guanine triplet repeats in response to 30-day hypoxic training (Wang et al., 2010). An increase in hematocrit has also been reported in males with relatively few cytosine-adenine-guanine triplet repeats in response to an elevation in testosterone levels caused by exercise or the administration of testosterone (Wang et al., 2010; Zitzmann & Nieschlag, 2007). These findings suggest that low R-L 2D:4D indicates a heightened sensitivity to elevations in postnatal testosterone levels which causes favorable changes in the human physiology (training effects). Hill's et al. (2012) findings could be explained by this sensitivity theory, despite the variety of sports which the boys played. High sensitivity to testosterone and pubertal elevations in serum testosterone might outweigh the effects of different types of training. For instance, a table tennis player with high testosterone sensitivity might have a higher $\dot{V}O_{2max}$

than a soccer player with low testosterone sensitivity. It is also possible that the boys with low R-L 2D:4D gravitated towards the sports that elicit large changes in and require high values of $\dot{V}O_{2max}$ for success. However, a similar study examining the influence of the relationship between 2D:4D and $\dot{V}O_{2max}$ on the relationship between 2D:4D and endurance running while controlling for training effects and fluctuations in serum testosterone levels is warranted. The gender differences in the relationship of digit ratios to $\dot{V}O_{2max}$ are also unknown.

Purpose of this Investigation

In summary, a commonly used putative measure of prenatal testosterone stimulation (2D:4D) did explain 25% of the variance in endurance running performances in trained runners (Manning et al., 2007). However, right or left 2D:4D did not explain differences in $\dot{V}O_{2max}$ whereas R-L 2D:4D did (Hill et al., 2012). The samples in previous studies were diverse, the evidence about the organizational effects of prenatal testosterone concentrations on the human physiology, in particular $\dot{V}O_{2max}$, are inconclusive, and the effects of long-term high volumes of endurance training on the relationship of maximal oxygen uptake to digit ratio are largely unknown. Therefore, the purpose of this investigation was to examine the influence of in-utero testosterone stimulation via 2D:4D on $\dot{V}O_{2max}$ and the effects of long-term endurance running training on this relationship by controlling for age and training. Due to the association of right and left 2D:4D with endurance running performance but the lack of association of right and left 2D:4D with $\dot{V}O_{2max}$, we also included an analysis of the relationship of 2D:4D with running economy (RE). RE is the volume of oxygen ($\dot{V}O_2$) consumed at a certain constant running speed. For instance, runner one consuming 30 $ml*kg^{-1}*min^{-1}$ of O_2 is more economical in absolute terms (RE_{abs}) than runner two consuming 34 $ml*kg^{-1}*min^{-1}$ of O_2 if both runners are running at the same speed and incline on two identical treadmills (Daniels & Daniels, 1992). However, if runner one has a $\dot{V}O_{2max}$ of 40 $ml*kg^{-1}*min^{-1}$ and runner two has a $\dot{V}O_{2max}$ of 68 $ml*kg^{-1}*min^{-1}$, it means that runner two displays better relative RE (RE_{rel}) running at 50% of his/her $\dot{V}O_{2max}$ than runner one running at 75% of his/her $\dot{V}O_{2max}$ (Daniels & Daniels, 1992). RE has been demonstrated to be a reliable predictor of endurance running performance among trained runners and it is also a sexually dimorphic trait, whereas men generally exhibit better RE_{rel} than women (Daniels & Daniels, 1992). Moreover, RE is dependent on a number of sexually dimorphic traits including body composition, height, weight, leg mass, and flexibility (Pate, Macera, Bailey, Bartoli, & Powell, 1992; Saunders, Pyne, Telford, & Hawley, 2004). This makes RE a potential correlate of digit ratio. Maximal respiratory exchange ratio (RER_{max}) will also be included as a dependent variable because of the association of R-L 2D:4D and maximal lactate concentrations in Hill's et al. (2012) investigation. While digit ratio is the independent variable, $\dot{V}O_{2max}$, RER_{max}, RE_{abs}, RE_{rel}, total time on the treadmill (ToT), and endurance running performance are the dependent variables that were manipulated through the

quasi-experimental (or categorical) variable of endurance running training. It is assumed based on past research that right and left 2D:4D reflect variations in prenatal testosterone levels while R-L 2D:4D is a stronger biomarker of testosterone sensitivity. Hence, we assumed that a negative correlation between measures of 2D:4D and $\dot{V}O_{2max}$ is moderated by prenatal testosterone and testosterone sensitivity. The investigation of the relationships between measures of 2D:4D, $\dot{V}O_{2max}$, RE, RER_{max}, and running performance within the same population allowed for a more direct comparison between the association of 2D:4D to $\dot{V}O_{2max}$, RE, and RER_{max} and the association of 2D:4D to endurance running performance. For this purpose, partial correlation analysis was used to explore which performance variable ($\dot{V}O_{2max}$, RE_{abs}, RE_{rel}, or RER_{max}) moderates the relationship between 2D:4D and endurance running performance.

Population

For this purpose, two different college aged post-pubertal populations were recruited: Highly endurance trained and sedentary. The highly trained population consisted of 26 (13 female) individuals presently involved in competitive collegiate athletics (cross country and track & field) while the sedentary population (n = 28; 15 female), serving as the control group in terms of long term endurance training, was recruited from the general student population. Both groups included males and females. All participants were between 18 and 25 years of age to control for pubertal changes in testosterone levels as testosterone levels tend to level off after age 14 (Crabbe, Christiansen, Rødbro, & Transbøl, 1979).

Hypotheses

We expected to find a negative correlation between right, left, and R-L 2D:4D and $\dot{V}O_{2max}$ in all samples. Frequent training stimuli may cause changes in $\dot{V}O_{2max}$ independent of training induced testosterone or prenatal testosterone stimulation due to other exercise induced hormonal changes and enzymatic activities. However, there should be a detectable negative relationship between 2D:4D and $\dot{V}O_{2max}$ in the highly trained runners as the physiological effects of serum testosterone spikes seem to be moderated by testosterone sensitivity (Wang et al., 2010) which is reflected in 2D:4D. Variations in $\dot{V}O_{2max}$ in the sedentary population, however, should be largely attributable to genetic variation such as prenatal testosterone exposure and testosterone sensitivity. In accordance with the previous report of a significant correlation between 2D:4D and endurance running performance (Manning et al., 2007), we expected to find a positive relationship of similar strength between measures of digit ratio and personal records in terms of time per race distance (PRs). In comparison, the relationship between $\dot{V}O_{2max}$ and 2D:4D was expected to be weaker due to other determinants of endurance running performance such as the lactate threshold, RE, as well as neuromuscular and biomechanical factors which could also be affected by prenatal testosterone

and/or testosterone sensitivity (Bassett & Howley, 2000; Kyröläinen, Belli, & Komi, 2001; Nummela et al., 2006). Due to these variables and because Hill et al. (2012) found a stronger association between 2D:4D and peak running velocity than between 2D:4D and $\dot{V}O_{2max}$, we expected to find a stronger relationship between 2D:4D and ToT compared to 2D:4D and $\dot{V}O_{2max}$ or RE. In accordance with Hill's et al. (2012) report of a negative relationship between R-L 2D:4D and LA_{max}, we also expected digit ratios to correlate negatively to RER_{max}. At equal running velocities, men generally exhibit greater oxygen consumption per kilogram of body weight ($\dot{V}O_2$) than women (RE_{abs}). Thus, we anticipated negative correlations between measures of 2D:4D and RE_{abs} and positive correlations between measures of 2D:4D and RE_{rel}, as RE_{rel} and $\dot{V}O_{2max}$ are inversely related. In summary, we expected negative correlations between measures of digit ratio (right 2D:4D, left 2D:4D, R-L 2D:4D) and $\dot{V}O_{2max}$, RE_{abs}, RER_{max}, and ToT, and positive correlations between digit ratios, RE_{rel}, and PRs.

Limitations

The intercollegiate runners have been subjected to the selective process of recruitment which is likely to eliminate those with poor genetic predispositions. This might cause the polymorphism of 2D:4D in the runners to be relatively small. However, the 2D:4D polymorphism in the sedentary population could be comparably small as well, as they might have poor genetic predispositions in common which might be a reason for their relative inactivity. This potential lack of relative variation may make it hard to detect associations between digit ratios and dependent variables. Another limitation is that highly trained endurance runners tend to have fairly homogenous $\dot{V}O_{2max}$ values (Daniels & Daniels, 1992). This decreased diversity in $\dot{V}O_{2max}$ values makes it less likely to detect differences and to correlate them with potentially small differences in 2D:4D. The sampling of female collegiate distance runners from one mid-sized southeastern university and one small southeastern college presents a limitation as the training stimuli between the runners from these two institutions are not equal. The male runners were all recruited from one mid-sized southeastern university. Furthermore, the years of exposure to college level training was diverse among the runners as their status varied from first year college students to fourth year college students. This difference in training status could cause difference in $\dot{V}O_{2max}$ which would not be explainable by 2D:4D. Moreover, we will not measure peak lactate concentrations or any other lactate variable. In depth analyses of the relationship between 2D:4D and blood lactate concentrations during and after exercise are warranted as Hill et al. (2012) found the strongest correlation to be between R-L 2D:4D and LA_{max}.

Delimitations

Delimitations in this study include homogenous groups of participants in regard to age (18-25 years), the limitation of training modalities to running, and the limitation of runners to intercollegiate runners. This allows age and training status to be fairly controlled because age and years of training have been demonstrated to influence $\dot{V}O_{2max}$ (Evans, Davy, Stevenson, & Seals, 1995; Franch, Madsen, Djurhuus, & Pedersen, 1998; Jones, 1998; Ogawa et al., 1992). Despite the large differences in cardiovascular fitness between groups, the $\dot{V}O_{2max}$ of all participants will be measured with the Bruce treadmill protocol to allow comparison of $\dot{V}O_{2max}$, RE_{max}, RE_{abs}, RE_{rel}, and ToT between groups. Digit length will be measured directly with a digital offset steel vernier caliper to reduce the distortion of soft tissue in the fingers which is often associated with measures obtained from photocopies of the ventral surface of the hand due to the pressure of the hand exerted against the glass of the photocopy machine. In order to control possible extraneous variables in the form of biochemical substances and physical fatigue, participants will be asked not to change their training routines, not to exercise the day before or the day of testing, not take any new medications or discontinue the use of routine medication unless the doctor told them to do so, and not to consume any alcohol, nicotine, or caffeine during the 24 hours prior to testing. Those who use performance enhancing drugs will also be excluded.

CHAPTER 2

LITERATURE REVIEW

2D:4D – A Sexually Dimorphic Trait

Dr. John T. Manning is known as the pioneer of research employing digit ratios as a putative measure of prenatal sex steriods. The likelihood of a predictive relationship between digit ratio and prenatal sex steroids seemed promising due to the chronological overlap in digit and urino-genital system development and the control of the development of both types of tissue by the same group of Hox genes (Kondo et al., 1997; McIntyre, 2006; Peichel, Prabhakaran, & Voght, 1997). In 1998, Manning, Scutt, Wilson, and Lewis-Jones were the first to explore the relationship between the second to fourth digit length ratio (2D:4D) and prenatal testosterone as well as estrogen among other measures. For the first part of this 1998 study, the length of the second (2D) and fourth digit (4D) from the basal crease proximal to the palm to the tip of the finger on both hands was measured in 340 male and 340 female participants whose numbers were equally distributed between the ages of two years to 18 years. In addition, 60 male and 60 female participants between the ages of 19 and 25 were included. These researchers reported a 2D:4D of 0.98 ± 0.002 (Mean ± SD) for men and a 2D:4D of 1.00 ± 0.002 for women in the right hand. Similar results were found for the left hand. An unpaired *t*-test revealed that the difference in 2D:4D between men and women is highly significant. These results have been supported by numerous studies since (Hönekopp & Watson, 2010). Hence, this ratio is well established as a sexually dimorphic trait with men tending to have a shorter index finger relative to the ring finger of the same hand compared to women. This data indicates that digit ratios, specifically 2D:4D, are stable after two years of age. However, a long term study was needed to provide evidence for the stability of digit ratio after birth. Data from the Fels Longitudinal Study supports the relative stability of digit ratios from age one through 18 in 52 female and 59 male participants (McIntyre, Ellison, Lieberman, Demerath, & Towne, 2005). Sex differences in digit ratio in children correlate to sex differences after puberty ($r^2 = 0.20$) indicating that digit ratios remain stable throughout puberty (McIntyre et al., 2005). Further evidence for the postnatal stability of digit ratios is provided by Trivers, Manning, and Jacobson (2006). The digit ratios of 54 Afro-Caribbean girls and 54 Afro-Caribbean boys aged 9.68 ± 1.39 years were compared to their digit ratios four years later. Their 2D:4D was sexually dimorphic and average 2D:4D decreased a little over the four years. Right 2D:4D ($r = 0.78$) and left 2D:4D ($r = 0.79$) remained stable from the first to the second measurement (Trivers et al., 2006). Therefore, digit ratio seems to be determined *in utero* and/or within the first year after birth and remains stable throughout puberty. In fact, digit ratios have been shown to be sexually dimorphic *in utero*

(Malas, Dogan, Evcil, & Desdicioglu, 2006). Malas et al. (2006) demonstrated the stability of 2D:4D from week 9 through week 40 of gestation in 161 human fetuses (78 female) and they found significantly greater 2D:4D in the female fetuses than the male fetuses.

The digit lengths of 69 men and 62 women were analyzed for the second part of Manning's and his colleagues' (1998) study. Blood samples of 58 men and 40 women were used to assay testosterone concentration in men and luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogen, and prolactin in both genders. The results indicate that testosterone concentration in men significantly and negatively correlates with 2D:4D. Thus, low 2D:4D seems to predict high serum testosterone concentrations in adult men. LH, estrogen, and prolactin significantly and positively correlated with 2D:4D across both genders. Based on Jamison and colleagues' (1993) theory that fetal testosterone concentrations positively correlate with adult concentrations, Manning et al. (1998) argues that men with low 2D:4D must have had high fetal gonadal activity, meaning high *in utero* testosterone production relates to low 2D:4D. However, a more in depth discussion of the relationship between 2D:4D and adult testosterone levels follows later.

In 2010, Hönekopp and Watson published a meta-analysis summarizing the differences in male and female 2D:4D of 116 reports. 107 of those studies compared right 2D:4D in a total of 12,507 females and 11,017 males while 99 studies compared left 2D:4D in a total of 11,610 females and 10,125 males. The authors found that the effect size for the sex differences in the right hand is $d = 0.35$ and $d = 0.28$ for the left hand when direct digit measurement methods (steel Vernier caliper measurements) were used, whereas men have lower 2D:4D values than women. Indirect measurement methods (photocopies, photographs, or scans of the ventral hand surface) yielded an effect size that is 0.13 higher in both hands. These are relatively weak effect sizes. The standard deviation for the sex difference in the right hand was 0.13 larger than in the left hand and statistically significant. It seems that greater gender differences can be found in right 2D:4D than in left 2D:4D as proposed by Manning and colleagues in 1998. This bilateral effect of fetal testosterone is supported by Geschwind and Galaburda (1985) who suggested that the growth of the left hemisphere of the brain may be slowed by high testosterone concentrations and that the growth of the right hemisphere may be accelerated by testosterone. However, despite the low effect sizes of gender differences in digit ratio, Hönekopp and Watson (2010) suggest that the comparison of 2D:4D correlation values across different variables is useful as stronger correlations do suggest a stronger influence of prenatal testosterone on the variable. However, this does not exclude the influence of a non-testosterone related variable on digit ratio. For example, estrogen plays a role in the 2D:4D (Manning et al., 1998) and it could also affect psychological as well as physiological measures.

The early research by Manning and his colleagues (1998) begged the question whether prenatal testosterone does indeed influence digit ratio development or if other moderating mechanisms are involved. Linking low 2D:4D to high adult testosterone concentrations, while presuming a positive relationship

between adult and fetal testosterone concentrations, did not suffice as evidence for the role of prenatal androgen concentrations in digit development. However, digit ratio remains undisputedly a sexually dimorphic trait.

Links between Prenatal Sex Steroid Concentrations and Digit Ratios

Experimental studies done on rats show that an artificial increase in prenatal testosterone reduces 2D:4D (Talarovicová, Krsková & Blazeková, 2009). Experiments done on mice also provide powerful evidence for the influence of sex steroid activation *in utero* on digit development (Zheng & Cohn, 2011). Zheng and Cohn (2011) found that the inactivation of androgen receptor genes leads to shortened 4D length while the inactivation of estrogen receptor genes results in lengthened 4D. The artificial elevation of *in utero* testosterone levels also led to longer 4D, while the artificial elevation of estrogen caused shortened 4D. Thus, both the inhibition of estrogen receptor genes and the elevation of *in utero* testosterone levels resulted in lower 2D:4D, independently. Both the inhibition of androgen receptor genes and the elevation of *in utero* estrogen levels resulted in higher 2D:4D. Thus, Zheng and Cohn (2011) have shown that 2D:4D is regulated by both androgen and estrogen stimulation and that sex steroid receptor genes are more active in the fourth digit than the second digit. Keeping the results of Hönekopp's and Watson's (2010) meta-analysis in mind, the finding by Zheng and Cohn (2011) that the sexual dimorphism of 2D:4D in mice was stronger in the right than the left paw increases the applicability of experimental data from rodents to humans.

As mentioned in the introduction, experimental manipulation of *in utero* sex steroid stimulation to examine their effects on digit development and digit ratios is unethical in humans. However, as researchers have employed more powerful methods to study the effects of prenatal testosterone on 2D:4D and as more and more evidence on the effects of prenatal sex steroids on digit development emerges, confidence in the validity of 2D:4D as a putative measure of prenatal sex steroid action grows. Five methods have typically been used to produce indirect yet overall convincing evidence of the influence of prenatal sex steroids on 2D:4D. These methods include amniocentesis (analysis of the amniotic fluid), the comparison of 2D:4D between females with congenital adrenal hyperplasia (CAH) and healthy controls, the comparison of 2D:4D between dizygotic twins of the same and opposite sex, the comparison of 2D:4D between women with polycystic ovary syndrome and healthy controls, and the comparison of 2D:4D between men with Klinefelter's syndrome (KS) and healthy controls.

Evidence for the influence of *in utero* testosterone and estrogen on digit ratios via amniocentesis is provided by Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, and Manning (2004). A sample of amniotic fluid of 18 male and 15 female fetuses was extracted during the second trimester of pregnancy; a time of peak fetal testosterone during gestation (McIntyre, 2006). The fluid was analyzed for its testosterone and

estradiol, a major estrogen, content. The digit lengths were recoded from the basal crease to the tip of the finger when the children were two years old. Significantly higher testosterone concentrations were found for the male children, while no differences in estrogen between the genders was found. Males presented non-significantly smaller 2D:4D than females. Associations between fetal testosterone or fetal estrogen and 2D:4D were not significant but were in the predicted direction. Higher testosterone concentrations coincided with smaller 2D:4D and higher estradiol concentrations coincided with larger 2D:4D. However, the relationship between the ratio of fetal testosterone to fetal estrogen and 2D:4D was significant and negative for both genders combined and separated. Interestingly, 2D:4D of the right hand showed stronger associations with fetal testosterone and estrogen than left 2D:4D. It is evident from these results that estrogen and androgen both are likely to play a role in the determination of digit ratio and that the right hand is more sensitive to testosterone stimulation.

Hönekopp's and Watson's (2010) meta-analysis of sex differences in 2D:4D included the analysis of three reports, including the one discussed above, on the relationship between sex steroids in the amniotic fluid and digit ratio. It revealed that the effect size of sex differences in amniotic testosterone is much larger (d = 1.4) than the effect size of sex differences in 2D:4D (d = 0.35). Moreover, differences in testosterone concentrations between weeks 11 and 18 of gestation have been found to be at least three standard deviations greater between genders than within genders. However, the between gender variation in 2D:4D is only about 0.48 standard deviations as reported by Forstmeier, Mueller, and Kempenaers (2010). A plausible explanation for these findings is that factors other than prenatal testosterone play a significant role in digit development.

Buck, Williams, Hughes, and Acerini (2003) examined 2D:4D of the left hand of 69 female controls, 77 male controls, and 66 females with CAH. Female fetuses with CAH have hyperactive adrenal glands which secret testosterone above normal levels and they often display masculinized external genitalia. The authors therefore hypothesized that females with CAH have lower 2D:4D than their control females. It was found that left 2D:4D was significantly lower in males than in the healthy females and females with CAH. CAH females did not have significantly lower 2D:4D than control females. These findings could be due to the X-ray method used to measure digit lengths. Usually digit lengths are either measured directly with steel Vernier calipers or they are determined from photo copies of the ventral surface of the hands. For example, Manning, Trivers, Thornhill, and Singh (2000) found sexual dimorphic 2D:4D in Jamacian children when determining digit length via photocopies but not via X-rays. Buck et al. (2003) also argue that estrogen influences bone development because osteoclast and osteoblasts have been shown to possess estrogen receptors. Furthermore, estrogens regulate the expression of *Hox* genes which are responsible for bone development (Taylor, Igarashi, Olive, and Arici, 1999). The development of 2D:4D of the left hand in females with CAH may thus be controlled by the estrogen concentration which

seems unaltered by the condition. The results of this study also indicate that prenatal testosterone has only minimal influence on left 2D:4D.

Brown, Hines, Fane, and Breedlove (2002) examined 2D:4D of both hands in 13 females and 16 males with CAH as well as 44 control females and 28 control males. Photocopies of the ventral surfaces of the hands were used for this study in order to determine 2D:4D. CAH females showed significantly lower 2D:4D compared to the control females only in the right hand while CAH males showed significantly lower 2D:4D compared to the control males only in the left hand. As mentioned, this finding supports the theory that prenatal androgen exerts stronger effects on the right hand than the left hand in women. The lack of significant difference in right 2D:4D among the male samples suggests that androgen levels within the normal range saturate androgen receptors (Zitzmann & Nieschlag, 2003). Thus, testosterone levels above normal would not lead to increased masculinization with androgen sensitivity being equal. It may also be possible that the feedback loop of the fetuses with CAH causes decreased androgen secretion by the gonads in response to high androgen levels caused by the adrenal cortex. Brown et al. (2002) also point out the possibility that increased concentrations of adrenocorticotropic hormone or decreased concentrations of corticosteroids caused decreased 2D:4D in people with CAH. Overall, participants with CAH displayed lower 2D:4D in both hands across both genders. This finding is supported by Ökten, Kalyoncu, and Yris (2002) who found that girls with CAH (n = 17) have lower right and left 2D:4D than control girls (n = 34) but not control boys (n = 34). Additionally, boys with CAH (n = 9) had significantly lower right 2D:4D than control girls (n = 18) and control boys (n = 18) and they had significant lower left 2D:4D than the control girls but not the control boys.

A meta-analysis of five studies (including the two previously discussed) examining 2D:4D differences between participants with CAH and healthy controls revealed that individuals with CAH display 2D:4D that is 0.8 standard deviations lower than the 2D:4D of their gender-matched controls (Hönekopp & Watson, 2010). This difference was significant in three out of four studies. As mentioned, Hönekopp and Watson (2010) also reported that the overall effect size of the differences in 2D:4D in healthy populations, as determined through meta-analysis, is $d = 0.35$. It is suggested that the difference in amniotic testosterone concentration between subjects with CAH and healthy controls is larger than the difference in amniotic testosterone levels between men and women. Indeed, amniotic testosterone concentrations for men are roughly twice as high as those for women (Auyeung et al., 2009; Knickmeyer et al., 2005; Lutchmaya et al., 2004), while the amniotic concentrations of testosterone in CAH females was five times as high compared to control females (Pang et al., 1980).

Anders, Vernon, and Wilbur (2005) conducted a study with dizygotic twins to find evidence for the effect of prenatal testosterone on digit ratios. These investigators based their hypothesis that females with an opposite-sex (OS) twin will have lower 2D:4D than females with a same-sex (SS) twin on Miller's (1994) theory that hormones transfer between OS twins during gestation. This theory finds support in a

study done on rats (Clemens, Gladue, & Coniglio, 1978). Anders et al. (2005) used (humans) 16 SS females, nine OS females, 22 SS males, and nine OS males between the ages of four and 15 years. All subjects combined, males had lower 2D:4D than females only in the left hand. Considering previously discussed findings and the small sample size of this study, it remains that prenatal testosterone exerts its effect mostly on the right hand. Assuming that the hormone transfer between OS twins led to a masculinization of the right hand digits in the OS females, this could potentially leave 40 participants (nine OS females + nine OS males + 22 SS males) of which nine are female with masculinized right 2D:4D versus only 16 females with feminine 2D:4D. Thus, the difference in right 2D:4D between the genders is non-significant. The significant gender difference in left 2D:4D could be due to the greater number of male controls (22) versus female controls (16) or a weaker effect of estrogen (if transferred to the male twin at all) on left 2D:4D in males than testosterone on right 2D:4D in females. The latter speculation is supported by the finding of no significant difference in left 2D:4D between OS and SS males. Interestingly, left 2D:4D was significantly lower in OS females compared to SS females but not right 2D:4D. This finding is consistent with the overall sex difference in left 2D:4D but it stands in opposition to the theory that prenatal testosterone exerts stronger effects on the right hand than the left hand. Moreover, OS females displayed 2D:4D that was similar to that of OS males which stands in support of the hormonal transfer theory and the organizational effect of *in utero* sex steroids on digit ratio. This evidence is supported by the finding that OS females showed lower left 2D:4D than the female average of other studies. The SS males differed from the SS females only in left 2D:4D which also weakens the laterality theory of prenatal testosterone's influence on digit ratio. Possible methodological explanations for these unusual findings include the relatively small sample size and the fact that some photocopies of the participants' hands were obtained by one of the investigators with a portable photocopier while other photocopies were done and mailed in by the participants themselves.

Based on their findings, Anders et al. (2005) suggest that fetal androgen concentrations are high in males at the same time when female digit development is taking place. This strengthens the argument for the influence of *in utero* androgen on digit ratio. Considering the latter argument and the masculinization of genitalia in females with CAH, it seems likely that 2D:4D can be used to predict other measures that developed during periods of high fetal androgen concentrations. For instance, brain structure, rough play, and other male-typical behaviors are influenced by prenatal androgen concentrations in animals (Breedlove, 1994; Goy & Phoenix, 1972). However, McIntyre (2006) also suggests that postnatal testosterone levels have a strong organizational effect. During infancy, the testes undergo a period of rapid growth. In summary, there is a high probability of development of the testes and digits occurring at the same time of gestational peaks of testosterone levels. Additionally, the tested undergo a growth spurt during infancy. The lack of evidence about the stability of digit ratios before two years of age affords the possibility that finger ratio continues to change during infancy chronologically corresponding to the growth of the testes. This

hypothesis is supported by evidence for the influence of the same pair of genes, *Hoxa and Hoxd*, on genital as well as digit development (Peichel, Prabhakaran, & Voght, 1997).

To further investigate the link of prenatal testosterone to 2D:4D, investigators have examined women with polycystic ovary syndrome. One such study reported that women with polycystic ovary syndrome tend to have higher prenatal and adult testosterone levels and lower 2D:4D in comparison to healthy controls (Cattrall, Vollenhoven, & Weston, 2005). In addition to supporting the relationship between 2D:4D and prenatal testosterone concentrations, this is also evidence in support of a positive relationship between *in utero* and adult testosterone levels.

Men with KS have an additional X-sex-chromosome (XXY). Fetuses and infants with KS have testosterone levels typical of their female counterparts (Künzig, Meyer, Schmitz-Roeckerath, & Broer, 1977). Adolescents with KS also have below average testosterone concentrations (Forti, Corona, Vignozzi, Krausz, & Maggi, 2010). Manning, Kilduff, and Trivers (2013) found that their sample of 51 individuals with KS had significantly greater right 2D:4D than their fathers ($d = 1.43$) and male controls ($d = 0.74$). They also had significantly greater left 2D:4D than their fathers ($d = 1.03$), their mothers ($d = 0.72$), and male controls ($d = 0.85$). There were no significant differences in R-L 2D:4D across the groups.

McIntyre (2006) also points out that the role of estrogen in bone development through estrogen receptors in the metaphyseal tissue (growth plates) is better understood than the role of testosterone. It is understood that the fusion of growth plates is accelerated by the stimulation of estrogen receptors (Weise et al., 2001) providing evidence for the growth-inhibiting effects of estrogen. Thus, extended growth after sexual maturation and above average heights have been reported in men with estrogen receptor deficiency or aromatase deficiency (Rochira, Balesrieri, Madeo, Spaggiari, & Carani, 2002). It seems that either bones of different fingers have different ratios of androgen and estrogen receptor genes or that bones have similar ratios of sex steroid receptor genes but different temporal growth patterns (McIntyre, 2006). Zheng and Cohn (2011) provide evidence through research on mice that the fourth digit possesses higher numbers of both androgen and estrogen receptor genes.

While the production of and sensitivity to hormones is governed by genes, other genetic factors that influence digit development and that could possibly undermine the role of sex steroids as the cause for the sexual dimorphism in 2D:4D have been pointed out. Winchester (1976) proposed that a skeletal structure gene which is recessive in men and dominant in women might be responsible for the relatively longer index finger in women. The Y-chromosome may also play a part in the sexual dimorphism of 2D:4D because of its important influence on other sexually dimorphic traits (Arnold, 1996). However, prenatal testosterone concentrations as opposed to postnatal testosterone levels seem to be a reliable predictor of 2D:4D.

Association of Digit Ratios with Postnatal Testosterone Concentrations

Based on Jamison's et al. (1993) theory that prenatal testosterone concentrations relate to adult serum testosterone concentrations, a discussion of the relationship of 2D:4D to postnatal testosterone levels will be included here. Hönekopp's, Bartholdt's, Meier's, and Liebert's (2007) meta-analysis including 17 samples revealed that there is no association between adult (postnatal) serum testosterone levels and 2D:4D. The investigations included in the meta-analysis analyzed the relationship between digit ratios and either bioavailable testosterone or total testosterone. Among all possible combinations of the forms of testosterone, digit ratios, and gender, correlations ranged from $r = 0.09$ to $r = -0.20$. Only, the correlation between total testosterone and R-L 2D:4D in men reached statistical significance. However, one of the three samples included in the analysis of the relationship between total testosterone and R-L 2D:4D in men was from a clinical population. The effect size of this relationship did not reach statistical significance upon removal of this sample from the meta-analysis (Hönekopp et al., 2007). Folland, Cauley, Phypers, Hansen, and Mastana (2012) found no association between right or left 2D:4D and free or total testosterone in 77 men (all $r < 0.12$, $p > 0.34$). Additionally, there was no relationship between right 2D:4D ($r = -0.01$), left 2D:4D ($r = -0.14$), or R-L 2D:4D ($r = -0.09$) and free salivary testosterone concentrations at rest in 54 professional rugby players (Kilduff et al., 2012). Thus, 2D:4D does not seem to be related to adult serum testosterone concentrations at rest.

However, there is evidence that 2D:4D relates to rises in serum testosterone associated with exercise. Kilduff et al. (2012) examined the relationship of digit ratios with levels of free salivary testosterone in 25 professional male rugby players immediately preceding, five minutes after, and 20 minutes after completion of a repeated sprint agility test. R-L 2D:4D related significantly to testosterone concentrations at all three times (prior: $r = -0.50$; 5 min post: $r = -0.54$; 20 min post: $r = -0.40$) and to the average testosterone concentrations across all three time ($r = -0.49$). The relationship of R-L 2D:4D was mainly mediated by positive and significant correlations between left 2D:4D and free testosterone ($0.48 \leq 0.41$) and a flat relationship between right 2D:4D and free testosterone ($0.15 \leq 0.05$) across all three measurements (Kilduff et al., 2012).

Androgen Sensitivity and Digit Ratios

The fetal development of digits is not only dependent on *in utero* androgen concentrations but also on the androgen sensitivity of receptor genes. To a degree, these two factors act independently on the development of digit ratios (Manning, Bundred, Newton, & Flanagan, 2003). It has been proposed that digit ratios are regulated by posterior Hox genes and their transcription in the metaphyseal tissue of the digits. The transcriptional activity of these Hox genes is in turn regulated by androgen and estrogen

receptors whose level of activity depends on the plasma concentration of these hormones (Forstmeier, Mueller, & Kempenaers, 2010). Evidence of the link between high 2D:4D and androgen insensitivity represents strong support for the association between low 2D:4D and high prenatal androgen stimulation, as

digit ratios appear stable early after birth. Thus, digit ratios could serve as indicators of adult traits provided that those traits are influenced by prenatal sex steroid concentrations (Figure B-1*).

A study of people with complete androgen insensitivity syndrome (CAIS) provides such evidence (Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009). People with CAIS do produce testosterone but due to absent or dysfunctional androgen receptors, there is no effective exposure to androgens prenatally and postnatally. Such individuals have female external genitalia, undergo aromatization of sex steroids at puberty (Grumbach, Hughes, & Conte, 2003), and have female gender identity and psychological characteristics (Hines, Ahmed, & Hughes, 2003; Mazur, 2005). Consequently, they are considered female. Berenbaum et al. (2009) used 16 women with CAIS, 90 control women, and 66 control men and found that CAIS women had significantly greater 2D:4D in both hands than men but not control women. The differences in digit ratio were more pronounced in the right hand than the left hand (Table A-1**).

These findings support the validity of 2D:4D as a proxy for androgen exposure and sensitivity and they also support the theory that androgens play a greater role in the development of the digits of the right hand than the left hand. However, the importance of the role of androgens in the development of the digits is diminished by the moderate difference ($d = 0.48$) in 2D:4D of both hands between CAIS women and control men and the lack of significant difference in 2D:4D between CAIS women and control women, as healthy women with functional androgen receptor genes are exposed to testosterone *in utero* (Berenbaum et al., 2009).

Forstmeier and colleagues (2010) studied polymorphism in an estrogen receptor gene in zebra finches, *Taeniopygia guttata,* on the grounds that animals and humans share "molecular mechanisms" that are more than 300 million years old because relationship between digit ratios and sex steroids similar to those in humans have also been found in mammals, birds, reptiles, and amphibians. The polymorphism of an estrogen receptor gene explained 11.3 % of the variation in digit ratio of 1156 birds. Polymorphism of an androgen receptor gene did not explain any variation in digit ratio. These results emphasize the influence of estrogen and receptor response on digit ratio. While the importance of the overall role of sex steroids in digit development is strengthened by this report, inferences about the proportionate roles of either estrogen or androgen in mammals, specifically humans, is not possible.

The number of CAG triplet repeats in the androgen receptor gene of humans has been shown to negatively correlate with testosterone sensitivity. The ability of testosterone to bind to the receptor gene is not influenced by the receptor gene's number of its CAG triplet repeats, but the ability of the androgen receptor gene to bind to DNA is inversely related to the number of CAG triplet repeats. Eleven to 30 triplet

repeats is considered a normal range, the average is around 20 to 22 repeats (Chamberlain, Driver, & Miesfeld, 1994; Kazemi-Esfarjani, Trifiro, & Pinski, 1995), and a number of more than 40 triplet repeats is

* all figures appear in Appendix B; ** all tables appear in Appendix A

associated with testosterone insensitivity (La Spada, Wilson, Lubahn, Harding, & Fishbeck, 1991). An association among more than 38 triplet repeats, spinal and bulbar muscular atrophy, defective spermatogenesis, and undervirilization has been reported (Dejager et al., 2002). Manning, Bundred, Newton, and Flanagan (2003) hypothesized that there is a positive correlation between right 2D:4D and the number of CAG triplet repeats as well as a positive correlation between R-L 2D:4D. The latter relationship is based on the stronger association between 2D:4D and prenatal testosterone in the right hand compared to the left hand. As the androgen receptor gene is found in the X-sex-chromosome and men have only one X-sex-chromosome unlike women, Manning et al. (2003) limited their study to 50 male participants to eliminate uncertainty about which X-chromosome was activated. Digit length was measured with steel Vernier calipers to the nearest 0.05 mm and 5 ml of blood was drawn from each participant for the analysis of CAG triplet repeats in the DNA. A significant positive correlation was found between the number of CAG triplet repeats and right 2D:4D ($r = 0.29$; $P = 0.02$, $r^2 = 0.09$) as well as between the number CAG triplet repeats and R-L 2D:4D ($r = 0.36$, $P = 0.005$, $r^2 = 0.13$). No relationship was found between the number of CAG triplet repeats and left 2D:4D. These results indicate that R-L 2D:4D is a slightly better proxy of testosterone sensitivity than right 2D:4D.

An investigation by Folland et al. (2012) including 77 male participants does not support right or left 2D:4D as a correlate of the number of CAG triplet repeats (all, $r < 0.20$, $p > 0.10$). However, the results of this investigation do not weaken the theory of R-L 2D:4D as a proxy of testosterone sensitivity as Folland et al. (2012) did not include R-L 2D:4D as a variable in their investigation. Manning, Bundred, et al. (2003) attribute the relatively low variances in right 2D:4D and R-L 2D:4D explained by the number of CAG triplet repeats to four separate factors influencing digit ratio: (1) prenatal testosterone concentration; (2) testosterone sensitivity; (3) prenatal estrogen concentration; (4) estrogen sensitivity.

Moreover, KS is characterized by low testosterone sensitivity as determined by a high number of CAG triplet repeats. Specifically, higher numbers of CAG triplet repeats in men with KS have been associated with gynecomastia, small testes, and above average height (Zitzmann, Depenbusch, Gromoll, & Nieschlag, 2004; Bojesen, Hertz, Gravholt, 2011). As mentioned above, Manning et al. (2013) compared measures of digit ratios between 51 men with KS and their fathers, mothers, as well as healthy male controls. Men with KS had significantly higher right 2D:4D than their fathers and controls and significantly higher left 2D:4D than their fathers, mothers, and controls. No differences between groups were found in R-L 2D:4D. These results, based on a clinical sample, support right and left 2D:4D but not R-L 2D:4D as proxies of testosterone sensitivity.

It is evident that the variation of prenatal testosterone concentrations will only be reflected in 2D:4D to the extent that the androgen receptor genes are able to translate the androgen levels into bone development. Conversely, the extent to which androgen sensitivity is reflected in 2D:4D depends on the levels of prenatal testosterone concentrations. For instance, very high *in utero* testosterone concentrations will most likely not result in low 2D:4D if the androgen receptor gene possesses a very high number of CAG triplet repeats. Conversely, a low number of triplet repeats in the androgen receptor gene will not be evident in 2D:4D if low testosterone levels do not provide sufficient stimulation to the receptors. In summary, low R-L 2D:4D seems to be the best biomarker of higher testosterone sensitivity as determined by the lower number of CAG triplet repeats contained in the androgen receptor gene.

Athletic Performance and 2D:4D

Based on evidence presented above linking digit ratio and prenatal testosterone stimulation, researchers have used 2D:4D as proxy of prenatal androgen exposure to explore the influence of prenatal fluctuations in testosterone on other sexually dimorphic traits. Of course, studies directly linking prenatal testosterone variations to sexually dimorphic traits would be preferable, but the longitudinal designs required to do so make such investigations very impractical. Digit ratio measures have emerged as the most widely accepted and used proxy of prenatal androgen exposure. Digit ratio has been used extensively in correlational research on the link between athletic performance, a sexually dimorphic trait, and genetic endowment in the form of prenatal androgen stimulation. Predictive relationships between 2D:4D and athletic performance would indicate a permanent influence of prenatal sex steroids on components of physical fitness. The spectrum of practical ability of 2D:4D would also be broadened if it reliably predicts athletic performance and physical fitness. Consistent negative relationships between 2D:4D and athletic performances would allow health professionals to gain insight into genetic predispositions for cardiovascular risk factors and the benefit related to physiological health components which can be expected from training interventions, through the quick and inexpensive field method of determining 2D:4D. It would also provide coaches with a method of assessing the probability of their athlete's athletic success and trainability.

Evidence for the predictive ability of 2D:4D in regards to athletic performance and related cardiovascular fitness variables is provided by Manning, Morris, and Caswell (2007). Manning and associates (2007) reasoned that 2D:4D should be related to distance running performance because of the sexually dimorphic nature of both distance running performance and digit ratio. Manning et al. (2007) conducted three separate studies to explore the relationship among endurance running performance, training frequency, and 2D:4D. The first study involved 16 male and 11 female endurance runners with a mean age of 24.04 ± 8.82 years whose endurance running performance was recorded as finishing position

among the training group at the end of bursts during fartlek type runs. The endurance running performance of 43 male runners with a mean age of 27.63 ± 10.25 years was determined by their average finishing position in a series of five cross country races during the second study. The third study was comprised of 40 female runners with a mean age of 33.58 ± 9.25 years whose running performance was determined from their finishing time in a one mile race. After the effects of age had been removed, study one yielded significant positive correlations between right 2D:4D and finishing position and between training frequency and finishing position. Moreover, a negative relationship between 2D:4D and training frequency almost reached significance. Simply put, those with lower 2D:4D and thus greater testosterone exposure *in utero* and most likely greater testosterone sensitivity tended to train more frequently and run faster than those with higher 2D:4D. Study two produced the same results as study one except that the correlations between 2D:4D and mean finishing position were significant for both hands and the relationship between 2D:4D and training frequency reached significance for both hands as well. Study three showed a significant positive correlation between right 2D:4D and finishing time in the one mile race. The relationship between training frequency and race time was also significant, however, the association of 2D:4D to training frequency was less clear in this purely female sample of runners. Combined, 2D:4D explained approximately 25% of the variance in endurance running performance.

These results support the link between prenatal testosterone, endurance running performance, physiological determinants of endurance running performance and digit ratio. It should be noted that 2D:4D correlated with running performance independent of training frequency, age, or gender. It seems that prenatal testosterone has a distinct influence on the structure and function of the cardiovascular system, which is the primary system determining endurance performance (Bassett & Howley, 2000). This link substantiates testosterone's importance for cardiovascular health.

Manning and Hill (2009) also studied the relationship between 2D:4D and sprinting speed. The 2D and 4D digit lengths and 50m sprinting times of 241 Middle-Eastern boys between the ages of 10 and 17 years were measured. Right 2D:4D showed a significant positive correlation with sprinting times ($r = 0.15$, $P = 0.02$). The correlation between left 2D:4D and sprinting time failed to reach statistical significance ($r = 0.12$, $P = 0.06$). However, age, BMI, and maturation index also significantly correlated with sprinting time and therefore, the authors removed the influence of those variables to re-examine the 2D:4D and sprinting speed relationship. The correlation between right 2D:4D and sprinting time remained largely unaltered ($r = 0.14$, $P = 0.02$) and the correlation between left 2D:4D and sprinting times reached statistical significance ($r = 0.15$, $P = 0.01$). The very low correlation coefficients and relatively large sample size of this investigation do not provide conclusive evidence for the influence of prenatal testosterone stimulation on sprinting ability. Folland et al. (2012) investigated the association of digit ratio to isometric and isokinetic knee extensor strength in 77 men aged 20.1 ± 2.2 years. There was no association between any measure of strength and right or left 2D:4D (all, $r < 0.12$, $p > 0.32$).

It appears that the link between 2D:4D and sports performance is not moderated by maturational or anthropometric measures, but by internal physiological variables whose development seems to be determined early on in life. The association between 2D:4D and sprinting speed but the lack of association between 2D:4D and knee extensor strength do not seem to support the role of prenatal testosterone as a determinant of strength, speed, or power. The stronger correlation between 2D:4D and endurance running performance (r = 0.25) (Manning et al., 2007) as well as the slightly stronger anabolic influence of androgenic-anabolic steroids (AAS) on slow twitch muscle fibers (Type I) than on fast twitch fibers (Type II) (Hartgens & Kuipers, 2004), suggest that Type I muscle fibers and the cardiovascular system combined are more sensitive to changes in perinatal testosterone levels than Type II muscle fibers.

Running, however, is not the only type of sport or physical activity that showed an association with 2D:4D. Researchers found that right 2D:4D predicts better performances in fencing (Voracek, Reimer, Ertl, & Dressler, 2006), skiing (Manning, 2002b), soccer (Manning & Taylor, 2001), field-based fitness tests (Hönekopp et al., 2006), sumo wrestling (Tamiya, Lee, & Ohtake, 2011), basketball (Tester & Campell, 2007), 50m dash (Manning & Hill, 2009), a hand-grip strength test (Fink, Thanzami, Seydel, & Manning, 2006), and 2,000m ergometer rowing (Longman, Stock, & Wells, 2011). Sometimes R-L 2D:4D was significantly and negatively correlated with performance (Bennett, Manning, Cook, & Kilduff, 2010; Manning, 2002a). These results are not surprising as testosterone seems to moderate a variety of physiological variables associated with endurance, strength, and digit ratio, as discussed above.

In fact, Hönekopp and Schuster (2010) found that 2D:4D is more reliable and powerful predictor of athletic performance than other variables such as behavior, health, and morphology. A meta-analysis including 24 studies revealed a significant effect size of r = -0.28 for the relationship between right 2D:4D and athletic prowess in a variety of sports and measures of athleticism (for details see Hönekopp & Schuster, 2010). The effect size for the left hand, based on 22 studies, was also significant (r = -0.26). It seems that neither hand is a significantly better predictor of athletic performances than the other. Analyzing the results of five studies involving running for distances of 50m, 800m, and longer, Hönekopp and Schuster (2010) found that the strength of the correlation between running performance and right 2D:4D increased in linear fashion with increasing race distances (Figure B-2). Although the relationship is not as linear, similar results were found for the left hand.

Hönekopp's and Schuster's (2010) meta-analysis reveals that 2D:4D and distance running performances show by far the strongest correlations (r = -0.51), whereas 2D:4D and sports requiring mostly strength and speed show the weakest correlations (r = -0.32 to r = -0.14). Endurance running shares the strongest relationship with 2D:4D compared to other athletic disciplines. The strength of this relationship seems to increase with increasing race distances. The average variance in athletic performances explained by 2D:4D falls between 1% and 16% (Hönekopp & Schuster, 2012), whereas 2D:4D seems to account for approximately 25% of the variance in endurance running performance (Manning et al., 2007).

Consequently, it seems plausible that physiological factors determining endurance performance, such as $\dot{V}O_{2max}$, lactate threshold, and running economy (Bassett & Howley, 2000), are influenced by *in utero* testosterone levels, testosterone sensitivity, and possibly adult testosterone levels. In Summary, Hönekopp & Schuster (2010) found considerable heterogeneity in the relationship between athletic prowess and 2D:4D. However, age and gender did not contribute to this heterogeneity which diminishes the likelihood that adult serum testosterone levels moderate the effects of prenatal testosterone on fitness related components (Hönekopp & Schuster, 2010). A factor that seems to significantly add to the heterogeneity of the aforementioned relationship is the distances of foot races. However, significant variability was found for the predictive power of right 2D:4D and left 2D:4D. It seems that certain activities, settings, and populations cause right 2D:4D to be a better predictor of performance than left 2D:4D and vice-versa. However, the exact activities, circumstances, and population characteristics remain unclear (Hönekopp & Schuster, 2010).

It is important to note that the relationship between 2D:4D and athletic performances does not seem to be moderated by personality variables (Tester & Campbell, 2007). Tester and Campbell (2007) tested the relationships between digit ratio and the personality constructs of social potency, achievement, control, and harm avoidance which have all been associated with sporting success. These relationships were also compared to the rankings of rugby, soccer, and basketball players. The rakings ranged from (1) no involvement in sports to (10) national representation. Height, weight, time spent training per week, years playing, social potency, and 2D:4D all significantly correlated with ranking. The association between 2D:4D and ranking (r = -0.35) was of the same magnitude as the association between years playing and ranking (r = 0.35). Height, years playing the sport, and social potency also correlated significantly with digit ratio. None of personality constructs were associated with 2D:4D. The association between 2D:4D and ranking remained significant (r = -0.24) even after the effects of weight, years playing the sport, and hours per week training had been entered into the regression equation as random factors.

Extensive research exploring the relationship between aggression and 2D:4D has also been completed (Hönekopp & Watson, 2011). A significant association between 2D:4D and physical aggression seemed plausible to many investigators because of the sexually dimorphic nature of physical aggression. However, Hönekopp's and Watson's (2011) meta-analysis revealed that 2D:4D is not associated with physical aggression in women and the same relationship is very small in men ($r \approx 0.06$). Lemieux, McKelvie, and Stout (2002) reported that there is no difference in aggression between athletes and non-athletes.

Consequently, it seems that physiological variables rather than personality variables establish the link between 2D:4D and success in sports (Manning et al., 2007). Interestingly, variables such as years of sports participation and training frequency seem to strengthen the link association of 2D:4D to sporting success (Tester & Campbell, 2007). Testosterone sensitivity seems responsible for reinforcing this

association as it facilitates physiological adaptations to training (see discussion of Wang et al., 2010, below) and because it is reflected in digit ratios (Manning et al., 2003).

2D:4D as a Biomarker of Cardiovascular Fitness

A logical extension of research on the association between digit ratio and athletic prowess was the investigation of the relationship between digit ratio and underlying physiological parameters to shed light on the permanent organizational effects of prenatal sex steroids on the human physiology.

Hill, Simpson, Manning, and Kilduff (2012) were the first to investigate the ability of 2D:4D to predict cardiovascular fitness as it relates to athletic performance. These investigators expected to find a significant negative correlation between 2D:4D and $\dot{V}O_{2max}$ given the strong correlation between $\dot{V}O_{2max}$ and distance running performance (r = -0.95) (Paliczka, Nichols, & Boreham, 1987), the relationship between distance running performance and 2D:4D, and the increasingly larger effect size of correlations between running speed and 2D:4D with increasing race distances (Hönekopp & Schuster, 2010; Manning et al., 2007). Hill et al. (2012) explored the relationship between digit ratios and $\dot{V}O_{2max}$, running velocity at $\dot{V}O_{2max}$ (v-$\dot{V}O_{2max}$), and peak lactate concentration (LA_{max}) in 41 young athletic Middle-Eastern teenage boys (age: 13.9 ± 1.3 years) during an incremental treadmill test. All of these variables have been shown to be sexually dimorphic, whereas men reach consistently higher values than women (Bouchard et al., 1998; Daniels & Daniels, 1991; Esbjörnsson-Liljedahl, Sundberg, Norman, & Jansson, 1999). Therefore, as with $\dot{V}O_{2max}$, a significant negative relationship between 2D:4D and these variables was expected. However, Hill and colleagues (2012) did not find a significant relationship between right or left hand 2D:4D and $\dot{V}O_{2max}$, v-$\dot{V}O_{2max}$, or LA_{max}. These findings are somewhat surprising as Manning and colleagues (2007) found that right hand 2D:4D correlated significantly with endurance running performance and that overall 2D:4D explained 25% of the variance in endurance running performance. As lower right and left hand 2D:4D is associated with higher prenatal testosterone concentrations, these findings do not support strong favorable organizational effects conducive to cardiovascular health and fitness of prenatal testosterone on the human physiology. The lack of a significant correlation between 2D:4D and $\dot{V}O_{2max}$ in Hill's and colleagues' (2012) study could be explained by the variety of sports, including soccer, squash, table tennis, and athletics, which the participants played. These sports require different amounts of running and movement which elicit varying acute cardiovascular responses and therefore varying chronic adaptations of the cardiovascular and neuromuscular systems. For example, table tennis might not elicit heart rates as high as athletics (track & field) and it certainly does not involve a comparable amount of locomotion in the sagittal plane (running). Therefore, table tennis players with low 2D:4D might have a low $\dot{V}O_{2max}$, as measured on the treadmill, compared to track & field runners with greater 2D:4D. Furthermore, keeping in mind that postnatal testosterone sensitivity may be reflected in 2D:4D, the difference in testosterone

secretion caused by these sports has never been studied in the same sample and is therefore unknown and a possible confounder. The age of Hill's et al. (2012) sample (13.9 ± 1.3 years) allows the assumption that most boys were in the stages of puberty whereas some boys might have matured to post-pubertal stages and others might have still been in a pre-pubertal phase (Mantzoros, Flier, & Rogol, 1997). The possibility of heterogeneity in terms of pubertal stages and puberty itself present possible confounders due to the increasing levels of testosterone and the accelerated growth and maturation during puberty (Mantzoros et al., 1997). Testosterone levels do generally not level off until age 14 (Crabbe, Christiansen, Rødbro, & Transbøl, 1979).

However, Hill and colleagues (2012) did find significant negative correlations between R-L 2D:4D and $\dot{V}O_{2max}$ (b = -0.33), v-$\dot{V}O_{2max}$ (b = -0.47), and LA_{max} (b = -0.50). As mentioned, low R-L 2D:4D has been associated with low numbers of CAG triplet repeats, which in turn are associated with high testosterone sensitivity (Manning et al., 2003). Hill's et al. (2012) findings could be explained by the significantly greater post-training increases in $\dot{V}O_{2max}$ and hematocrit in individuals with fewer CAG triplet repeats as reported by other investigators (see section titled *Testosterone Sensitivity and Cardiovascular Fitness* for discussion) (Wang et al., 2010; Zitzmann & Nieschlag, 2007). Despite the variety of sports which the boys played, high sensitivity to testosterone might outweigh the effects of different types of training due to the pubertal elevations in serum testosterone. For instance, a table tennis player with high testosterone sensitivity might have a higher $\dot{V}O_{2max}$ than a soccer player with low testosterone sensitivity. This conclusion would support R-L 2D:4D as a marker of testosterone sensitivity. It is also possible that the boys with low R-L 2D:4D gravitated towards the sports that elicit large changes in and require high values of $\dot{V}O_{2max}$ for success. However, a similar study controlling for training effects and temporary fluctuations in testosterone levels is warranted. If $\dot{V}O_{2max}$ is influenced by postnatal testosterone levels mediated via testosterone sensitivity, then long term fluctuations in serum testosterone levels need to be controlled if a more accurate relationship between 2D:4D and maximal oxygen uptake is to be determined. Moreover, gender differences in the strength of the 2D:4D to $\dot{V}O_{2max}$ relationship are unknown as this relationship has not been investigated in women.

In summary, Hill's et al. (2012) report suggests that prenatal testosterone does not play a significant role in the development of $\dot{V}O_{2max}$ related physiological traits but that postnatal testosterone levels mediated by testosterone sensitivity might determine inter-individual differences in $\dot{V}O_{2max}$. There seems to be a relationship between R-L 2D:4D, the number CAG triplet repeats, and $\dot{V}O_{2max}$. However, we cannot exclude the possibility that factors determining $\dot{V}O_{2max}$ were developed prenatally. It is possible that differences in $\dot{V}O_{2max}$ did not correlate significantly with 2D:4D in Hill's et al. (2012) sample because of the heterogeneity of the sample in terms of training. Investigations with more homogenous samples in regard to confounders such as training status in terms of cardiovascular fitness could shed more light on the relationship of digit ratio to the number of CAG triplet repeats.

The stronger correlations between R-L 2D:4D and v-$\dot{V}O_{2max}$ (b = -0.47) and R-L 2D:4D and LA_{max} (b = -0.50) compared to R-L 2D:4D and $\dot{V}O_{2max}$ (b = -0.33) suggest that androgen sensitivity also has an influence on physiological factors other than those determining $\dot{V}O_{2max}$. Other determinants of endurance running performance include the lactate threshold, running economy, as well as neuromuscular and biomechanical factors (Bassett & Howley, 2000; Kyröläinen, Belli, & Komi, 2001; Nummela et al., 2006). The argument that at least some of these factors are partially controlled through testosterone stimulation is also supported by the significant negative correlation between 2D:4D and distance running performance (Manning et al., 2007), and the finding that peak running velocity during a $\dot{V}O_{2max}$ test is the best predictor of race performance (Noakes, Myburgh, & Schall, 1990).

Additionally, a high ratio of Type IIa muscle fibers might be associated with high testosterone concentrations and explain the relatively strong relationship between 2D:4D and LA_{max} in Hill's et al. (2012) study. However, this assumption contradicts the hypertrophy and relative increase in number of Type I muscle fibers compared to Type II muscle fibers found as a result of AAS use (Hartgens & Kuipers, 2004).

In summary, the lack of association between $\dot{V}O_{2max}$ and 2D:4D in Hill and colleagues' (2012) investigation is unexpected, as $\dot{V}O_{2max}$ has been found to be the best predictor of endurance running performance among heterogeneous groups in regard to their $\dot{V}O_{2max}$ (Daniels & Daniels, 1992).

Fink, Neave, and Manning (2003) compared body mass index (BMI), waist-to-hip ratio (WHR), and waist-to chest ratio (WCR) to digit ratio. BMI is well established as an indicator of cardiovascular risk factors (ACSM, 2010) and very high WHR, independent of total body fat, is associated with diabetes, hypertension, heart attack, and stroke among other diseases (Singh, 1993). BMI, WHR, and WCR are all sexually dimorphic traits and largely developed during puberty and determined by estrogen and testosterone stimulation. In general, men have lower BMI while women have lower WHR and WCR. Higher pubertal testosterone levels are associated with more android (masculine) body types while higher pubertal estrogen and lower testosterone levels are associated with more gynoid (female) body types (Fink et al., 2003). Fink et al. (2003) expected to find positive relationships between 2D:4D and gynoid body characteristics and negative relationships between 2D:4D and android body characteristics. These hypotheses are supported by the findings that women with high WHR have low 2D:4D and tend to have children with low 2D:4D (Manning, Trivers, Singh, and Thronhill, 1999). The purpose of Fink's et al. (2003) investigation was to explore to what degree sexually dimorphic traits determined via testosterone and estrogen during puberty (BMI, WHR, and WCR) are associated with sexually dimorphic traits determined largely *in utero* by testosterone and estrogen (2D:4D).

Using 30 male and 50 female adult participants, Fink et al. (2003) found significantly larger values for BMI, WHR, and WCR in males than in females. Men also had significantly lower 2D:4D than women on both hands. A significant positive relationship (r = 0.475, p = 0.004) between left 2D:4D and BMI was

found for the men. A marginally significant negative relationship was found between right 2D:4D and WHR in men, but no further significant relationships between 2D:4D and any of the anthropometric measures were found for men. Women, however, presented three significant negative correlations for each hand between 2D:4D and anthropometric measures. Correlations for the right and left hand included waist circumference, hip circumference, and WCR. The significant positive relationship between 2D:4D and BMI in men presents evidence for the positive relationship between prenatal testosterone levels and cardiovascular health in adulthood. However, this argument is weakened by the marginally significant negative correlation between 2D:4D and WHR which indicates that abdominal adiposity may be more likely in individuals who were exposed to more testosterone *in utero*. The negative relationship between 2D:4D and WCR in women is in the expected direction as estrogen should be positively associated with chest circumference in women (Fink et al., 2003).

Further evidence for the positive impact of prenatal testosterone on adult cardiovascular health is provided by Fink, Manning, and Neave (2006). Fink and associates (2006) compared neck circumference (NC) to digit ratio in 117 women and 127 men. NC was previously found to positively correlate with BMI, weight, waist circumference, hip circumference, WHR, and systolic and diastolic blood pressure (Ben-Noun & Laor, 2004; Ben-Noun, Sohar, & Laor, 2001). Thus, NC is deemed a viable field method to assess cardiovascular health through the indirect assessment of obesity and the risk of metabolic syndrome (Fink et al., 2006). Fink et al. (2006) found right 2D:4D to significantly negatively correlate with WHR in men, but NC did not significantly correlate with 2D:4D. Body weight and NC correlated significantly and positively in both genders (men: $r = 0.709$; women: $r = 0.648$). When controlling for weight, the NC to 2D:4D correlation for both hands remained positive and reached statistical significance in men. After controlling for weight, NC also positively and significantly correlated with WHR in men. None of the correlations reached statistical significance in women. These results support the importance of prenatal and postnatal testosterone in the formation and maintenance of a healthier body composition and cardiovascular system (Fink et al., 2006). It also seems that the cardiovascular system is more sensitive to testosterone than estrogen as women, who possess lower testosterone levels than men, did not exhibit significant parallel variations in anthropometric measures with 2D:4D. The association between 2D:4D and NC in women is likely to be further diminished by the sexually dimorphic nature of body fat distribution particularly around the neck, whereas men have larger NC (Fink et al., 2006), which points towards an organizational role of testosterone in fat storage. In summary, Fink's et al. (2006) results support the positive impact of testosterone on cardiovascular health and the validity of 2D:4D as a proxy of cardiovascular risk factors. The overall effect of prenatal testosterone stimulation seems to be a masculinization of anthropometric measures and parameters of cardiovascular fitness. In terms of BMI and NC these effects are favorable for cardiovascular health but in terms of WHR the effects on cardiovascular health seem negative. The latter observation is consistent with the masculinizing effects of prenatal

testosterone as higher WHRs are characteristic of the android body shape. In terms of $\dot{V}O_{2max}$, we should expect higher prenatal testosterone levels (lower 2D:4D) to relate to higher $\dot{V}O_{2max}$ values as men typically have higher $\dot{V}O_{2max}$ values than women.

Influence of Exercise on Serum Testosterone Levels

As 2D:4D relates to prenatal testosterone stimulation and markers of cardiovascular fitness, we are interested in the effects of postnatal testosterone stimulation, as a possible confounder, on the relationship of 2D:4D to measures of cardiovascular fitness. In order to shed light on this question we must first understand the stimuli which induce changes in adult serum testosterone levels. Next, we must find out how these changes affect the organization of a cardiovascular parameter. Then, we can attempt to link these changes in a measure of cardiovascular fitness brought forth by testosterone stimulation to 2D:4D. Lastly, we can compare this manipulated relationship between a measure of cardiovascular fitness and 2D:4D to the relationship between the same variables under the 'unaltered' testosterone condition, whereas we would remove the testosterone stimulus to leave the cardiovascular system unchanged in regard to this postnatal testosterone stimulus.

Exercise is a natural, safe, and likely one of the most common stimuli causing changes in serum testosterone concentrations. Improvements in cardiovascular fitness are linked to exercise and if testosterone is to play a role in the improvements of cardiovascular fitness components, then there must be a link connecting serum testosterone levels to exercise. This relationship is well established in the literature.

Testosterone secretion by the gonads, namely the testes in men and the ovaries in women, is regulated by the hypothalamic-pituitary-gonadal axis. Simply put, the hypothalamus periodically releases gonadotropin-releasing hormone (GRH) which stimulates LH release by the anterior pituitary gland. LH prompts the gonads to produce and secrete testosterone. Increased serum testosterone levels in return have inhibitory effects on the hypothalamus and pituitary gland. The factors determining serum testosterone levels are thus circulating LH, the number of LH receptors in the testes or ovaries, the availability and production of testosterone in the gonads and the rate at which testosterone is cleared from the blood (Hackney, 1989). It seems that only five to 10% of all available LH receptors need to be occupied to achieve maximal testosterone secretion (Genuth, 1983). Vasoconstriction and vasodilation of the blood vessels around the gonads also affects the amount of testosterone that can dissolve into the blood. Therefore, catecholamines, such as adrenaline and norepinephrine, contribute to the regulation of serum testosterone levels (Eik-Nes, 1964). Most serum testosterone (97%) is combined testosterone which is bound to carrier proteins. The remaining 3% of testosterone, called free testosterone, is unbound. Both types of testosterone are referred to as total testosterone. As much as 5% of total testosterone can be contributed by the adrenal glands (Hackney, 1989).

Jensen et al. (1991) compared serum testosterone levels following strength training to the levels following endurance training. Strength training consisted of 90 minutes of hard to very hard (6-20 point Borg RPE scale) weight training and endurance training consisted of 90 minutes of hard to very hard cross country running. Training sessions occurred at the same time of the day and the weight training consisted of nine exercises with three sets of eight repetitions each at 80% of the participant's 1-RM and no more than two minutes rest between exercises. The running session was performed within ± 5 bpm of the heart rate corresponding to 70% of predetermined $\dot{V}O_{2max}$. The participants were all experienced in both exercise modalities with average $\dot{V}O_{2max}$ values of 66.1 $ml*kg^{-1}*min^{-1}$ and average bench press 1-RM of 182 lbs. All participants performed both types of training sessions separated by seven days to limit interference between the tests. Three participants performed the strength training session first and the remaining four participants performed the endurance training session first. Blood for testosterone concentration analysis was drawn 30 minutes before the start of each training session, immediately following the training session, as well as two, four, six, and 16 hours post-training. The seven participants were between 23 and 29 years old. A standardized breakfast was administered between 90 to 60 minutes prior to training sessions and participants were not allowed to train, consume any nicotine, alcohol, or medication during the 48 hours prior to the training sessions.

Strength training post-exercise testosterone concentration was elevated by 27% over the resting values. This increase was significantly larger than the 9.3% increase in serum testosterone during the same hours of day during non-exercise days. A mean increase of 37% in serum testosterone levels was observed following the endurance exercise treatment. The authors report that there was no significant difference in serum testosterone level elevations between post-strength training and post-endurance training. The serum levels of testosterone returned to resting values within two hours following both exercise modalities. There was no significant difference in pre- or post-exercise resting testosterone levels between the two exercise modes. Neither one of the exercise modes led to a chronic change in resting testosterone concentrations. Inter-individual differences in testosterone responses to training were large. This observation points towards the influence of genetic factors on testosterone production and secretion. Intra-individual responses to both exercise modalities showed a very strong correlation ($r = 0.98$) allowing the conclusion that both strength and endurance exercises cause the same acute physiological adaptation responsible for either stimulation of testosterone secretion or impaired clearance of serum testosterone.

A study of 10 sedentary, non-obese men yielded comparable results to the ones discussed above (Vogel, Books, Ketchum, Zauner, & Murray, 1985). Vogel et al. (1985) measured serum testosterone levels during the 30 minutes preceding exercise, during 45 minutes of ergometer cycling at 49.4% of $\dot{V}O_{2max}$, and during the first 30 minutes following exercise. Mean testosterone levels peaked at 18% above resting levels at 15 minutes into the exercise bout. There was no significant difference between resting and recovery levels of testosterone. Interestingly, no significant relationships were found between peak or resting

testosterone levels and $\dot{V}O_{2max}$. This seems intuitive, as sedentary individuals are generally not exposed to exercise stimuli that would cause a rise in testosterone levels and lasting changes in $\dot{V}O_{2max}$. In other words, those with inherently greater testosterone responses do not exhibit higher $\dot{V}O_{2max}$ values because, as sedentary individuals, they have not been taking advantage of their relatively strong responses to testosterone elevated due to exercise. However, these results do not support the theory that individuals who had been exposed to relatively high *in utero* testosterone concentrations are equipped with better cardiovascular efficiency.

Other studies have reported similar acute increases in serum testosterone levels in response to submaximal endurance training (Galbo, Hummer, Petersen, Christensen, & Bie, 1977; Guglielmini, Poalini, & Conconi, 1984; Kindermann, Schmitt, Schnabel, Berg, & Biro, 1985; MacConnie, Barkan, Lampman, Schork, & Beitins, 1986; Webb, Wallace, Hamill, Hodgson, & Mashaly, 1984; Wilkerson, Horvath, & Gutin, 1980). A comparison of serum testosterone levels between trained endurance athletes and sedentary individuals during and after submaximal exercise revealed a significant serum testosterone increase only in the athletes. The athletes also had significantly greater total and relative increases following exercise (Bunt, Bahr, & Bemben, 1987). Remes, Kuoppasalmi, and Adlercreutz (1979) analyzed plasma testosterone levels in Finnish military recruits before and after six months of boot camp training consisting of up to 40 hours per week of moderate to vigorous physical activities. The recruits were separated into to two groups based on increases in $\dot{V}O_{2max}$ from pre- to post-training. Those with greater increases in $\dot{V}O_{2max}$ had greater increases in plasma testosterone concentrations (28% vs. 15%). Cumming, Brunsting, Strich, Ries, and Rebar (1986) provide evidence that incremental maximal exercise also results in significant increases in serum testosterone levels which peak at approximately 20 minutes into the exercise. Experiments comparing testosterone responses during maximal exercise of trained endurance athletes to those of sedentary controls have shown similar testosterone responses in both groups (Hackney, 1989).

However, it has been shown that two hours of training twice per day lead to a decrease in resting testosterone levels and that male marathon runners have decreased levels of GRH (Häkkinen, Pakarinen, Alén, Kauhanen, & Komi, 1988; MacConnie et al., 1986). There seems to be a volume threshold or continuum characterized by decreasing testosterone levels with increasing training volumes. Souza, Arce, Pescatello, Scherzer, and Luciona (1994) produced evidence in support of the threshold theory by showing that high milage runners (108 km per week) have decreased free and total testosterone levels compared to moderate milage runners (54 km per week) and sedentary controls, while no difference existed between the moderate milage runners and the control group. Endurance athletes consistently present lower resting serum testosterone levels and fewer LH secretions per time interval compared to sedentary controls as long as they have been training for about five years or longer (for a review see Hackney, 1989). Distance runners also have suppressed LH and testosterone secretions respective to exogenous GRH and LH administration. The duration of single exercise bouts also seems to influence serum testosterone levels.

Submaximal or maximal exercise lasting longer than 90 minutes generally leads to serum testosterone concentrations below resting levels (Hackney, 1989). It appears that testosterone levels continue to drop after approximately 20 minutes of exercise. Decreased testicular blood flow caused by catecholamines and increased absorption of testosterone by skeletal muscle are possible mechanism for the depression in serum testosterone levels (Hackney, 1989). Hence, endurance athletes may not be suffering from decreased secretion of testosterone at rest provided that serum catechomaline levels return to normal resting levels after the cessation of exercise. The depressed serum testosterone levels associated with high milage endurance training may be due to an increased absorption of serum testosterone by skeletal muscle. Thus, individuals with high testosterone sensitivity (low R-L 2D:4D) may respond to endurance training with larger increases in $\dot{V}O_{2max}$ (Hill et al., 2012). However, Remes et al. (1979) found increases in serum testosterone (21%), androstenedoine (25%), and LH (25%) in 39 military recruits following a six month boot camp training program of up to 40 hours of exercise per week. It seems that more than six months of consistent high volume endurance training may be required to cause depressions in serum testosterone levels. Conversely, the rise in serum testosterone levels in military recruits following boot camp training may be in part due to a very competitive environment.

Besides exercise, genetic factors, as shown by Manning et al. (1998), are also responsible for intra-individual fluctuations in testosterone concentrations. LH is released in a pulsating fashion, with two to four secretions every six to eight hours (Hackney, 1998). Large nocturnal secretions of testosterone, regulated by the circadian cycle have also been observed (Bardin, 1978). The amount of LH or testosterone secreted during those cycles is most likely genetically regulated. Other factors contributing to serum testosterone levels include psychological stress, sleep loss, diet, and weight loss (McGrady, 1984; Opstad & Aakvaag, 1983).

It is apparent that endurance and other types of exercise lead to acute spikes in serum testosterone concentrations. Endurance training may thus lead to increases in $\dot{V}O_{2max}$, if testosterone does indeed have favorable effects on $\dot{V}O_{2max}$ (see discussion below). In regards to the stimuli causing fluctuations in serum testosterone, as discussed above, it can be argued that exercise and sensitivity to testosterone may be the most important determinants of $\dot{V}O_{2max}$ if the exercise stimulus is frequent, intense, and consistent enough. It seems logical that individuals endowed with greater testosterone sensitivity would respond with greater increases in $\dot{V}O_{2max}$ compared to those with lower sensitivity provided the training stimuli are equal (see discussion of Wang et al., 2010, below). As higher testosterone sensitivity is reflected in lower 2D:4D and lower R-L 2D:4D it seems plausible that those with lower digit ratios will have greater increase in $\dot{V}O_{2max}$ values during an exercise intervention program. However, if digit ratio reflects variations in prenatal testosterone concentrations more strongly than testosterone sensitivity then variations in digit ratio may be reflected more strongly in baseline $\dot{V}O_{2max}$ prior to training than in increases in $\dot{V}O_{2max}$ post-training. This last statement is based on the assumption that other genetic factors also contribute to increases in $\dot{V}O_{2max}$

following endurance training. Unfortunately, no investigation to date has included measures of both fetal testosterone concentrations and testosterone sensitivity as independent variables and digit ratio as the dependent variable. Hence, we do not know which factor explains more of the variation in 2D:4D. Moreover, reported effect sizes of prenatal androgen concentrations on digit ratios and effect sizes of androgen sensitivity on digit ratios are usually not reported or cannot be compared due to methodological differences. All published reports include only one of the various ways to determine variations in prenatal androgen concentrations or androgen sensitivity (i.e. amniocentesis, CAH, dizygotic twins, polycystic ovary syndrome, CAIS, CAG triplet repeat count, etc.) thus limiting comparability between studies. Additionally, some investigators combined female and male participants to investigate the relationship of androgen stimulation to 2D:4D, whereas others used participants of only one gender. Overall, there are not enough studies with similar methodology and statistical analyses to compare the effects of prenatal androgen levels to those of androgen sensitivity on digit ratio.

Postnatal Testosterone's Influence on Components of Cardiovascular Fitness

The mid-gestational peak in testosterone concentration is said to be responsible for the sex differences at birth (McIntyre, 2006). This evidence for the organizational effects of testosterone on the human body suggests the possibility that high postnatal testosterone concentrations exert measurable effects on the human physiology if testosterone sensitivity affords it. Of particular interest is the relationship between postnatal testosterone levels and the physiological determinants of maximal oxygen uptake ($\dot{V}O_{2max}$). Cardiovascular fitness, as estimated either by $\dot{V}O_{2max}$ or the time to exhaustion on graded treadmill exercise tests, is well established as an independent risk factor for cardiovascular disease, coronary heart disease, and all-cause mortality (Blair & Kohl, 1989; Blair, Kohl, & Barlow, 1995; Blair, Kohl, & Paffenbarger, 1989), whereas all-cause mortality refers to all causes of mortality combined into one statistic. $\dot{V}O_{2max}$ is widely accepted as the best measure of cardiovascular fitness (ACSM, 2010; Bassett & Howley, 2000; Mitchel & Blomqvist, 1971). $\dot{V}O_{2max}$ refers to the maximum amount of oxygen an individual can consume for energy production during vigorous exercise (Mitchel & Blomqvist, 1971) and is thus a measure of the ability of the cardiovascular system to deliver oxygen to exercising muscle tissue and the ability of the muscle tissue to consume oxygen. The limiting factors of $\dot{V}O_{2max}$ are pulmonary diffusion capacity, cardiac output, oxygen carrying capacity of blood, peripheral diffusion gradients, mitochondrial enzyme concentrations, and capillary density in muscle tissue (Bassett & Howley, 2000). Higher percentages of Type I muscle fibers allow for higher relative $\dot{V}O_{2max}$ values as oxygen consumption by muscle tissue depends largely on mitochondrial enzyme activity which is elevated in Type I compared to Type II fibers (Ivy, Withers, Van Handel, Elger, & Costill, 1980). Relative $\dot{V}O_{2max}$ values and percentages

of body fat also share an inverse relationship because adipose tissue has much lower oxygen consumption capacities than skeletal muscle (Czajkowska, Lutosławska, Mazurek, Keska, & Zmijewski, 2009).

As mentioned, one of testosterone's functions is the development of secondary sex characteristics including the development of more lean tissue mass in men compared to women. Testosterone has an anabolic function promoting lean tissue growth. Hartgens and Kuipers (2004) published a review about the effects of androgenic-anabolic steroids (AAS) on the human body. AAS are synthetic a version of testosterone. Some versions have stronger androgenic (masculinizing) effects while other versions have stronger anabolic effects depending on the particular steroid's affinity to bind to androgen receptors. The anabolic effects of AAS consistently produced 2-5 kg increases in body mass as a result of administration periods of less than 10 weeks in non-exercising adults. This increase is largely attributed to increases in lean body mass while fat mass tends to stay the same and the percentage of body fat decreases (Hartgens & Kuipers, 2004). Age shares a negative relationship with serum testosterone concentrations and lean body weight while total abdominal fat increases with age (Fink et al., 2003). Lean body mass has been shown to increase and WHR has been shown to decrease in elderly and healthy eugonadal men as a result of testosterone administration (Rebuffe-Scrive et al., 1991; Vermeulen et al., 1999), signifying a decrease in abdominal adipose tissue and healthier body composition. The decrease in WHR in elderly men is considered beneficial as total abdominal fat increases with age. It seems that testosterone might play an active and positive role in the development cardiovascular health, as higher percentages of fat free weight and lower percentages of fat weight are associated with improved cardiovascular health. This argument is supported by the regression to pre-intervention body composition values within three months after cessation of AAS administration.

The positive effects of testosterone supplementation on cardiovascular health are also supported by Zitzmann and Nieschlag (2007), who found decreased low-density lipoprotein (LDL) serum levels, increased high-density lipoprotein (HDL) serum levels, lower resting diastolic and systolic blood pressure, and lower resting heart rates in 66 hypogonadal men who were treated with a longitudinal intramuscular testosterone undeccanoate therapy (TU). Hypogonadism is a condition characterized by insufficient testosterone production of the gonads and TU is a newer form of testosterone replacement therapy causing fewer or different side effects and more favorable physiological changes than traditional testosterone substitutes (Zitzmann & Nieschlag, 2007).

Increases in muscle mass in a variety of leg, trunk, shoulder, and arm muscles were observed directly in a number of experimental studies involving the administration of androgenic steroids. These increases were observed in non-exercising and strength training participants, while the strength training participants exhibited larger increases. Interestingly, Type I (slow-twitch) muscle fibers exhibit greater hypertrophy and increases in number than Type II muscle fibers as a result of the use of AAS in some studies (Hartgens & Kuipers, 2004). Consequently, elevated serum testosterone levels might yield

beneficial effects for endurance athletes who largely rely on Type I muscle fibers. Type I muscle fibers have higher oxidative capacities than Type II fibers. This characteristic makes them an important contributor to cardiovascular fitness and maximal oxygen consumption capabilities. However, the use of different types of AAS, the small number of studies, and the somewhat mixed results do not allow a firm conclusion regarding the influence of natural testosterone on cardiovascular efficiency through its effect on the different muscle fiber types.

The anabolic effects are not limited to skeletal muscle but include hematopoiesis as well as glycogen synthesis and storage (Hackney, 1989). Testosterone's influence on hematopoiesis points towards beneficial effects of testosterone on cardiovascular fitness. Long-term administration of AAS has been shown to increase serum hemoglobin concentrations (Hartgens & Kuipers, 2004), a protein responsible for the transport of 99% of serum oxygen. Therefore, AAS are registered for the treatment of different types of anemia. However, only one investigation involving athletes found a significant increase in hemoglobin levels. Changes in the amount of erythrocytes have not been reported, although the anabolic trait of AAS is said to stimulate direct erythropoiesis, erythropoietin synthesis in the kidneys, the differentiation of stem cells into erythropoietic cells, and sensitization of erythroid progenitors. The majority of studies have failed to show improvements in endurance performance paralleling AAS treatment. Curiously, two studies involving strength athletes found improvements in aerobic capacity following AAS administration (Hartgens & Kuipers, 2004). Additionally, Zitzmann and Nieschlag (2007) found their long-term treatment of hypogonadal men with TU to stimulate erythropoiesis.

A number of researchers detected increased left ventricular wall thickness in AAS users, but only two cross-sectional investigations and one longitudinal investigation yielded increased left ventricular end-diastolic diameters. No decreases in any echocardiographic measures are associated with AAS use. Overall, AAS do not seem to benefit users in terms of altered heart function and structure (Hartgens & Kuipers, 2004). However, natural elevations in serum testosterone have been associated with an increased efficiency of the heart. Remes et al. (1979) found the increases in predicted $\dot{V}O_{2max}$ following boot camp training to be larger in those military recruits with greater increases in serum testosterone levels following boot camp compared to pre-camp levels. The investigators labeled those with larger increases in predicted $\dot{V}O_{2max}$ (n = 19) "well-conditioned" and those with smaller increases in predicted $\dot{V}O_{2max}$ (n = 20) "poorly conditioned". The "well-conditioned" recruits had a mean increase in serum testosterone levels of 28%, whereas the "poorly-conditioned" group showed an increase of 15%. In fact, the 10 "best-conditioned" recruits had an elevation in serum testosterone of 43%, compared to 13% in "worst-conditioned" group. Predicted $\dot{V}O_{2max}$ values were based on heart rate responses to submaximal ergometer tests (Remes et al., 1979). Hence, it seems that elevations in serum testosterone levels relate positively to increased stroke volume and most likely improved $\dot{V}O_{2max}$ values.

In the interest of cautioning the reader, it should be mentioned that the abuse of AAS can have severe and fatal side effects, including cardiomyopathy, atrial fibrillation, and myocardial infarction. The cause of these side effects is unclear. However, the suppression of the hypothalamic-pituitary-gonadal axis by the exogenous administration of derived testosterone (AAS), leads to the inhibition of hypothalamic and pituitary activity which causes decreased secretion of LH and FSH (Hartgens & Kuipers, 2004). These hormones are vital for healthy reproductive function. Further discussion of the negative side effects of AAS is irrelevant to this investigation because research involving digit ratios is limited to natural elevations in testosterone which do not have reported harmful side effects. However, the investigations involving AAS provide insight into the possible influence of testosterone on components of the cardiovascular system.

It seems that postnatal testosterone stimulation has a favorable effect on the physiological determinants of $\dot{V}O_{2max}$. A negative correlation between digit ratio and $\dot{V}O_{2max}$ can be as digit ratio probably reflects postnatal testosterone sensitivity. Equal training stimuli across a sample should not change this relationship as the spikes in serum testosterone will be translated into greater increases in $\dot{V}O_{2max}$ in those with greater testosterone sensitivity as indicated by lower 2D:4D.

Testosterone Sensitivity and Cardiovascular Fitness

McIntyre (2006) pointed out that *in utero* peaks of testosterone concentrations rise to the level of concentrations in male adults. Given the physiological organizational effects of *in utero* testosterone mediated by androgen sensitivity, it seems likely that the serum level of testosterone in adults is responsible for organizational effects as longs as androgen sensitivity is adequate. Evidence for the effects of androgen sensitivity coupled with frequent and consistent spikes in serum testosterone concentrations due to training on cardiovascular fitness is provided by Wang et al. (2010). CAG triplet repeat length was used as an indicator of testosterone sensitivity and $\dot{V}O_{2max}$ was the measure of choice for cardiovascular fitness. Sixty-five healthy men underwent a 30-day training consisting of 30 minutes of hypoxic exercise at 75% of $\dot{V}O_{2max}$ (simulating 2,500m altitude) three times a week interspersed by routine sea-level training. The participants also slept in hypoxic conditions (simulating 2,800-3,000m altitude). When dividing the number of participants equally into a group with few CAG triplet repeats (≤ 22) and into a group with more CAG triplet repeats (> 22), then the increase in absolute $\dot{V}O_{2max}$ of the ≤ 22 group was 5.6 greater than the increase in $\dot{V}O_{2max}$ of the > 22 group. This increase in $\dot{V}O_{2max}$ can best be explained by increases in hematocrit and hemoglobin count due to the exercise induced spikes in serum testosterone levels (Hartgens & Kuipers, 2004; Zitzmann & Nieschlag, 2007). High altitude has also been shown to cause elevations in hematocrit and increases in hemoglobin concentrations (Stray-Gundersen, Chapman, & Levine, 2001). It seems that the effects of hypoxic conditions on erythropoiesis are mediated by testosterone as those individuals with higher testosterone sensitivity responded with larger elevations $\dot{V}O_{2max}$. Surprisingly,

baseline absolute $\dot{V}O_{2max}$ values were significantly higher for those with > 22 CAG triplet repeats than those ≤ 22 CAG triplet repeats. This could be explained by the significantly higher body weight of those with > 22 CAG triplet repeats than those with ≤ 22 CAG triplet repeats. However, BMI did not differ between those groups. This means that men with less CAG triplet repeats must have been shorter than those with more CAG triplet repeats, as BMI is a measure of the body weight divided by the square of the height. This result stands in contrast to the findings that androgen sensitivity promotes bone development and that estrogen insensitivity results in continued growth after puberty (Rochira, Balesrieri, Madeo, Spaggiari, & Carani, 2002; Zheng & Cohn, 2011), but it is supported by the positive correlation between the number of CAG repeats and fat free mass found by Walsh et al. (2005). However, as the fusion of growth plates coincides with the stimulation of estrogen receptors (Weise et al., 2001), it is possible that those men with fewer CAG triplet repeats also had greater estrogen sensitivity and/or serum concentrations. The heavier weight of those with > 22, but similar BMI to those with ≤ 22 CAG triplet repeats could also be explained by higher percentages of body fat in those with > 22 CAG triplet repeats. Positive correlations between CAG repeat length and BMI as well as body fat content, obesity, waist circumference, and leptin in men with type 2 diabetes have been reported (Stanworth, Kapoor, Channer, & Jones, 2008; Zitzmann, Gromoll, von Eckardstein, & Nieschlag, 2003). Nielsen et al. (2010), examining 783 men aged 20-29 years, found a negative relationship between CAG triplet repeat length in the androgen receptor gene and absolute muscle mass in the thigh (r = -0.108) and lower trunk (r = -0.132) as well as relative muscle mass in the thigh (r = -0.128), lower trunk (r = -0.126) and relative lean tissue mass in the lower extremities (r = -0.108) and whole body (r = -0.082). Additionally, CAG repeat polymorphism related positively to relative subcutaneous fat mass in the thigh (r = 0.137) and lower trunk (r = 0.188) as well as relative fat mass in the lower extremities (r = 0.107) and whole body (r = 0.082). However, a strong negative correlation has also been found between the number of CAG triplet repeats and central obesity in post-menopausal women and older adults (Gustafson, Wen, & Koppanati, 2003). It seems that cardiovascular disease risk associated with unusually long or short CAG triplet repeat chains is most likely mediated by cofactors (Zitzmann et al., 2003).

Zitzmann and Nieschlag (2007) found increases in hematocrit to levels approaching 54% during long-term TU treatment in hypogonadal men with low CAG repeat length. Hematocrit concentration increased in a non-linear fashion with increases in serum testosterone concentrations and decreases in CAG triplet repeat length. The increase in hematocrit is due to increased erythropoiesis, which is the reason for testosterone supplementation in hypogonadal patients who typically suffer from anemia and accompanying symptoms of weakness and fatigue (Zitzmann & Nieschlag, 2007).

Testosterone sensitivity seems to play an important part in the development and maintenance of cardiovascular health, especially those factors determining maximal oxygen uptake including the blood's

capacity for oxygen transport. Consequently, digit ratios could prove to be a useful biomarker of predispositions for cardiovascular risk factors and trainability of the cardiovascular system.

Conclusion

Digit ratio as a biomarker of prenatal testosterone stimulation is associated with athletic performance, whereas low digit ratios indicating higher prenatal testosterone stimulation relate to better athletic performances. This relationship is strongest in endurance running. The most widely used physiological correlate of endurance running performance is $\dot{V}O_{2max}$. However, conclusive evidence linking lower digit ratios to higher $\dot{V}O_{2max}$ values does not exist. Confounders in Hill's et al. (2012) investigation on 2D:4D and $\dot{V}O_{2max}$ include the pubertal age of the participants and the diverse range of sports which the participants played. These are argued to be confounders because prenatal testosterone levels may relate positively to postnatal testosterone levels (Cattrall, Vollenhoven, & Weston, 2005) and fluctuations in postnatal testosterone levels have been shown to influence physiological parameters which determine $\dot{V}O_{2max}$. However, 2D:4D has not been established as a biomarker of postnatal testosterone levels at rest (Hönekopp et al., 2007). Conversely, R-L 2D:4D does seem to correlate negatively to rises in free testosterone levels associate with physical challenges (Kilduff et al., 2012). This investigation sought to examine the relationship between right, left, and R-L 2D:4D and $\dot{V}O_{2max}$, RER_{max}, RE, and endurance running performance in female and male healthy, sedentary individuals and highly trained endurance runners aged 18 to 25. The aim was to control for fluctuations in adult serum testosterone concentrations caused by puberty and differences in training stimuli. The exercise stimuli in the sedentary population were assumed to be too infrequent and not intense enough for significant alterations in $\dot{V}O_{2max}$ values. The endurance runners were recruited from intercollegiate track & field teams which served to ensure consistency in terms of training stimuli. The post-pubertal age of all participants ensured stable resting serum testosterone concentrations (Crabbe, Christiansen, Rødbro, & Transbøl, 1979). Hence, the main purpose of this investigation was to examine the relationship of total prenatal testosterone stimulation via 2D:4D to $\dot{V}O_{2max}$, RER_{max}, RE_{abs}, RE_{rel}, and ToT, as well as the effects of long-term endurance running training on these relationships. The relationship between 2D:4D and $\dot{V}O_{2max}$ in the trained runners was expected to be negative as Wang et al. (2010) found significant increase in $\dot{V}O_{2max}$ in those with high androgen sensitivity compared to those with lower androgen sensitivity and because 2D:4D is an indicator of testosterone sensitivity (Manning et al., 2003). The investigation of the relationships between measures of 2D:4D, $\dot{V}O_{2max}$, RE, RER_{max}, and running performance in the form of PRs within the same population allowed for a more direct comparison between the association of 2D:4D to $\dot{V}O_{2max}$, RE, and RER_{max} and the association of 2D:4D to endurance running performance. For this purpose, partial correlation analysis

was used to explore which performance variable ($\dot{V}O_{2max}$, RE_{abs}, RE_{rel}, or RER_{max}) moderates the relationship between 2D:4D and endurance running performance.

CHAPTER 3

METHODS

Introduction

These methods were employed to examine the relationships between measures of 2D:4D and performance variables ($\dot{V}O_{2max}$, RER_{max}, RE_{abs}, RE_{rel}, and ToT), and to examine the influence of the relationships of 2D:4D to performance variables on the relationship between 2D:4D and endurance running performance, while controlling for a number of potential confounders such as age and weight. The relationship between 2D:4D and endurance running in itself will also be examined. The age of the participants will be early adulthood age, homogeneous, and post-pubertal to limit age associated fluctuations in testosterone levels. The influence of endurance running training on the relationship of 2D:4D to $\dot{V}O_{2max}$ will also be explored by comparing highly trained endurance runners to sedentary individuals based on this relationship. Thus, the training modality will be restricted to one, namely endurance running. The training experience is also controlled by the recruitment of distance runners from intercollegiate track and field/cross country teams. For the first time, the relationship of 2D:4D to $\dot{V}O_{2max}$ in women will be explored.

Participants

Thirteen male intercollegiate cross country and/or track and field runners were recruited from a mid-sized southeastern university in the USA. Seven female intercollegiate cross country and/or track and field runners were recruited from the mid-sized southeastern university and six female intercollegiate runners were recruited from a small southeastern college. In total, 13 female intercollegiate long distance runners participated in this study. Additionally, 13 male and 15 female sedentary college students were recruited from the general student population of the southeastern university. All participants were between the age of 18 and 25 years. General exclusion criteria included: (1) responding "YES" to one or more questions on the Physical Activity Readiness Questionnaire (PAR-Q & YOU) (Appendix D) or the Pre-Test Questionnaire (Appendix E) which includes general exclusion criteria 2-4 as well as questions to determine adherence to the pre-test instructions; (2) knowingly or possibly pregnant, or knowingly pregnant within the previous six months; (3) knowingly taking any ergogenic or performance enhancing drugs; (4) smoking; (5) being younger than 18 years or older than 25 years; (6) Body Mass Index of ≥ 30 $kg*m^{-2}$ (ACSM, 2010); (7) systolic blood pressure of ≥ 140 mm Hg or diastolic blood pressure of ≥ 90 mm

Hg (ACSM, 2010); (8) not having followed the pre-test instructions (Appendix F). The pretest instructions asked the participants not to consume any alcohol, caffeine, or nicotine during the 24 hours prior to testing, not to exercise the day before and the day of the testing session, not change their exercise regimen, not to change their medication regimen unless recommended by a doctor, and not to eat anything 2.5 hours prior to testing. Additional exclusion criteria for the sedentary individuals included meeting the minimal physical activity recommendations from the U.S. Surgeon General's Report, which amount to two hours or more per week of moderate to vigorous physical activity (ACSM, 2010; U.S. Department of Health and Human Services, 1996). Exclusion criteria particular to the runners included any injury within the last two months that has resulted in a reduction of training quantity, intensity, or frequency for more than two weeks.

Instruments and Apparatuses

The participants were asked to fill out a brief demographics questionnaire (Appendix G). The runners also answered questions about their personal records on the track, years of training, and average mileage run per week on the demographics questionnaire. Using a stainless steel offset LCD digital Vernier caliper, Model K01-101 (Kbd Tools Co., Ltd., Jiangsu, China), the length of the second (index) and the fourth (ring) finger on both hands were measured twice to the nearest 0.01 mm by an investigator. Body composition was assessed using a Tanita SC-331S Body Composition Analyzer (Tanita Corporation of America, Inc., Arlington Heights, Illinois). $\dot{V}O_{2max}$ was measured using the Bruce Protocol for the treadmill (see Pilot Study below; ACSM, 2010). Prior to the commencement of the $\dot{V}O_{2max}$ test the investigator read the $\dot{V}O_{2max}$ test instructional script to the participant. A Desmo HP Woodway treadmill (Woodway, Waukesha, WI), a Physio-Dyne, MAX-1 model, computerized gas analyzer with Physio-Dyne Metabolic System software (Physio-Dyne Instruments Corp., Quogue, NY), and a Polar Monitor System (Polar Electro Inc., Woodbury, NY) to measure heart rate were used during this test.

Reliability and Validity of Apparatuses

Using 30 male and 30 female subjects, Allaway, Bloski, Pierson, & Lujan (2009) analyzed intratester and intertester reliability of four different techniques commonly used to determine digit ratio. These techniques included (1) physical measurements, (2) photocopies, (3) printed and scanned images, and (4) computer-based image analysis. Three experienced testers made two different measurements of each participant's fingers using all four techniques. Interobserver reliability was highest for the computer assisted technique ($r = 0.892$), second highest for the photocopies ($r = 0.858$), third highest for the physical measurements ($r = 0.795$), and lowest for the printed scans ($r = 0.761$). Intraobserver reliability was also highest for computer assisted measurements ($r = 0.957$), second highest for the photocopies ($r = 0.939$),

third highest for the physical measurements ($r = 0.925$), and lowest for the printed scans ($r = 0.842$). Thus, physical digit length measurements are associated with satisfactory reliability. Allaway et al. (2009) as well as Manning, Fink, Neave, and Caswell (2005) found that physical measurements consistently produce higher 2D:4D values compared to other measurement techniques, especially photocopies. Manning et al. (2005) attributed these differences to the distortion of soft tissue in the fingers when the hands are placed on the glass panel of photocopy machines. Manning et al. (2005) thus recommend using physical measurement techniques. Cattrall et al. (2005) reported intraclass correlation coefficients (*ICC*) for repeated measurements of the same digit by the same investigator taking physical digit measurements with a steel Vernier caliper. The *ICC* for right 2D and 4D were both 0.99 and between participant variation was greater than within participant variation. Voracek and Dressler (2007) used a digital vernier caliper to measure digit lengths from printed scans of the ventral surface of participants' hands. The intraobserver repeatability ranged from *ICC* = 0.997 for right 2D to *ICC* = 0.995 for left 4D.

Digit measurements in the present study will be taken by the same investigator to remove interobserver error. The investigator will be blind to participants' $\dot{V}O_{2max}$ values as, chronologically, digit measurements will be taken before the $\dot{V}O_{2max}$ test will be conducted. During the pilot study, the second and fourth finger of both hands of five female and four male participants were measured twice for a total of 72 measurements by the investigator who will measure the digit lengths for the present study. The *ICC* was high ($r = 0.99$, df = 7, $P < 0.0005$) indicating that the observer is able to obtain reliable digit length measures.

Rutherford, Diemer, & Scott (2011) found that foot-to-foot bioelectrical impedance (BIA) measurements shared a r = 0.63 correlation with hydrodensitometry and tended to overestimate body fat percentages compared to hydrodensitometry, sum of three skinfold method, sum of seven skinfold method, and hand held bioelectrical impedance measurements in college populations. However, Rutherford et al. (2011) used the Tanita Body Fat Monitor, Model TBF-315, for foot to foot measurements, whereas we will use the Tanita SC-331S Body Composition Analyzer. The TBF-315 model is not in production anymore, while the SC-331S model is one of the newest body composition analyzers from Tanita. The manufacturer does not report any measures of reliability or validity. Other investigators compared the Tanita TBF 305 foot to foot analyzer to a hand to foot BIA analyzer and hydrodensitometry (Dias, Veiga, da Silva, & Monteiro, 2001). No significant difference was found between the two BIA analyzers but both overestimated body fat percentages compared to hydrostatic weighing. One study used the Tanita SC-331S model to assess body composition (Wang, Reed, Goli, & Goswami, 2011), but validational work has not been performed with this model.

A study comparing the Physio-Dyne MAX-1 metabolic cart to the gold standard Douglas bag method across four different work rates yielded no significant difference between the systems in regard to $\dot{V}O_2$, $\dot{V}CO_2$, FEO_2, or $FECO_2$ (Cullum, Welch, & Yates, 1999). However, the average $\dot{V}O_2$ value produced

by the Physio-Dyne MAX-1 systems was 87 ml/min less than the values produced by the Douglas bag system. When tested for repeatability, the Physio-Dyne system produced a relative error of 3.2% in $\dot{V}O_2$ measurements which was only slightly greater than that of the Douglas bag system (2.5%). Another investigation found that the Physio-Dyne MAX-1 cart non-significantly underestimated $\dot{V}O_2$ values with an error of 3.1 to 6.1% depending on the flow rate (Yates & Cullum, 2001). Overall, the Phsyio-Dyne MAX-1 system provides accurate and reliable measurements of oxygen consumption.

Procedures

Testing consisted of one session lasting approximately 50 minutes. Upon arrival at the exercise physiology laboratory eligibility for participation was determined through the PAR-Q & YOU questionnaire and the Pre-Test Questionnaire. The eligible participant then filled out the demographics questionnaire. Personal records (PRs) per race distance were self-reported by the runners on the demographics questionnaire. Next, the participant's blood pressure was measured with a standard sphygmomanometer and stethoscope. The participant's height was measured and the body composition and body mass index were assessed by the TANITA Body Composition Analyzer. The participant stepped on the body composition analyzer barefoot wearing the clothes to be worn for the treadmill test and his or her gender, age, height, and level of physical activity were entered. One half to one pound were entered into the body composition analyzer for the weight of the clothes depending on the participant's size and type of clothing. Then, an investigator measured and recorded the lengths of the second and fourth digit on both hands by measuring all four fingers in no particular order once, followed by a second measurement of all four fingers. The Vernier caliper was calibrated before each measurement. Participants were asked to remove rings and jewelry on all fingers as it could interfere with the measurements and they were then instructed to place the dorsal surface of their hand on a table while stretching their fingers and spreading them slightly as well as aligning the direction in which forearm and middle finger were pointing. The lower tip of the caliper was placed in the center of the basal crease proximal to the palm of the hand on the ventral surface. The caliper was then extended and the upper arm of the caliper was placed against the soft tissue tip of the finger without the exertion of pressure as described by Manning et al. (1998) (Figure B-3). Participants with injuries to any of the second or fourth fingers were excluded.

Then the principal investigator orally provided the participant with the standardized instructions for the incremental treadmill test (Appendix H). After the participant's questions had been answered, he or she was fitted with a heart rate monitor and a face mask and asked to step on the treadmill for the commencement of the test. The metabolic cart was calibrated before each testing session according to the manufacturer's guidelines. The participant's data including weight and height as well as the ambient temperature and atmospheric pressure were entered into the Physio-Dyne MAX-1 software.

The incremental treadmill test was terminated at the point of voluntary exhaustion of the participant. The criteria for $\dot{V}O_{2max}$ achievement included (1) a plateau in $\dot{V}O_2$ signified by a failure of $\dot{V}O_2$ to increase despite increases in work rate or an increase in $\dot{V}O_2$ of $\overline{x}$ – 2SD or less between the last two measurements, whereas $\overline{x}$ is the average increase in $\dot{V}O_2$ from measurement to measurement and SD is the standard deviation of increases in $\dot{V}O_2$ (Taylor, Buskirk, & Henschel, 1955), (2) a respiratory exchange ratio (RER) of ≥ 1.15 (Issekutz, Birckhead, & Rodahl, 1962; Issekutz & Rodahl, 1961), (3) a heart rate within 95% of the age adjusted maximal as determined through the following formula: 208 – 0.70*age (Tanaka, Monahan, Douglas, & Seals, 2001) and a maximal rating of 9 or 10 on the OMNI Rating of Perceived Exertion Scale (Utter et al., 2004). Breath by breath gas samples were automatically averaged over 30 seconds by the computerized metabolic cart. $\dot{V}O_{2max}$ was recorded as the highest 30s average $\dot{V}O_2$ and expressed in $ml*kg^{-1}*min^{-1}$. Two out of four criteria had to be satisfied for the achievement of $\dot{V}O_{2max}$.

Absolute RE (RE_{abs}) was determined as the average of two $\dot{V}O_2$ ($ml*kg^{-1}*min^{-1}$) measurements with one measurement immediately prior to and one measurement immediately after the 8:00 minute mark during the treadmill test. $\dot{V}O_2$ measurements were automatically averaged over 30 seconds by the computerized metabolic cart. RE_{rel} was expressed as the percentage of RE_{abs} in relation to the $\dot{V}O_{2max}$. The RER was calculated from measurements of VO_2 and VCO_2. The highest RER value recorded was considered the RER_{max}.

Statistical Analyses

Statistical analyses were performed with the Statistical Package for the Social Sciences v.19.0 (SPSS, Inc., Chicago, IL). One-way ANOVA and Tukey's post-hoc tests were used to test for differences between groups in all variables. Linear contrasting was performed to test for gender differences in measures of digit ratio. Pearson's *r* correlation coefficient was used to examine relationships between variables by group. First order partial correlation analyses were used to remove the effects of a third variable from the relationships between measures of digit ratio and dependent variables. Results were accepted as significant at $p \leq 0.05$.

Pilot Study

The purpose of this pilot work was to explore the relationship between digit ratio and maximal oxygen uptake in a sample of college aged male and female individuals and to determine intra-tester reliability in regard to digit length measurements. Three college aged women and four college aged men from a mid-sized southeastern university participated in this study. Using a stainless steel LCD digital Vernier caliper, the length of the second (index) and the fourth (ring) finger on both hands were measured

twice to the nearest 0.01 mm. $\dot{V}O_{2max}$ was assessed using the following treadmill protocol: Stage 1: 3 min at 3 mph and 0 % grade; Stage 2: 3 min at 5 mph and 0 % grade; Stage 3 and all subsequent stages were 2 minutes long at 5 mph with 3 % increase in grade at the beginning of each stage. Two out of three criteria had to be satisfied in order to achieve $\dot{V}O_{2max}$. These criteria included (1) a plateau in $\dot{V}O_{2max}$ signified by a failure of $\dot{V}O_2$ to increase despite increases in work rate, (2) a RER of at least 1.15, and (3) a heart rate within 95% of the age adjusted maximal as determined through the following formula: 208 – 0.70*age (Tanaka, Monahan, Douglas, & Seals, 2001). The same apparatuses as described above were used for this pilot investigation.

The age of the participants was 24.11 ± 2.09 years (Mean ± SD). An independent samples *t*-test revealed no significant difference between male right 2D:4D (0.963 ± 0.049) and female right 2D:4D (0.987 ± 0.026) although men presented with lower right 2D:4D than women, as expected. Men also had non-significantly lower left 2D:4D (0.947 ± 0.042) and R-L 2D:4D (0.016 ± 0.033) than women (0.967 ± 0.019, 0.020 ± 0.013 respectively). The mean relative $\dot{V}O_{2max}$ of the participants was 42.3 ± 13.7 $ml*min^{-1}*kg^{-1}$. Using Pearson's *r* correlation coefficient, $\dot{V}O_{2max}$ was significantly negatively correlated to right 2D:4D ($r = -0.69$, $d.f. = 5$, $p < 0.05$). Negative correlations were also found between VO_{2max} and left 2D:4D ($r = -0.52$, $d.f. = 5$, $p > 0.05$) as well as R-L 2D:4D ($r = -0.42$, $d.f. = 5$, $p > 0.05$). These relationships are stronger than those found by Hill et al. (2012), however the sample size of this pilot work was very small and both genders were combined into one group.

CHAPTER 4

MANUSCRIPT

Introduction

The ratio of the length of the second finger to the length of the fourth finger (2D:4D) is firmly established as a sexually dimorphic trait, whereas men generally have smaller ratios than women (Hönekopp & Watson, 2010; Manning, Scutt, Wilson, & Lewis-Jones, 1998). This ratio seems to be determined *in utero* by prenatal testosterone levels (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004; Manning et al., 1998) and by testosterone sensitivity (Manning, Bundred, Newton, & Flanagan, 2003), whereas higher prenatal testosterone levels and greater testosterone sensitivity are associated with lower 2D:4D (Manning et al., 2003).

Usually, clinical populations with altered prenatal testosterone levels, including samples with congenital adrenal hyperplasia (i.e. Brown, Hines, Frane, & Breedlove, 2002), polycystic ovary syndrome (Cattrall, Vollenhoven, & Weston, 2005), and Klinefelter's syndrome (Manning, Kilduff, & Trivers, 2013) have been used to demonstrate that greater androgen exposure is associated with lower 2D:4D. Amniocentesis, the analysis of hormone concentrations in the amniotic fluid, has also been employed to link higher prenatal testosterone levels to lower digit ratios (Lutchmaya et al., 2004).

Cytosine-adenine-guanine (CAG) triplet repeat length in exon 1 of the androgen receptor gene (AR) located on the X-sex-chromosome is a determinant of androgen sensitivity (Chamberlain, Driver, & Miesfeld, 1994). CAG polymorphism is inversely related to sensitivity to testosterone and thus positively related to 2D:4D (Manning et al., 2003). Significant positive correlations between right 2D:4D ($r = 0.29$; $p = 0.02$) as well as R-L 2D:4D (subtracting left 2D:4D from right 2D:4D) ($r = 0.36$, $p = 0.005$) and CAG repeat polymorphism have been reported in 50 healthy men (Manning et al., 2003). Individuals with complete androgen insensitivity syndrome, characterized by absent or dysfunctional androgen receptors, presented with greater 2D:4D then men but not women. These differences were more pronounced in the right hand than the left hand (Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009).

Besides digit ratio, many other gender differences are determined *in utero* by prenatal testosterone (Cohen-Bendahan, Buitelaar, Van Goozen, Orlebeke, & Cohen-Kettenis, 2005; Hönekopp, Manning, & Müller, 2006). 2D:4D is a practical biomarker of prenatal testosterone when investigating the relationship of prenatal testosterone levels to sexually dimorphic traits in adults. One such family of sexually dimorphic traits seems to be athletic prowess (Hönekopp & Schuster, 2010). Lower 2D:4D has been associated with superior performances in fencing (Voracek, Reimer, Ertl, & Dressler, 2006), skiing (Manning, 2002b),

soccer (Manning & Taylor, 2001), field-based fitness tests (Hönekopp et al., 2006), sumo wrestling (Tamiya, Lee, & Ohtake, 2011), basketball (Tester & Campell, 2007), 50m dash (Manning & Hill, 2009), a hand-grip strength test (Fink, Thanzami, Seydel, & Manning, 2006), and 2,000m ergometer rowing (Longman, Stock, & Wells, 2011). The strongest reported association has been found between 2D:4D and endurance running performance, whereas the strength of the association between 2D:4D and endurance running performance increases as race distance increases. The average variance in sports performance explained by 2D:4D falls between 1% and 16%, the shared variance between 2D:4D and 50m dash times was reported to be 2%, and the variance in foot races of one to four miles shared with 2D:4D reached 25% (Hönekopp & Schuster, 2010; Manning, Morris, & Caswell, 2007). A meta-analysis of 24 studies revealed an effect size of r = -0.28 for the relationship between right 2D:4D and athletic prowess in a variety of sports and measures of athleticism while an analysis of 22 studies revealed an effect size of r = -0.26 for the relationship of left 2D:4D with measures of athletic prowess. Hence, the strength of association between athletic performances and digit ratios is not significantly different between hands (Hönekopp & Schuster, 2010).

Maximal oxygen uptake ($\dot{V}O_{2max}$) correlates strongly to endurance running performance (Bassett & Howley, 2000; Paliczka, Nichols, & Boreham, 1987) and is a sexually dimorphic trait with higher values displayed by men as compared to women (Bouchard et al., 1998). Hence, Hill, Simpson, Manning, and Kilduff (2012) investigated the relationship between 2D:4D and $\dot{V}O_{2max}$ and found a significant relationship only between R-L 2D:4D and $\dot{V}O_{2max}$ (b = -0.33). In the same study, significant relationships were also found between running velocity at $\dot{V}O_{2max}$ (b = -0.47) as well as maximal lactate concentrations (b = -0.50) and R-L 2D:4D. A potential confounder in Hill's et al. (2012) investigation is the pubertal age (13.9 ± 1.3 years) of the 41 boys tested. During puberty, testosterone levels rise and do not level off until after the age of 14 years in boys (Crabbe, Christiansen, Rødbro, & Transbøl, 1979). During this time, accelerated growth and maturation of the skeletal, nervous, and cardiovascular system takes place (Mantzoros, Flier, & Rogol, 1997). Moreover, the onset and end of puberty is not chronologically synchronous between individuals, which means that some boys might have matured to post-pubertal stages and others might have still been in a pre-pubertal phase at the time of data collection (Mantzoros et al., 1997). Thus, the association between 2D:4D, a biomarker of prenatal testosterone, and $\dot{V}O_{2max}$, which is subject to change during puberty, may be weakened during puberty. The association between 2D:4D and $\dot{V}O_{2max}$ may thus be stronger in post-pubertal populations. Furthermore, the participants in Hill's et al. (2012) investigation were involved in a variety of sports, including table tennis, squash, soccer, and track and field. The diverse training stimuli associated with these sports likely led to differing cardiovascular and muscular adaptations. Hence, the $\dot{V}O_{2max}$ adaptations were dependent on the sport, which confounds the influence of prenatal testosterone on $\dot{V}O_{2max}$. Consequently, the nature of the relationship of 2D:4D to

$\dot{V}O_{2max}$ in post-pubertal populations with homogeneous training stimuli or relative lack of training stimuli remains unclear.

Due to the association of right and left 2D:4D with endurance running performance but the lack of association of right and left 2D:4D with $\dot{V}O_{2max}$, it is warranted to conduct an analysis of the relationship of 2D:4D with running economy (RE), as RE is a sexually dimorphic trait dependent on a number of sex-specific traits including body composition, height, weight, leg mass, and flexibility (Pate, Macera, Bailey, Bartoli, & Powell, 1992; Saunders, Pyne, Telford, & Hawley, 2004). RE refers to the amount of oxygen consumed per kilogram of body weight ($\dot{V}O_2$) at a certain running velocity. At equal running velocities, men generally exhibit a higher $\dot{V}O_2$ than women (RE_{abs}). However, men typically perform at a lower percentage of their $\dot{V}O_{2max}$ (RE_{rel}) (Daniels & Daniels, 1992). Moreover, maximal respiratory exchange ratio (RER_{max}), an indicator of buffered lactic acid, may also be a moderator of the relationship between digit ratios and endurance running due to the association of R-L 2D:4D and maximal lactate concentrations in Hill's et al. (2012) investigation.

Superior increases in $\dot{V}O_{2max}$ following endurance training under hypoxic and normoxic conditions in men with relatively short CAG triplet repeat chains has been reported (Wang et al., 2010). Hence, testosterone sensitivity relates to increases in $\dot{V}O_{2max}$ following training. Additionally, Remes, Kuoppasalmi, and Adlercreutz (1979) found that those with larger increases in serum testosterone after six months of military boot camp training also had larger increases in $\dot{V}O_{2max}$ compared to those with smaller increases in serum testosterone. As CAG repeat polymorphism is positively associated with 2D:4D it can be hypothesized that 2D:4D relates negatively to $\dot{V}O_{2max}$ in cohorts exposed to frequent, consistent, and equal endurance training stimuli.

First of all, the purpose of this investigation was to examine the influence of *in-utero* testosterone stimulation (concentration of and sensitivity to testosterone) via 2D:4D on $\dot{V}O_{2max}$, RE_{abs}, RE_{rel}, RER_{max}, and total time on the treadmill (ToT) during an incremental treadmill test as well as the effects of long-term endurance running training on these relationship by controlling for age and training in a quasi-experimental study design. Secondly, we explored which performance variable ($\dot{V}O_{2max}$, RE_{abs}, RE_{rel}, or RER_{max}) is the most likely moderator of the relationship between 2D:4D and endurance running performance. The term 'performance variables' will be used interchangeably with $\dot{V}O_{2max}$, RER_{max}, RE_{abs}, RE_{rel}, and ToT collectively.

Negative correlations between measures of digit ratio (right 2D:4D, left 2D:4D, R-L 2D:4D) and $\dot{V}O_{2max}$, RE_{abs}, RER_{max}, and ToT were expected. Positive correlations between digit ratios, RE_{rel}, and endurance running performance measured as personal record time (PR) per race distance were also anticipated.

Methods

Participants

Thirteen male (MR) and 13 female (FR) intercollegiate cross country and/or track and field runners along with 13 male (MC) and 15 female (FC) sedentary college students from the general student population were recruited from a mid-sized southeastern university and college in the USA. At the time of data collection all runners were in the competitive phase of their cross country and/or track & field seasons. Exclusion criteria particular to the runners included any injury within the last two months that has resulted in a reduction of training quantity, intensity, or frequency for more than two weeks. To control for training effects in the control group, sedentary participants were excluded if they met or exceeded the minimal physical activity recommendations by the U.S. Surgeon General's Report (ACSM, 2010; U.S. Department of Health and Human Services, 1996). The recommendations amount to moderate to vigorous physical activity for a total of two or more hours per week. All participants were between the age of 18 and 25 years. The university's institutional review board approved the study and all participants provided written informed consent to participate prior to the start of any testing procedures.

Finger Length Measurements

All testing was completed in one session lasting approximately 50 minutes and all sessions took place between 13:00 and 17:00 hours. First, participants' second and fourth finger lengths were measured twice on the ventral surface of each hand by the principal investigator. Direct finger measurements were taken with a stainless steel digital Vernier offset caliper with a reported accuracy of 0.01mm (Model K01-101, Kbd Tools Co., Ltd., Jiangsu, China). Finger lengths were measured from the most proximal basal crease of each finger to the soft tissue tip of the finger. Minimal pressure was exerted with the caliper against the soft tissue of the fingertip in order to avoid distortion of the soft tissue (Manning et al., 1998).

Measurement of Performance Variables and PRs

The MR and FR groups self-reported personal records (PR) for the 800m, 1.5km, 1.6km, 3km, 5km, and 10km race distances. Body composition was assessed using a Tanita SC-331S Body Composition Analyzer (Tanita Corporation of America, Inc., Arlington Heights, Illinois). $\dot{V}O_2$max, RERmax, and ToT were determined with the Bruce Protocol (ACSM, 2010) on a Desmo HP Woodway treadmill (Woodway, Waukesha, WI). A Physio-Dyne, MAX-1 model, computerized gas analyzer with Physio-Dyne Metabolic System software (Physio-Dyne Instruments Corp., Quogue, NY) was used for the gas analysis and the system was calibrated before each testing session according to the manufacturer's guidelines. Two out of four criteria had to be satisfied for the achievement of $\dot{V}O_{2max}$. These criteria included (1) a plateau in

$\dot{V}O_{2max}$ signified by a failure of $\dot{V}O_2$ to increase despite increases in work rate or an increase in $\dot{V}O_2$ of $\overline{x}$ – 2SD or less between the last two measurements, whereas $\overline{x}$ is the average increase in $\dot{V}O_2$ from measurement to measurement and SD is the standard deviation of increases in $\dot{V}O_2$ (Taylor, Buskirk, & Henschel, 1955), (2) a respiratory exchange ratio (RER) of ≥ 1.15 (Issekutz, Birckhead, & Rodahl, 1962; Issekutz & Rodahl, 1961), (3) a heart rate within 95% of the age adjusted maximal heart rate as determined through the following formula: 208 – 0.70*age (Tanaka, Monahan, Douglas, & Seals, 2001), and (4) a maximal rating of 9 or 10 on the OMNI Rating of Perceived Exertion Scale (Utter et. al., 2004). The treadmill test ended at the point of voluntary exhaustion of the participant. Breath by breath gas samples were automatically averaged over 30 seconds by the computerized metabolic cart. $\dot{V}O_{2max}$ was recorded as the highest 30s average $\dot{V}O_2$ and expressed in $ml*kg^{-1}*min^{-1}$. The RER was calculated from measurements of $\dot{V}O_2$ and $\dot{V}CO_2$. The highest RER value recorded was considered the RER_{max}. RE_{abs} was determined as the average of two $\dot{V}O_2$ ($ml*kg^{-1}*min^{-1}$) measurements with one measurement immediately prior to and one measurement immediately after the 8:00 minute mark during the treadmill test. RE_{rel} was expressed as the percentage of RE_{abs} in relation to the $\dot{V}O_{2max}$.

Statistical Analyses

Statistical analyses were performed with the Statistical Package for the Social Sciences v.19.0 (SPSS, Inc., Chicago, IL). One-way ANOVA and Tukey's post-hoc tests were used to test for differences between groups in all variables. Linear contrasting was performed to test for gender differences in measures of digit ratio. Pearson's *r* correlation coefficient was used to examine relationships between variables by group. First order partial correlation analyses were performed individually by group to remove the effects of a third variable from the relationships between measures of digit ratio and dependent variables. The variable correlating most strongly to digit ratios and the dependent variable was chosen as the control variable. Results were accepted as significant at $p \leq 0.05$.

Results

Intraobserver Reliability of Finger Length Measurements and 2D:4D Descriptive Statistics

The intraclass correlation coefficient (*ICC*) between all first and second finger length measurements of all participants combined was 0.996 ($d.f.(1) = 215$, $d.f.(2) = 215$; $p \leq 0.001$). This intraobserver reliability score is consistent with the literature (Voracek & Dressler, 2007) and we concluded that differences in finger lengths are not attributable to measurement error. Thus, the average of the first

and second finger length measurements was used in the calculation of digit ratios. The descriptive statistics for digit ratios by group and total sample are summarized in Table A-2.

One-way ANOVA revealed no differences between the groups in right 2D:4D ($F(3, 50) = 0.56$, $p = 0.645$), left 2D:4D ($F(3, 50) = 1.01$, $p = 0.396$), or R-L 2D:4D ($F(3, 50) = 0.83$, $p = 0.487$). No overall gender difference in right 2D:4D ($t(50) = 1.27$, $p = 0.208$), left 2D:4D ($t(50) = 1.16$, $p = 0.252$), or R-L 2D:4D ($t(50) = 0.55$, $p = 0.583$) was found using linear contrasting. However, digit ratios differed in the expected with males having lower values than females. Runners had the same or higher right and R-L 2D:4D but lower left 2D:4D than their gender-matched sedentary counterparts.

Other Descriptive Statistics and Group Differences

The descriptive statistics of age, height, weight, body mass index (BMI), percentage of body fat (%BF), ToT, $\dot{V}O_{2max}$, RER_{max}, RE_{abs}, and RE_{rel} by group and total sample are summarized in Table A-3. One-way ANOVA revealed no difference between the groups in terms of age ($F(3, 50) = 2.61$, $p = 0.062$), RE_{abs} ($F(3, 50) = 1.98$, $p = 0.129$), and RER_{max} ($F(3, 50) = 2.54$, $p = 0.067$). There were anticipated significant differences between groups in height ($F(3, 50) = 12.24$, $p < 0.001$), weight ($F(3, 50) = 11.21$, $p < 0.001$), %BF ($F(3, 50) = 41.78$, $p < 0.001$), BMI ($F(3, 50) = 5.96$, $p = 0.001$), ToT ($F(3, 50) = 122.50$, $p < 0.001$), $\dot{V}O_{2max}$ ($F(3, 50) = 29.94$, $p < 0.001$), and RE_{rel} ($F(3, 50) = 49.39$, $p < 0.001$).

Tukey's post-hoc tests revealed the following: There was a difference in height between male groups and female groups (all $p \leq 0.004$). Sedentary men were significantly heavier than all other participants (all $p \leq 0.001$). There were no differences in weight between MR, FR, and FC ($p = 0.808$). The percentage of body fat (%BF) was significantly lower in MR than in all other groups ($p \leq 0.001$), and FC had significantly higher %BF than all other groups ($p = \leq 0.016$). %BF did not differ between MC and FR ($p = 0.496$). MC showed a higher BMI than MR ($p = 0.001$) and FR ($p = 0.032$). There was no difference in BMI between FC and all other groups ($p \geq 0.156$). ToT of MR was significantly greater than ToT of FR ($p < 0.001$), ToT of FR was significantly greater than ToT of MC ($p < 0.001$), and ToT of MC was significantly greater than ToT of FC ($p = 0.009$). MR achieved greater $\dot{V}O_{2max}$ values than all others ($p < 0.001$) while FR achieved greater $\dot{V}O_{2max}$ values than FC ($p = 0.003$). $\dot{V}O_{2max}$ values of MC were not different from $\dot{V}O_{2max}$ values of FR ($p = 0.304$) and FC ($p = 0.264$). MR displayed the lowest RE_{rel}, followed by FR ($p < 0.001$), which were followed by MC ($p = 0.002$), while FC displayed the highest RE_{rel} ($p = 0.014$). MR presented with significantly lower RER_{max} than FR ($p = 0.049$). No other group differences were present in RER_{max} ($p \geq 0.249$).

The descriptive statistics for PRs by race distance and group are summarized in Table A-4. As expected, the male runners had consistently faster PRs than the female runners (800m, $F(1, 22) = 54.77$, $p \leq$

0.001; 1.5km, $F(1, 18) = 62.12$, $p \leq 0.001$; 1.6km, $F(1, 16) = 61.05$, $p \leq 0.001$; 3km, $F(1, 17) = 69.02$, $p \leq 0.001$; 5km, $F(1, 22) = 76.85$, $p \leq 0.001$; 10km, $F(1, 14) = 63.98$, $p \leq 0.001$).

Correlations for MC

Table A-5 shows the correlations between digit ratios, height, weight, and age, and performance variables in MC. There was no association between digit ratios and $\dot{V}O_{2max}$. Right and left 2D:4D related negatively to RE_{abs} with the same trend in R-L 2D:4D. Right and R-L 2D:4D related negatively to RE_{rel} with the same trend in left 2D:4D. Contrary to our hypotheses, RER_{max} and ToT showed a trend to be positively related to right and left 2D:4D. Weight showed a trend to be negatively related to $\dot{V}O_{2max}$, RER_{max}, and ToT, and positively to RE_{rel}. Age and RE_{rel} showed a negative association with the same trend between age and RE_{abs}. Age also shared positive relationships (sig. 2-tailed) with right 2D:4D ($r = 0.55$, $p = 0.052$), left 2D:4D ($r = 0.29$, $p = 0.329$), and R-L 2D:4D ($r = 0.49$, $p = 0.087$). We used first order partial correlation analyses to remove the effects of age on the relationships between digit ratios and measures of running economy. The relationships between right 2D:4D and RE_{abs} ($r = -0.45$, $p = 0.071$) as well as right 2D:4D and RE_{rel} ($r = -0.42$, $p = 0.086$) did not remain significant. The relationship between left 2D:4D and RE_{abs} ($r = -0.42$, $p = 0.085$) as well as RE_{rel} ($r = -0.28$, $p = 0.192$) and the relationship between R-L 2D:4D and RE_{rel} ($r = -0.25$, $p = 0.215$) were also weakened.

Correlations for FC

Table A-6 shows the correlations between digit ratios, height, weight, and age, and performance variables in FC. There was no association between digit ratios and $\dot{V}O_{2max}$. Height and weight showed a trend to be negatively associated to $\dot{V}O_{2max}$. Consistent with the trend in MC, there was a positive correlation between left 2D:4D and RER_{max}. The relationship between left 2D:4D and RE_{rel} was positive and marginally significant. There was also a strong trend for height and weight to share a negative relationship with RE_{abs} and ToT. Age did not show a strong trend to be related to any of the performance variables. Weight showed a stronger association (sig. 2-tailed) with right 2D:4D ($r = -0.30$, $p = 0.284$) and left 2D:4D ($r = -0.40$, $p = 0.136$) than height did (right 2D:4D, $r = -0.15$, $p = 0.588$; left 2D:4D, $r = -0.14$, $p = 0.623$). Therefore, we used first order partial correlation analyses to remove the effects of weight on the relationships between digit ratios and performance variables (Table A-7).

After controlling for weight, right and left 2D:4D had become more strongly inversely related to $\dot{V}O_{2max}$, RE_{abs}, and ToT. Right and left 2D:4D and RER_{max} as well as RE_{rel} were more strongly positively related. The correlations between left 2D:4D and $\dot{V}O_{2max}$, RER_{max}, RE_{abs}, and RE_{rel} reached statistical significance. The correlations between R-L 2D:4D and performance variables were unchanged. The

correlations between right and left 2D:4D and performance variables were in the expected direction except for the correlations between right and left 2D:4D and RER_{max}.

Correlations for MR

Table A-8 shows the correlations between digit ratios, height, weight, and age, and performance variables in MR. Slight positive associations between measures of digit ratio and $\dot{V}O_{2max}$ emerged. There was a strong trend for negative correlations between right and left 2D:4D and RER_{max}. R-L 2D:4D also seemed to be inversely related to RER_{max}. R-L 2D:4D shared weak negative correlations with RE_{abs} and RE_{rel}. The same trend also appeared between right and left 2D:4D and RE_{rel}. There were also weak negative relationships between right and left 2D:4D and ToT. Age and RER_{max} showed a trend towards a positive relationship. Height and weight shared a significant positive relationship with $\dot{V}O_{2max}$ and RE_{abs}. Weight was also positively associated with ToT, while the association between height and ToT displayed the same trend. Height and weight also appeared to be negatively associated to RER_{max} and RE_{rel}. However, height (sig. 2-tailed: right 2D:4D, $r = 0.10$, $p = 0.743$; left 2D:4D, $r = 0.07$, $p = 0.826$; R-L 2D:4D, $r = 0.11$, $p = 0.710$) and weight (sig. 2-tailed: right 2D:4D, $r = 0.05$, $p = 0.875$; left 2D:4D, $r = -0.01$, $p = 0.980$; R-L 2D:4D, $r = 0.13$, $p = 0.667$) were not associated with digit ratios.

Table A-9 shows the correlations between digit ratios, performance variables, and age, and PRs in MR. There are consistent positive relationships between measures of digit ratio and PRs except for the relationships between left 2D:4D and 1.5km PRs as well as R-L 2D:4D and 10km PRs. The following relationships were statistically significant: Right and R-L 2D:4D with 800m PRs, R-L 2D:4D with 1.5km PRs, and right and left 2D:4D with 5km PRs. The relationships between $\dot{V}O_{2max}$ and 1.5km, 1.6km, and 3km PRs were negative but did not reach significance. RER_{max} generally showed a strong negative association to PRs with the association between RER_{max} and 5km PRs being significant. Relationships between RE_{abs} and PRs showed a weak tendency to be negative, however, the association of RE_{abs} with 10km PRs was significant and positive. Age seemed to be negatively related to PRs while reaching statistical significance when correlated with 3km and 5km PRs. Additionally, age showed trends to related negatively to right 2D:4D (sig. 2-tailed, $r = -0.22$, $p = 0.467$) and left 2D:4D (sig. 2-tailed, $r = -0.27$, $p = 0.377$). We used first order partial correlation analyses to remove the effects of age, and found an average change of $r = -0.02$ (range: $-0.12 \leq r \leq 0.08$) in the strength of the relationships between all measures of digit ratios and PRs combined. The correlations (sig. 1-tailed) between right 2D:4D and 5km PRs ($r = 0.53$, $p = 0.040$), R-L 2D:4D and 800m PRs ($r = 0.67$, $p = 0.009$) as well as 1.5km PRs ($r = 0.75$, $p = 0.010$) remained significant. Thus, age did not seem to moderate the relationship between digit ratios and endurance running performance. However, RER_{max} showed consistent trends to be negatively related to measures of digit ratios and PRs across all distances. First order partial correlation analyses were used to

remove the effects of RER_{max} on the relationship between digit ratios and PRs and we found that the directions of the relationships between digit ratios and PRs were generally reversed. Except for the relationships (sig. 1-tailed) between left 2D:4D and 800m PRs ($r = 0.01$, $p = 0.486$) as well as R-L 2D:4D and 10km PRs ($r = 0.60$, $p = 0.059$), all relationships between digit ratios and PRs ranged between $r = -0.05$ and $r = -0.71$. The relationship between right 2D:4D and 1.6km PRs ($r = 0.71$, $p = 0.016$) reached statistical significance.

Correlations for FR

Table A-10 shows the correlations between digit ratios, height, weight, and age, and performance variables in FR. Consistent with our findings in MR and contrary to our hypotheses, all measures of digit ratios showed a trend to relate positively to $\dot{V}O_{2max}$. Also consistent with MR, we found a trend for negative relationships between measures of digit ratios and RER_{max} as well as RE_{rel}. Digit ratios seemed to relate positively to RE_{abs} and ToT. In general, all relationships between weight and performance variables where reversed in FR compared to MR. Whereas weight was positively related to $\dot{V}O_{2max}$ in MR, the trend between these variables was negative in FR. Weight and RER_{max} shared a significant positive relationship and weight and RE_{abs} shared a significant negative relationship in FR. Moreover, $\dot{V}O_{2max}$ and ToT seemed to relate negatively to weight. There were weak trends for right 2D:4D (sig. 2-tailed, $r = -0.22$, $p = 0.481$) and left 2D:4D (sig. 2-tailed, $r = -0.31$, $p = 0.298$) to relate negatively to weight. There was no relationship between R-L 2D:4D and weight ($r = 0.08$, $p = 0.794$). We used first order partial correlation analyses to remove the effects of weight on the relationships between right and left 2D:4D and performance variables. The relationships between right and left 2D:4D and performance variables were weakened after controlling for weight except for the relationships between right and left 2D:4D and RE_{rel}, which did not change. The relationships between right and left 2D:4D and $\dot{V}O_{2max}$ changed by $r = -0.06$ and $r = -0.10$, respectively. The relationships between right and left 2D:4D and RER_{max} changed by $r = 0.11$ and $r = 0.18$, respectively. The relationships between right and left 2D:4D and RE_{abs} changed by $r = -0.05$ and $r = -0.16$, respectively, and the relationships between right and left 2D:4D and ToT changed $r = -0.09$ and $r = -0.16$, respectively.

Table A-11 shows the correlations between digit ratios, performance variables, and age, and PRs in FR. The relationships between all measures of digit ratios and PRs were highly inconsistent, with nine relationships being positive and nine relationships being negative. The relationship between R-L 2D:4D and 10km PRs was, contrary to our hypothesis, negative and the only one which reached statistical significance. Interestingly, the relationship between R-L 2D:4D and 10km PRs was also one of only two negative relationships among all relationships between digit ratios and PRs in MR. In FR, $\dot{V}O_{2max}$ showed strong negative relationships with PRs in all distances, which failed to reach statistical significance only in 3km and 10km PRs. Strong trends in terms of positive relationships between RER_{max} and PRs were also

present. The relationship between RER_{max} and 1.6km PRs was highly significant. RE_{abs} was negatively associated with PRs, reaching marginal significance in 800m, 1.6km, and 5km PRs. RE_{rel} and PRs across all distances shared positive relationships which failed to reach significance only in 3km and 10km PRs. ToT was significantly and negatively correlated to PRs in all distances. The latter were the strongest and most consistent correlations found in this study. As indicated above, $\dot{V}O_{2max}$ and RE_{abs} showed the strongest associations to measures of digit ratio. Moreover, $\dot{V}O_{2max}$ and RE_{abs} shared 83% of variance (r = 0.91, $r < 0.001$) in this sample. Hence, we used first order partial correlation analyses to remove the effects of $\dot{V}O_{2max}$ only from the relationship of digit ratios to PRs (Table A-12).

After controlling for $\dot{V}O_{2max}$, all relationship between digit ratios and PRs changed in the positive direction, except for the correlation between R-L 2D:4D and 10km PRs, which became a stronger negative relationship reaching statistical significance. The positive correlations between right and left 2D:4D and 1.5km PRs and the relationship between left 2D:4D and 5km PRs reached significance.

Discussion

Comparison of group differences in 2D:4D digit ratios in this investigation supports 2D:4D as a sexually dimorphic trait, however, the data do not indicate that endurance runners have lower ratios than their gender-matched sedentary counterparts.

We interpret our results in terms of the associations of right and left 2D:4D with prenatal testosterone concentrations and R-L 2D:4D with testosterone sensitivity. Unless otherwise indicated, the interpretations of these results are based on the correlations which are uncontrolled for a third variable. In the sedentary samples, right and left 2D:4D showed very weak trends, at best, to be negatively related to $\dot{V}O_{2max}$. After controlling for weight, the negative correlation between left 2D:4D and $\dot{V}O_{2max}$ reached significance in FC. In MR and especially in FR, right and left 2D:4D showed a slightly stronger trend but towards a positive relationship with $\dot{V}O_{2max}$. It appears that total body weight plays a mediating role in these positive relationships in FR as they were weakened after weight was controlled for. R-L 2D:4D and $\dot{V}O_{2max}$ shared a weak positive relationship across all samples. In summary, we cannot support the theory that prenatal testosterone or testosterone sensitivity exert organizational effects on the human physiology favoring $\dot{V}O_{2max}$. Our results stand in contrast to Hill's et al. (2012) results who found a negative relationship between R-L 2D:4D and $\dot{V}O_{2max}$. Hill et al. (2012), concluded that testosterone sensitivity, specifically CAG repeat polymorphism of the AR, may be the mediating factor between low R-L 2D:4D and $\dot{V}O_{2max}$ in conjunction with training as the testosterone stimulus. As the relationship of right and left 2D:4D with $\dot{V}O_{2max}$ was in the hypothesized direction in FC but not in MR and FR, and because the relationship between R-L 2D:4D and $\dot{V}O_{2max}$ was in the positive direction across all samples, it seems that

endurance training causes changes in $\dot{V}O_{2max}$ through pathways more powerful than CAG triplet repeat polymorphism provided the number of CAG triplet repeats is in the normal range. The reversal in the direction of the relationship of right and left 2D:4D to $\dot{V}O_{2max}$ from the sedentary population to the runners seems to indicate that training effects outweigh the effects of prenatal testosterone concentrations on the cardiovascular system.

RER_{max} related negatively to right, left, and R-L 2D:4D in the runners and negatively to R-L 2D:4D in the sedentary college students. Right and left 2D:4D showed a weak positive and somewhat inconsistent relationship to RER_{max} in the sedentary participants. After controlling for weight, the positive correlation between left 2D:4D and RER_{max} in FC reached statistical significance. The negative correlations between R-L 2D:4D and RER_{max} are consistent with Hill's et al. (2012) report of a negative relationship between maximal lactate concentrations and R-L 2D:4D, as RER_{max} is an indicator of the amount of buffered lactic acid. The underlying mediators of the relationship between R-L 2D:4D and peak lactate concentrations as well as lactic acid buffering capacity remain elusive. Testosterone sensitivity, specifically CAG triplet repeat length of which R-L 2D:4D seems to be a biomarker, has been linked to improvements in $\dot{V}O_{2max}$ (Wang et al., 2010) and improvements in $\dot{V}O_{2max}$ are linked to increased capillarisation of skeletal muscle and increased ratios of Type I muscle fibers both of which facilitate the clearance of lactic acid and lead to lower lactate thresholds (Bassett & Howley, 2000; Ivy, Withers, Van Handel, Elger, & Costill, 1980). Additionally, endurance training has been shown to increase the ratio of Type I to Type II muscle fibers (Howald, Hoppeler, Claassen, Mathieu, & Straub, 1985). Thus, lower R-L 2D:4D should be associated with lower RER_{max} and maximal lactate concentrations. Hence, the negative association between R-L 2D:4D and RER_{max} in endurance runners is somewhat counterintuitive. It seems that the factors controlling lactate accumulation respond to testosterone stimulation independently of the factors causing improvements in oxygen uptake capacities. Moreover, the relationships between right and left 2D:4D and RER_{max} seemed to be reversed by endurance training, whereas these relationships are in the expected direction in relative long term inactivity.

There was a trend in women for all measures of digit ratios, except for left 2D:4D in FC, to relate positively to RE_{abs}. In men, digit ratios, except for left 2D:4D in MR, generally related negatively to RE_{abs} with significant relationships between right and left 2D:4D and RE_{abs} in MC. Thus, the associations between measures of digit ratios and RE_{abs} were somewhat inconsistent in MR and FC. There were strong associations between RE_{abs} and $\dot{V}O_{2max}$ (MR, $r = 0.72$, $p = 0.003$; MC, $r = 0.48$, $p = 0.051$; FR, $r = 0.91$, $p < 0.001$; FC, $r = 0.93$, $p < 0.001$). Hence, the relationships between digit ratios and RE_{abs} should be similar to the relationships between digit ratios and $\dot{V}O_{2max}$ with stronger similarities in women compared to men. Indeed, in FC and FR the relationships between measures of digit ratio and RE_{abs} were in the same direction and generally stronger compared with the relationships between measures of 2D:4D and $\dot{V}O_{2max}$. The

relationships between measures of 2D:4D and RE_{abs} were in the expected direction in MC and in the opposite direction in MR. It appears that prenatal testosterone is associated with higher submaximal oxygen consumption in sedentary men but not women, and that prenatal testosterone is not associated with submaximal oxygen consumption in male runners. Age, however, seems to partially mediate this relationship in MC, whereas younger ages seem to be associated with lower 2D:4D and poor running economy (higher RE_{abs} and RE_{rel}). In female runners, prenatal testosterone seems to be associated with lower submaximal oxygen consumption. This finding is contrary to our hypothesis, as total muscle mass should mediate this relationship as it is positively associated with testosterone stimulation (Hartgens & Kuipers, 2004) and submaximal oxygen consumption (Morgan, Tseh, Caputo, Graig, Keefer, & Martin, 1999). This theory is supported by the positive relationship among $\dot{V}O_{2max}$, RE_{abs}, and weight in MR as their low average %BF suggests that differences in weight are attributable to differences in lean body mass and height.

The trends of the relationships between digit ratios and RE_{rel} were similar to the trends between digit ratios and RE_{abs}, whereas more negative and stronger relationships were found in men. In MC, right and R-L 2D:4D correlated significantly and negatively with RE_{rel}. These same relationships were second strongest among MR. FR also displayed weak negative associations between digit ratios and RE_{rel}. The relationship between digit ratios and RE_{rel} were trending in a positive direction only in right and left 2D:4D of FC, whereas the correlation between left 2D:4D and RE_{rel} reach statistical significance after controlling for weight. It appears that lower digit ratios and thus higher prenatal testosterone levels are associated with higher submaximal oxygen consumption relative to $\dot{V}O_{2max}$. This finding is in agreement with the positive correlations found between digit ratios and $\dot{V}O_{2max}$ in the runners as lower RE_{rel} partially depends on high $\dot{V}O_{2max}$ values. However, these results are another contraindicator of the positive influence of prenatal testosterone on the aerobic fitness of the cardiovascular system.

We found a trend for positive relationships between digit ratios and ToT in MC and FR. In FC and MR, there was a very weak trend for negative relationships between these variables. The relationship between digit ratios and ToT are not consistent with Hill et al. (2012), as they found a negative relationship between R-L 2D:4D and running velocity at $\dot{V}O_{2max}$. However, we found very small positive correlations between these variables in FR and MC and a weak negative correlation in FC and MR. Thus, the influence of prenatal testosterone and testosterone sensitivity on cardiovascular fitness may be highly gender and training specific.

Consistent with past reports, we found positive relationships between measures of digit ratios and PRs in 800m through 10km in MR. In fact, partial correlations analyses controlling for RER_{max} indicated that RER_{max} may be a physiological factor mediating the relationship between digit ratios and endurance running performance in male endurance runners. While 2D:4D seemed to be more strongly associated with

PRs than performance variables in MR, the opposite was true in FR. Moreover, the relationship between performance variables and PRs in FR were all in the expected direction and consistent with the literature (Daniels & Daniels, 1992). After controlling for $\dot{V}O_{2max}$, correlations between digit ratios and PRs, became more positive in FR indicating that $\dot{V}O_{2max}$ is a potential confounder of positive relationships between digit ratios and PRs. This finding does not support the positive effects of prenatal testosterone and testosterone sensitivity on maximal oxygen uptake capacity as the mediator of the relationship between prenatal testosterone stimulation and endurance running performance. It seems that positive associations between digit ratios and endurance running performance are mediated by other factors such as lactic acid buffering capacity. However, R-L 2D:4D and 10km PRs shared a negative relationship especially in FR, even after controlling for $\dot{V}O_{2max}$, indicating that higher R-L 2D:4D and thus lower testosterone sensitivity are associated with better endurance running performance as race distance increases. Overall, these findings, in part, support Manning's et al. (2007) report of a positive influence of prenatal testosterone on endurance running performance. It appears possible that prenatal testosterone exerts its positive effects on endurance performance by increasing the tolerance for lactate accumulation and not by increasing maximal aerobic capacities. Moreover, these relationships may be confounded or reversed when considering proxies of testosterone sensitivity (R-L 2D:4D) and longer race distances as indicated by the negative relationships between R-L 2D:4D and 10km PRs.

In the present investigation, all measures of digit ratio relate to endurance running performance, whereas lower ratios are associated with better performance. In female endurance runners, however, this only seems true after controlling for the relatively weak relationship of $\dot{V}O_{2max}$ to digit ratio and the strong relationship of $\dot{V}O_{2max}$ to race performance. Thus the relatively strong relationship between $\dot{V}O_{2max}$ and endurance running performance in FR masks the relationship of digit ratios to endurance running performance. This finding also underlines possible weaknesses of the associations between digit ratios and endurance running performance as well as digit ratios and $\dot{V}O_{2max}$ in female runners. It appears that prenatal testosterone does not have beneficial effects on adult maximal oxygen uptake capacities and, in contrast to previous reports (i.e. Wang et al, 2010) increases in $\dot{V}O_{2max}$ following training do not seem to be mediated by effects of testosterone sensitivity which means that endurance training further weakens the relationship between digit ratios and $\dot{V}O_{2max}$.

It seems also plausible that anthropometric measures which are correlates of RE might explain part of the relationship of 2D:4D to endurance running performance. Bone development in lower extremities may be predetermined by prenatal testosterone because of the effects of prenatal testosterone on finger bones.

It should be noted that controlling for age in MC weakened the relationships between digit ratios and performance variables, indicating that age may moderate those relationships to some degree. In MR,

controlling for age had no effect on these relationships. However, we recommend that future investigations of the relationships between digit ratios and physiological, anthropometric, or athletic performance measures limit the age of participants to a window of less than seven years as digit ratios and the aforementioned measures seem to change with age. Controlling for weight in FC strengthened the relationships between digit ratios and performance variables, indicating that total body weight may confound these relationships in inactive women. In contrast, body weight, to a small degree, seems to mediate these same relationships in female runners.

The generalizability of our results is limited due to sample sizes which were too small to allow for more consistent and significant relationships between measures of digit ratio, performance variables, and PRs. It is also possible that differences in training status between first year and fourth year collegiate runners accounted for more variance in $\dot{V}O_{2max}$ and PRs than digit ratios. Moreover, some runners were tested towards the beginning, some towards the middle, and some towards the end of their respective competitive season. Thus, differences in training status increase the error variance in performance variables. Additionally, the sampling of female collegiate distance runners from one mid-sized southeastern university and one small southeastern college presents a limitation as the training stimuli between the runners from these two institutions are not equal.

Conclusion

It seems that digit ratios and $\dot{V}O_{2max}$ may be inversely related only in sedentary populations, while this relationship may be confounded by total body weight in sedentary women. Endurance training may change the direction of relationship between digit ratios and $\dot{V}O_{2max}$. We found that R-L 2D:4D is negatively associated with RER_{max}. However, the mechanisms moderating the association of testosterone sensitivity to lactate accumulation and buffering capacity remain unclear. Prenatal testosterone seems to be associated with lower submaximal oxygen consumption in female runners and sedentary counterparts while it is associated with higher submaximal oxygen consumption in sedentary men. There is no apparent relationship between these relationships in male runners. Except in sedentary women, prenatal testosterone appears to relate to higher submaximal oxygen consumption relative to $\dot{V}O_{2max}$ indicating poorer cardiovascular fitness. ToT related positively to R-L 2D:4D in all groups except in sedentary women. Thus, testosterone sensitivity does not seem to have positive effects on physiological determinants of cardiovascular endurance.

The consistent and moderately strong relationships between digit ratios and endurance running performance do not seem to be mediated by $\dot{V}O_{2max}$ in female runners. Running economy, although related to $\dot{V}O_{2max}$, appears to be more strongly associated to digit ratios. However, the direction of the relationship seems to depend on gender and training status. We found stronger evidence for a relationship of lactate

accumulation and buffering capacity to digit ratios. The association between 2D:4D and endurance running performance may be mediated by the capacity to buffer and/or clear lactic acid in male runners. We recommend the investigation of the relationships among lactate threshold, endurance running performance and 2D:4D. Weight and age seem to partially mediate or confound the relationships between digit ratios and performance variables depending on gender and training status and may thus need to be controlled for when comparing the relationships between digit ratios and physiological performance variables among groups of differing body weights and ages.

We cannot support prenatal testosterone and testosterone sensitivity as a correlate of good cardiovascular fitness in terms of $\dot{V}O_{2max}$ and ToT except in sedentary women after controlling for weight. In highly trained runners, however, prenatal testosterone and testosterone sensitivity do relate to improved endurance running capabilities, however, this association does not seem to be moderated by absolute aerobic capacities ($\dot{V}O_{2max}$) but rather by lactate accumulation and the capacity to buffer it.

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APPENDIX A:

Tables

Table A-1. 2D:4D in Individuals With CAIS and Controls (Mean ± SD).

	CAIS (n = 16)	Control Women (n = 90)	Control Men (n = 66)
Right 2D:4D	0.972 ± 0.032	0.969 ± 0.037	0.952 ± 0.033
Left 2D:4D	0.972 ± 0.041	0.972 ± 0.037	0.960 ± 0.033

Source: Berenbaum et al. (2009). Fingers as a marker of prenatal androgen exposure. *Endocrinology, 150,* 5119-5124.

Table A-2. Descriptive Statistics of Digit Ratios by Group and Total Sample (Mean ± SD).

	MC (n = 13)	FC (n = 15)	MR (n = 13)	FR (n = 13)	Total (n = 54)
Right 2D:4D	0.972 ± 0.034	0.986 ± 0.030	0.973 ± 0.039	0.984 ± 0.038	0.979 ± 0.035
Left 2D:4D	0.975 ± 0.028	0.985 ± 0.024	0.966 ± 0.031	0.974 ± 0.032	0.975 ± 0.029
R-L 2D:4D	-0.002 ± 0.022	0.001 ± 0.025	0.007 ± 0.016	0.010 ± 0.022	0.004 ± 0.022

Table A-3. Descriptive Statistics of Age, Height, Weight, Body Mass Index (BMI), Percentage of Body Fat (%BF), and Performance Variables by Group and Total Sample (Mean ± SD).

	MC (n = 13)	FC (n = 15)	MR (n = 13)	FR (n = 13)	Total (n = 54)
Age [years]	20.2 ± 2.8	18.7 ± 1.0	20.5 ± 1.6	20.2 ± 1.6	19.9 ± 1.9
Height [cm]	176.8 ± 8.1	165.6 ± 6.3	174.5 ± 6.7	164.3 ± 4.7	170.1 ± 8.3
Weight [kg]	76.5 ± 14.6	59.9 ± 8.9	60.5 ± 5.6	57.2 ± 6.2	63.4 ± 11.9
BMI	24.3 ± 3.7	22.0 ± 3.3	19.8 ± 1.1	21.2 ± 2.1	21.8 ± 3.1
%BF	16.4 ± 6.2	25.1 ± 6.6	4.1 ± 1.7	19.2 ± 3.8	16.5 ± 9.2
ToT [sec]	704.8 ± 65.2	596.4 ± 71.8	1183.6 ± 99.8	914.3 ± 103.1	839.9 ± 242.5
$\dot{V}O_{2max}$	39.0 ± 5.1	33.0 ± 6.2	62.5 ± 11.2	44.9 ± 10.4	44.4 ± 13.9
RER_{max}	1.39 ± 0.16	1.34 ± 0.15	1.23 ± 0.13	1.45 ± 0.34	1.35 ± 0.22
RE_{abs}	30.2 ± 3.7	29.1 ± 3.3	32.3 ± 4.0	29.2 ± 4.4	30.2 ± 4.0
RE_{rel} [%]	78.2 ± 10.4	87.9 ± 7.1	52.5 ± 6.4	66.3 ± 7.5	71.5 ± 15.5

Table A-4. Descriptive Statistics of PRs by Race Distance and Group (Mean [min:sec] ± SD [sec]).

	800m	1.5km	1.6km	3km	5km	10km
MR	1:58.3 ± 4.5	3:54.6 ± 7.6	4:19.4 ± 11.3	8:27.6 ± 25.7	14:48.1 ± 48.9	31:04.7 ± 101.4
FR	2:28.3 ± 13.8	5:03.3 ± 26.5	5:30.8 ± 26.2	10:22 ± 34.2	19:10.2 ± 94.1	40:13.4 ± 171.9

Table A-5. Relationships (*r, d.f.* = 11) Between Right 2D:4D, Left 2D:4D, R-L 2D:4D, Height, Weight, and Age, and Performance Variables in Male Sedentary College Students (MC).

	$\dot{V}O_{2max}$		RER_{max}		RE_{abs}		RE_{rel}		ToT	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Right 2D:4D*	0.03	0.452	0.21	0.245	***-0.60***	0.014	***-0.62***	0.012	0.34	0.126
Left 2D:4D*	-0.11	0.360	0.36	0.116	***-0.50***	0.041	-0.39	0.093	0.25	0.201
R-L 2D:4D*	0.20	0.255	-0.12	0.346	-0.32	0.145	***-0.48***	0.048	0.22	0.237
Height**	0.09	0.389	0.06	0.424	-0.06	0.842	-0.14	0.648	0.08	0.788
Weight**	-0.28	0.178	-0.22	0.235	0.04	0.898	0.34	0.264	-0.34	0.261
Age**	0.13	0.678	0.21	0.498	-0.51	0.073	***-0.64***	0.019	0.34	0.256

*sig. 1-tailed; **sig. 2-tailed; significant correlations ($p \leq 0.05$) in ***bold italics***

Table A-6. Relationships (*r, d.f.* = 13) Between Right 2D:4D, Left 2D:4D, R-L 2D:4D, Height, Weight, and Age, and Performance Variables in Female Sedentary College Students (FC).

	$\dot{V}O_{2max}$		RER_{max}		RE_{abs}		RE_{rel}		ToT	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Right 2D:4D*	-0.05	0.437	0.16	0.290	0.06	0.418	0.23	0.216	-0.13	0.323
Left 2D:4D*	-0.22	0.220	***0.45***	0.048	-0.31	0.140	0.46	0.051	0.03	0.455
R-L 2D:4D*	0.15	0.295	-0.24	0.197	0.36	0.103	-0.12	0.339	-0.19	0.255
Height**	-0.39	0.153	-0.08	0.777	-0.51	0.065	0.25	0.388	-0.46	0.082
Weight**	-0.46	0.088	0.12	0.665	-0.43	0.124	0.18	0.546	-0.41	0.127
Age**	-0.20	0.482	0.24	0.388	-0.15	0.621	-0.21	0.479	-0.21	0.447

*sig. 1-tailed; **sig. 2-tailed; significant correlations ($p \leq 0.05$) in ***bold italics***

Table A-7. First Order Partial Correlations Between Right 2D:4D, Left 2D:4D, and R-L 2D:4D, and Performance Variables Controlled for Weight in Female Sedentary College Students (FC).

	$\dot{V}O_{2max}$		RER_{max}		RE_{abs}		RE_{rel}		ToT	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Right 2D:4D*	-0.21	0.234	0.20	0.244	-0.08	0.401	0.30	0.161	-0.29	0.158
Left 2D:4D*	***-0.49***	0.037	***0.55***	0.022	***-0.59***	0.018	***0.59***	0.018	-0.16	0.292
R-L 2D:4D*	0.19	0.263	-0.24	0.201	0.41	0.080	-0.13	0.337	-0.19	0.258

*sig. 1-tailed; significant correlations ($p \leq 0.05$) in ***bold italics***

Table A-8. Relationships (*r, d.f.* = 11) Between Right 2D:4D, Left 2D:4D, R-L 2D:4D, Height, Weight, and Age, and Performance Variables in Male Intercollegiate Long Distance Runners (MR).

	$\dot{V}O_{2max}$		RER_{max}		RE_{abs}		RE_{rel}		ToT	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Right 2D:4D*	0.14	0.322	-0.36	0.110	-0.09	0.386	-0.30	0.156	-0.22	0.239
Left 2D:4D*	0.15	0.310	-0.37	0.109	0.06	0.417	-0.18	0.277	-0.28	0.176
R-L 2D:4D*	0.05	0.433	-0.18	0.279	-0.34	0.129	-0.39	0.094	0.02	0.479
Height**	***0.61***	0.027	-0.37	0.212	***0.56***	0.049	-0.35	0.236	0.45	0.126
Weight**	***0.74***	0.004	-0.51	0.077	***0.66***	0.014	-0.45	0.126	***0.56***	0.046
Age**	-0.05	0.862	0.41	0.168	0.05	0.880	0.14	0.650	-0.05	0.868

*sig. 1-tailed; **sig. 2-tailed; significant correlations ($p \leq 0.05$) in ***bold italics***

Table A-9. Relationships Between Right 2D:4D, Left 2D:4D, R-L 2D:4D, Performance Variables, and Age, and PRs in Male Intercollegiate Long Distance Runners (MR).

	800m (*d.f.* = 11)		1.5km (*d.f.* = 8)		1.6km (*d.f.* = 8)		3km (*d.f.* = 10)		5km (*d.f.* = 11)		10km (*d.f.* = 7)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Right 2D:4D*	***0.52***	0.034	0.24	0.255	0.44	0.104	0.43	0.084	***0.54***	0.028	0.42	0.129
Left 2D:4D*	0.35	0.120	-0.26	0.235	0.51	0.067	0.29	0.179	***0.51***	0.037	0.45	0.113
R-L 2D:4D*	***0.59***	0.017	***0.75***	0.006	0.08	0.413	0.24	0.225	0.33	0.136	-0.08	0.419
$\dot{V}O_{2max}$*	-0.06	0.418	-0.19	0.304	-0.25	0.240	-0.18	0.285	-0.02	0.470	0.15	0.349
RER_{max}*	-0.33	0.137	-0.09	0.398	-0.50	0.073	-0.47	0.060	***-0.49***	0.043	-0.52	0.075
RE_{abs}*	-0.28	0.181	-0.22	0.270	0.00	0.499	-0.12	0.350	-0.06	0.418	***0.65***	0.030
RE_{rel}*	-0.17	0.294	0.14	0.350	0.35	0.161	0.16	0.306	-0.03	0.467	0.23	0.277
ToT*	0.07	0.413	-0.14	0.349	-0.30	0.204	-0.13	0.349	-0.16	0.300	-0.21	0.291
Age**	-0.51	0.074	-0.05	0.888	-0.60	0.068	***-0.59***	0.042	***-0.61***	0.026	-0.37	0.324

*sig. 1-tailed; **sig. 2-tailed; significant correlations ($p \leq 0.05$) in ***bold italics***

Table A-10. Relationships (*r, d.f.* = 11) Between Right 2D:4D, Left 2D:4D, R-L 2D:4D, Height, Weight, and Age, and Performance Variables in Female Intercollegiate Long Distance Runners (FR).

	$\dot{V}O_{2max}$		RER_{max}		RE_{abs}		RE_{rel}		ToT	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Right 2D:4D*	0.35	0.123	-0.20	0.259	0.38	0.098	-0.15	0.316	0.16	0.296
Left 2D:4D*	0.27	0.183	-0.20	0.257	0.32	0.146	-0.13	0.338	0.11	0.364
R-L 2D:4D*	0.21	0.251	-0.06	0.430	0.21	0.247	-0.07	0.411	0.13	0.334
Height**	-0.04	0.899	0.23	0.446	-0.17	0.582	-0.19	0.539	0.13	0.672
Weight**	-0.41	0.161	***0.60***	0.032	***-0.65***	0.017	0.00	0.995	-0.48	0.098
Age**	0.30	0.322	-0.11	0.725	0.12	0.703	-0.33	0.275	0.00	0.993

*sig. 1-tailed; **sig. 2-tailed; significant correlations ($p \leq 0.05$) in ***bold italics***

Table A-11. Relationships Between Right 2D:4D, Left 2D:4D, R-L 2D:4D, Performance Variables, and Age, and PRs in Female Intercollegiate Long Distance Runners (FR).

	800m (*d.f.* = 9)		1.5km (*d.f.* = 8)		1.6km (*d.f.* = 6)		3km (*d.f.* = 5)		5km (*d.f.* = 9)		10km (*d.f.* = 5)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Right 2D:4D*	-0.18	0.300	0.23	0.260	0.12	0.387	-0.03	0.473	0.03	0.463	-0.18	0.346
Left 2D:4D*	-0.28	0.205	0.34	0.170	-0.02	0.483	0.17	0.356	0.31	0.177	0.22	0.320
R-L 2D:4D*	0.04	0.449	-0.11	0.378	0.24	0.286	-0.54	0.107	-0.38	0.128	***-0.74***	0.028
$\dot{V}O_{2max}$*	***-0.56***	0.038	***-0.61***	0.032	***-0.73***	0.019	-0.52	0.116	***-0.70***	0.009	-0.67	0.052
RER_{max}*	0.47	0.075	0.55	0.051	***0.82***	0.007	0.20	0.332	0.43	0.093	0.65	0.059
RE_{abs}*	-0.44	0.086	-0.38	0.142	-0.56	0.075	-0.36	0.213	-0.49	0.063	-0.54	0.107
RE_{rel}*	***0.65***	0.015	***0.78***	0.004	***0.86***	0.003	0.59	0.081	***0.75***	0.004	0.61	0.071
ToT*	***-0.75***	0.004	***-0.86***	0.001	***-0.93***	0.000	***-0.93***	0.001	***-0.90***	0.000	***-0.70***	0.040
Age**	-0.10	0.760	0.20	0.585	-0.20	0.640	0.15	0.743	-0.09	0.785	-0.60	0.151

*sig. 1-tailed; **sig. 2-tailed; significant correlations ($p \leq 0.05$) in ***bold italics***

Table A-12. First Order Partial Correlations Between Right 2D:4D, Left 2D:4D, and R-L 2D:4D, and PRs Controlled for $\dot{V}O_{2max}$ in Female Intercollegiate Long Distance Runners (FR).

	800m (*d.f.* = 9)		1.5km (*d.f.* = 8)		1.6km (*d.f.* = 6)		3km (*d.f.* = 5)		5km (*d.f.* = 9)		10km (*d.f.* = 5)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Right 2D:4D*	0.02	0.480	***0.59***	0.047	0.59	0.082	0.19	0.363	0.40	0.123	0.07	0.451
Left 2D:4D*	-0.16	0.334	***0.66***	0.027	0.28	0.272	0.38	0.227	***0.72***	0.009	0.56	0.126
R-L 2D:4D*	0.20	0.295	0.01	0.486	0.58	0.085	-0.52	0.148	-0.33	0.176	***-0.83***	0.020

*sig. 1-tailed; significant correlations ($p \leq 0.05$) in ***bold italics***

APPENDIX B:

Figures

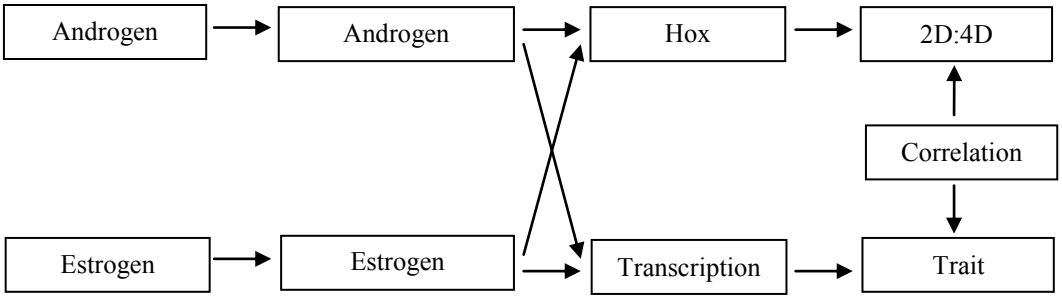

Figure B-1. Sex steroid receptors as link between 2D:4D and postnatal traits.
Adapted from: Forstmeier, W., Müller, J. C., & Kempenaers, B. (2010). A polymorphism in the oestrogen receptor gene explains covariance between digit ratio and mating behavior. *Proceedings of the Royal Society B, 277,* 3353-3361.

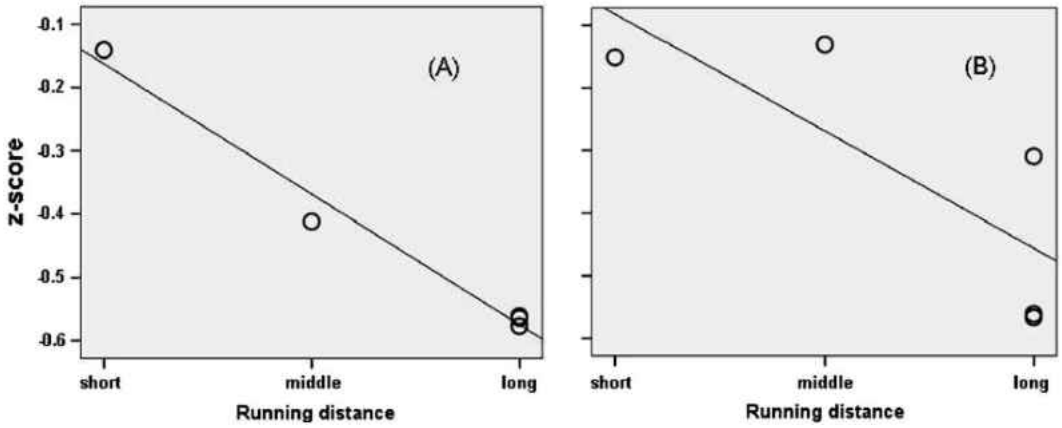

Figure B-2. Standardized correlations of right-hand (A) and left-hand (B) 2D:4D with running performance by distance.
Source: Hönekopp, J. & Schuster, M. (2010). A meta-analysis on 2D:4D and athletic prowess: Substantial relationships but neither hand outpredicts the other. *Personality and Individual Differences, 1,* 4–10.

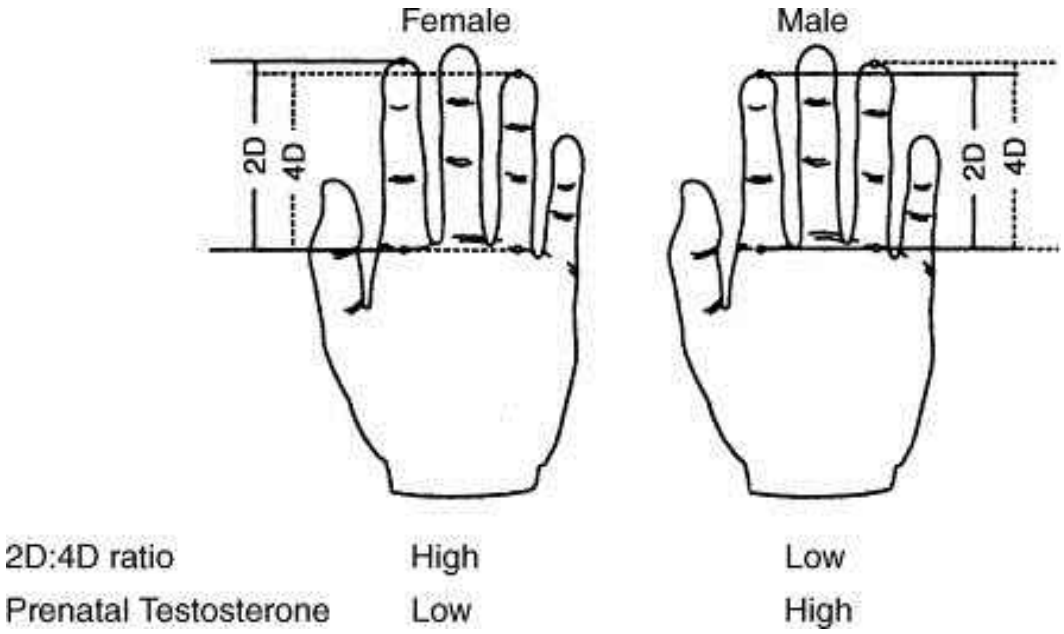

Figure B-3. A schematic representation of the determination of digit ratios. The length of the second digit (2D) and the fourth digit (4D) are measured from the basal crease of the digit to the soft tissue tip. The 2D:4D ratio is calculated by dividing the length of the second digit by that of the fourth
Source: Kallai, J., Csathó, Á., Kövér, F., Makány, T., Nemes, J., Horváth, K., Kovács, N., Manning, J. T., Nadel, L., & Nagy, F. (2005). MRI-assessed volume of left and right hippocampi in females correlates with the relative length of the second and fourth fingers (the 2D:4D ratio). *Psychiatry Research: Neuroimaging, 140*(2), 199-210.

APPENDIX C

Pre-Test Instructions

Eastern Kentucky University
Department of Exercise and Sports Science
223 Moberly Building
521 Lancaster Avenue, Richmond, KY 40475

Digit Ratio (2D:4D) and VO_2max Research Study

Pre-Test Instructions

NOTE: These **pre-test instructions must be strictly adhered to**. If you do not follow these instructions we must exclude you from the study. The purpose of these instructions is to minimize variables that would otherwise render the research results useless.
We ask that you:

1) Do not exercise the day before and the day of the testing session until the testing has been concluded

2) Do not change your exercising routine or usual amount of physical activity from now until the testing session has been concluded

3) Continue taking any medication that you take on a regular basis unless your doctor tells you to stop taking it

4) Do not take any new medication that you have not been taking on a regular basis prior to the testing unless your doctor tells you to do so

5) Do not consume any caffeine, nicotine, or alcohol during the 24 hours prior to the testing session

6) Do not eat anything 2½ hours prior to the testing session

7) Clip the nails of your index and ring fingers on both hands so that the nails do not extend beyond the fleshy part of your fingers

8) Wear comfortable sports apparel such as athletic or running shorts, a t-shirt, and gym shoes or preferably running shoes for the testing session

9) Arrive at Moberly 223 on _____ at _____ for the testing session

You may withdraw from the study at any time for any reason without penalty or any negative consequences. If you have any concerns or questions or you would like to withdraw from the study, please contact Simon Holzapfel at simon_holzapfel@mymail.eku.edu or 423-329-3038.

Thank you for your cooperation and willingness to participate in this study!

Eastern Kentucky University
Department of Exercise and Sports Science
223 Moberly Building
521 Lancaster Avenue, Richmond, KY 40475

Digit Ratio (2D:4D) and VO_2max Research Study

Pre-Test Instructions

NOTE: These **pre-test instructions must be strictly adhered to**. If you do not follow these instructions we must exclude you from the study. The purpose of these instructions is to minimize variables that would otherwise render the research results useless.
We ask that you:

1) Do not exercise and/or run the day before and the day of the testing session until the testing has been concluded

2) Run no more than 5 miles at an easy, conversational pace 2 days prior to the testing session

3) Continue taking any medication that you take on a regular basis unless your doctor tells you to stop taking it

4) Do not take any new medication that you have not been taking on a regular basis prior to the testing unless your doctor tells you to do so

5) Do not consume any caffeine, nicotine, or alcohol during the 24 hours prior to the testing session

6) Do not eat anything 2½ hours prior to the testing session

7) Clip the nails of your index and ring fingers on both hands so that the nails do not extend beyond the fleshy part of your fingers

8) Wear comfortable sports apparel such as athletic or running shorts, a t-shirt, and gym shoes or preferably running shoes for the testing session

9) Arrive at Moberly 223 on _____ at _____ for the testing session

You may withdraw from the study at any time for any reason without penalty or any negative consequences. If you have any concerns or questions or you would like to withdraw from the study, please contact Simon Holzapfel at simon_holzapfel@mymail.eku.edu or 423-329-3038.

Thank you for your cooperation and willingness to participate in this study!

Eastern Kentucky University
Department of Exercise and Sports Science
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521 Lancaster Avenue, Richmond, KY 40475

Digit Ratio (2D:4D) and VO_2max Research Study

Pre-Test Instructions

NOTE: These **pre-test instructions must be strictly adhered to**. If you do not follow these instructions we must exclude you from the study. The purpose of these instructions is to minimize variables that would otherwise render the research results useless.
We ask that you:

1) Do not exercise and/or run the day before and the day of the testing session until the testing has been concluded

2) Run no more than 4 miles at an easy, conversational pace 2 days prior to the testing session

3) Continue taking any medication that you take on a regular basis unless your doctor tells you to stop taking it

4) Do not take any new medication that you have not been taking on a regular basis prior to the testing unless your doctor tells you to do so

5) Do not consume any caffeine, nicotine, or alcohol during the 24 hours prior to the testing session

6) Do not eat anything 2½ hours prior to the testing session

7) Clip the nails of your index and ring fingers on both hands so that the nails do not extend beyond the fleshy part of your fingers

8) Wear comfortable sports apparel such as athletic or running shorts, a t-shirt, and gym shoes or preferably running shoes for the testing session

9) Arrive at Moberly 223 on _____ at _____ for the testing session

You may withdraw from the study at any time for any reason without penalty or any negative consequences. If you have any concerns or questions or you would like to withdraw from the study, please contact Simon Holzapfel at simon_holzapfel@mymail.eku.edu or 423-329-3038.

Thank you for your cooperation and willingness to participate in this study!

APPENDIX D:

Informed Consent Form

Graduate Education and Research
Division of Sponsored Programs
Institutional Review Board

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NOTICE OF IRB APPROVAL
Protocol Number: 12-107
Institutional Review Board IRB00002836, DHHS FWA00003332
Review Type: ☒Full ☐Expedited
Approval Type: ☒New ☐Extension of Time ☐Revision ☐Continuing Review

Principal Investigator:	**Simon Holzpafel**	Faculty Advisor:	**Dr. Matthew Sabin**
Project Title:	**VERDICT (The VO_2max, Endurance Running, and Digit-Ratio Connection to Testosterone) Study**		
Approval Date:	**02/28/2012**	Expiration Date:	**05/04/2013, subject to annual continuing review**
Approved by:	**Dr. Megan Purcell, IRB Chair**		

This document confirms that the Institutional Review Board (IRB) has approved the above referenced research project as outlined in the application submitted for IRB review with an immediate effective date.

Principal Investigator Responsibilities: It is the responsibility of the principal investigator to ensure that all investigators and staff associated with this study meet the training requirements for conducting research involving human subjects, follow the approved protocol, use only the approved forms, keep appropriate research records, and comply with applicable University policies and state and federal regulations.

Consent Forms: All subjects must receive a copy of the consent form as approved with the EKU IRB approval stamp. Copies of the signed consent forms must be kept on file unless a waiver has been granted by the IRB.

Adverse Events: Any adverse or unexpected events that occur in conjunction with this study must be reported to the IRB within ten calendar days of the occurrence.

Research Records: Accurate and detailed research records must be maintained for a minimum of three years following the completion of the research and are subject to audit.

Changes to Approved Research Protocol: If changes to the approved research protocol become necessary, a description of those changes must be submitted for IRB review and approval prior to implementation. Some changes may be approved by expedited review while others may require full IRB review. Changes include, but are not limited to, those involving study personnel, consent forms, subjects, and procedures.

Annual IRB Continuing Review: This approval is valid through the expiration date noted above and is subject to continuing IRB review on an annual basis for as long as the study is active. It is the responsibility of the principal investigator to submit the annual continuing review request and receive approval prior to the anniversary date of the approval. Continuing reviews may be used to continue a project for up to three years from the original approval date, after which time a new application must be filed for IRB review and approval.

Final Report: Within 30 days from the expiration of the project, a final report must be filed with the IRB. A copy of the research results or an abstract from a resulting publication or presentation must be attached. If copies of significant new findings are provided to the research subjects, a copy must be also be provided to the IRB with the final report.

Other Provisions of Approval, if applicable: None

Please contact Sponsored Programs at 859-622-3636 or send email to tiffany.hamblin@eku.edu or lisa.royalty@eku.edu with questions about this approval or reporting requirements.

Kentucky UNBRIDLED SPIRIT

Eastern Kentucky University
Department of Exercise and Sports Science
223 Moberly Building
521 Lancaster Avenue, Richmond, KY 40475

Digit Ratio (2D:4D) and VO_2max Study

Consent to Participate in a Research Study

Why am I being asked to participate in this research?

You are invited to take part in a research study about the effects of fetal testosterone levels and testosterone sensitivity on maximal oxygen uptake during running. You are invited to participate in this research study because you are a healthy individual between 18 and 25 years of age and you have never been a competitive runner. If you take part in this study, you will be one of about 80 people to do so.

Who is doing the study?

The principal investigator is Simon Holzapfel, B.A., at Eastern Kentucky University. He is being guided in this research by Matthew Sabin, Ph.D., Peter Chomentowski, Ph.D., and Lousia Summers, Ph.D. There may be other people on the research team assisting at different times during the study.

What is the purpose of the study?

It has been shown that the ratio of the length of second finger (index finger) to the fourth finger (ring finger) (2D:4D) negatively correlates with prenatal testosterone levels. In other words, the more testosterone an individual has been exposed to as a fetus the shorter the second finger is compared the fourth finger. Prenatal testosterone levels measured by 2D:4D have been reported to positively influence the performance in a variety of sport including long distance running. However, 2D:4D has not been shown to affect maximal oxygen uptake by the only study investigating this relationship. Maximal oxygen uptake is the maximal amount of oxygen your body can consume during maximal efforts and is regarded as one of the best determinants of endurance performance. However, the previously mentioned study reported that the difference between right hand and left hand 2D:4D (right-left 2D:4D) seems to predict maximal oxygen uptake. Evidence supports right-left 2D:4D as an indicator of testosterone sensitivity even after birth. This means that the lower right 2D:4D is compared to left 2D:4D, the more sensitive the genes are to increases in testosterone concentrations. Exercise stimulates testosterone secretion and it has been reported that individuals with high testosterone sensitivity develop larger capacities for oxygen consumption in response to exercise compared to individuals of low testosterone sensitivity. However, no study has used right-left 2D:4D to investigate the effects of long-term endurance training and testosterone sensitivity on maximal oxygen uptake. The influence of prenatal testosterone levels measured via 2D:4D on maximal oxygen uptake in inactive individuals compared with highly trained endurance runners has never been investigated either. This study is designed to examine those relationships.

We are interested in the aforementioned relationships because of the importance of maximal oxygen uptake as an indicator of cardiovascular risks factors and all causes of mortality associated with inactivity. The knowledge gained about the influence of prenatal testosterone levels and testosterone sensitivity on maximal oxygen uptake mediated by endurance exercise could be used in the evaluation of cardiovascular risk factors from a genetic viewpoint. This information would be useful for the prescription of appropriate exercise interventions and outcome expectations. The measurement of 2D:4D would provide health and wellness professionals with a simple and inexpensive filed based method to assess the genetic predisposition to cardiovascular disease and the potential trainability for the reduction of cardiovascular risk factors.

Where is the study going to take place and how long will it last?

The research procedures will be conducted during a single session of approximately 50 minutes at the Harry Moberly building, Room 223, on the campus of Eastern Kentucky University in Richmond, KY.

What will I be asked to do?

You will be asked to:

- Read the *Informed Consent Form* (this document) and sign and date it if you agree to participate in the study
- Fill out a *Demographics Questionnaire*

During this testing session (approx. 50 min):

- Your blood pressure will be measured
- The length of your index (second) and ring (fourth) finger on both hands will be measured with a vernier caliper by two investigators
- Your percent body fat will be measured using the Tanita Body Composition Analyzer. It looks similar to a bathroom scale. You will be asked to step on it barefoot and the device measures your body composition using a small electrical current that you cannot feel.
- Your VO_2max will be measured using the Bruce treadmill protocol. Your maximal oxygen uptake (VO_2max) is a measure of the largest possible amount of oxygen your body can consume during maximal exercise. For this test, you will start out walking on a 10% grade. Every 3 minutes, the speed and incline of the treadmill will increase. You will eventually have to start jogging and/or running. You will be fitted with a mouthpiece, a face mask, a nose clip, and a heart rate monitor. These are necessary devices to measure the amount of oxygen you are consuming during the test and to determine your level of effort. You will be asked to do the best that you can on this test by going as long as possible on the treadmill until you are too tired to continue. At that point, you will grab hold of the bar directly in front of you and the investigator will slow down the treadmill immediately.

Are there reasons why I should not take part in this study?

At this point, we have determined that you are eligible to participate in the study. If you feel uncomfortable with the testing procedures you may withdraw from the study at any time.

What are the possible risks and discomforts?

Abnormal responses during the treadmill test, such as shortness of breath, chest pain, heart rhythm irregularity, collapse requiring treatment and death in young healthy females and males are

rare – expected to occur in less than 1% of people (less than 1 out of 100 people). However, some common risks – expected to occur in 10-25% of people (10-25 out of 100 people) of maximal exercise testing include heavy breathing, dizziness, nose clip discomfort, anxiousness or claustrophobia due to the nose clip or mouthpiece, muscle fatigue and overall fatigue.

You may experience muscle soreness during the hours or days following the treadmill test. Muscle soreness occurs regardless of the person's general fitness level. No evidence exists to support that muscle soreness resulting from exercise is associated with long-term damage or reduced function in the muscles. Muscle soreness begins 8-24 hours after exercise and peaks 24-72 hours post-exercise.

Although we have made every effort to minimize these risks, some procedures we ask you to do may be upsetting or stressful. If so, we can tell you about some people who may be able to help you with these feelings. You may also experience a previously unknown risk or side effect.

Will I benefit from taking part in this study?

The results of the body composition assessment and VO_2max test will be disclosed to you if you complete the testing procedures. This can increase your knowledge of your current cardiorespiratory fitness level. Additional benefits from participating in this study are not expected.

Do I have to take part in this study?

If you decide to take part in the study, it should be because you really want to volunteer. You will not lose any benefits or rights you would normally have if you choose not to volunteer. You can stop at any time during the study for any reason and still keep the benefits and rights you had before volunteering.

What will it cost me to participate?

There are no costs associated with taking part in this study.

Will I receive any payment or rewards for taking part in the study?

You will not receive any payment or reward for taking part in this study.

Who will see the information I give?

Your information will be combined with information from other people taking part in the study. When we write up the study to share it with other researchers, we will write about this combined information. You will not be identified in these written materials. The only document linking your name to data is the ID assignment master list, which will be destroyed upon conclusion of the study.

We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For that purpose, all forms with your name on them will be stored separately under lock and key from the de-identified data forms.

However, there are some circumstances in which we may have to show your information to other people. We may be required to show information that identifies you to people who need to be sure we have done the research correctly; these would be people from such organizations as Eastern Kentucky University.

Can my taking part in the study end early?

If you decide to take part in the study, you still have the right to decide at any time that you no longer want to participate. You will not be treated differently if you decide to stop taking part in the study.

The individuals conducting the study may need to end your participation in the study. They may do this if you are not able to follow the directions they give you, or if you are experiencing shortness of breath, chest pain, dizziness, nausea, collapse, claustrophobia, or other potentially harmful conditions.

What happens if I get hurt or sick during the study?

If you believe you are hurt or if you get sick because of something that is done during the study, you should call Simon Holzapfel at 423-329-3038 immediately. It is important for you to understand that Eastern Kentucky University will not pay for the cost of any care or treatment that might be necessary because you get hurt or sick while taking part in this study. That cost will be your responsibility. Also, Eastern Kentucky University will not pay for any wages you may lose if you are harmed by this study.

Usually, medical costs that result from research-related harm cannot be included as regular medical costs. Therefore, the costs related to your care and treatment because of something that is done during the study will be your responsibility. You should ask your insurer if you have any questions about your insurer's willingness to pay under these circumstances.

What if I have questions?

Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have questions about the study, you can contact the investigator, Simon Holzapfel at simon_holzapfel@mymail.eku.edu. If you have any questions about your rights as a research volunteer, contact the staff in the Division of Sponsored Programs at Eastern Kentucky University at 859-622-3636. We will give you a copy of this consent form to take with you.

What else do I need to know?

You will be told if any new information is learned which may affect your condition or influence your willingness to continue taking part in this study.

I have thoroughly read this document, understand its contents, have been given an opportunity to have my questions answered, and agree to participate in this research project.

_____ _____

Signature of person agreeing to take part in the study Date

_____

Printed name of person taking part in the study

_____

Name of person providing information to subject

Eastern Kentucky University
Department of Exercise and Sports Science
223 Moberly Building
521 Lancaster Avenue, Richmond, KY 40475

Digit Ratio (2D:4D) and VO_2max Study

Consent to Participate in a Research Study

Why am I being asked to participate in this research?

You are invited to take part in a research study about the effects of fetal testosterone levels and testosterone sensitivity on maximal oxygen uptake during running. You are invited to participate in this research study because you are a highly trained, competitive endurance runner between 18 and 25 years of age. If you take part in this study, you will be one of about 80 people to do so.

Who is doing the study?

The principal investigator is Simon Holzapfel, B.A., at Eastern Kentucky University. He is being guided in this research by Matthew Sabin, Ph.D., Peter Chomentowski, Ph.D., and Lousia Summers, Ph.D. There may be other people on the research team assisting at different times during the study.

What is the purpose of the study?

It has been shown that the ratio of the length of second finger (index finger) to the fourth finger (ring finger) (2D:4D) negatively correlates with prenatal testosterone levels. In other words, the more testosterone an individual has been exposed to as a fetus the shorter the second finger is compared the fourth finger. Prenatal testosterone levels measured by 2D:4D have been reported to positively influence the performance in a variety of sport including long distance running. However, 2D:4D has not been shown to affect maximal oxygen uptake by the only study investigating this relationship. Maximal oxygen uptake is the maximal amount of oxygen your body can consume during maximal efforts and is regarded as one of the best determinants of endurance performance. However, the previously mentioned study reported that the difference between right hand and left hand 2D:4D (right-left 2D:4D) seems to predict maximal oxygen uptake. Evidence supports right-left 2D:4D as an indicator of testosterone sensitivity even after birth. This means that the lower right 2D:4D is compared to left 2D:4D, the more sensitive the genes are to increases in testosterone concentrations. Exercise stimulates testosterone secretion and it has been reported that individuals with high testosterone sensitivity develop larger capacities for oxygen consumption in response to exercise compared to individuals of low testosterone sensitivity. However, no study has used right-left 2D:4D to investigate the effects of long-term endurance training and testosterone sensitivity on maximal oxygen uptake. The influence of prenatal testosterone levels measured via 2D:4D on maximal oxygen uptake in

inactive individuals compared with highly trained endurance runners has never been investigated either. This study is designed to examine those relationships.

We are interested in the aforementioned relationships because of the importance of maximal oxygen uptake as an indicator of cardiovascular risks factors and all causes of mortality associated with inactivity. The knowledge gained about the influence of prenatal testosterone levels and testosterone sensitivity on maximal oxygen uptake mediated by endurance exercise could be used in the evaluation of cardiovascular risk factors from a genetic viewpoint. This information would be useful for the prescription of appropriate exercise interventions and outcome expectations. The measurement of 2D:4D would provide health and wellness professionals with a simple and inexpensive filed based method to assess the genetic predisposition to cardiovascular disease and the potential trainability for the reduction of cardiovascular risk factors.

Where is the study going to take place and how long will it last?

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- Read the *Informed Consent Form* (this document) and sign and date it if you agree to participate in the study
- Fill out a *Demographics Questionnaire*

During the testing session (approx. 50 min):

- Your blood pressure will be measured
- The length of your index (second) and ring (fourth) finger on both hands will be measured with a vernier caliper by two investigators
- Your percent body fat will be measured using the Tanita Body Composition Analyzer. It looks similar to a bathroom scale. You will be asked to step on it barefoot and the device measures your body composition using a small electrical current that you cannot feel.
- Your VO_2max will be measured using the Bruce treadmill protocol. Your maximal oxygen uptake (VO_2max) is a measure of the largest possible amount of oxygen your body can consume during maximal exercise. For this test, you will start out walking on a 10% grade. Every 3 minutes, the speed and incline of the treadmill will increase. You will eventually have to start jogging and/or running. You will be fitted with a mouthpiece, a face mask, a nose clip, and a heart rate monitor. These are necessary devices to measure the amount of oxygen you are consuming during the test and to determine your level of effort. You will be asked to do the best that you can on this test by going as long as possible on the treadmill until you are too tired to continue. At that point, you will grab hold of the bar directly in front of you and the investigator will slow down the treadmill immediately.

Are there reasons why I should not take part in this study?

At this point, we have determined that you are eligible to participate in the study. If you feel uncomfortable with the testing procedures you may withdraw from the study at any time.

What are the possible risks and discomforts?

Abnormal responses during the treadmill test, such as shortness of breath, chest pain, heart rhythm irregularity, collapse requiring treatment and death in young healthy females and males are rare – expected to occur in less than 1% of people (less than 1 out of 100 people). However, some common risks – expected to occur in 10-25% of people (10-25 out of 100 people) of maximal exercise testing include heavy breathing, dizziness, nose clip discomfort, anxiousness or claustrophobia due to the nose clip or mouthpiece, muscle fatigue and overall fatigue.

You may experience muscle soreness during the hours or days following the treadmill test. Muscle soreness occurs regardless of the person's general fitness level. No evidence exists to support that muscle soreness resulting from exercise is associated with long-term damage or reduced function in the muscles. Muscle soreness begins 8-24 hours after exercise and peaks 24-72 hours post-exercise.

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Do I have to take part in this study?

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What will it cost me to participate?

There are no costs associated with taking part in this study.

Will I receive any payment or rewards for taking part in the study?

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Usually, medical costs that result from research-related harm cannot be included as regular medical costs. Therefore, the costs related to your care and treatment because of something that is done during the study will be your responsibility. You should ask your insurer if you have any questions about your insurer's willingness to pay under these circumstances.

What if I have questions?

Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have questions about the study, you can contact the investigator, Simon Holzapfel at simon_holzapfel@mymail.eku.edu. If you have any questions about your rights as a research volunteer, contact the staff in the Division of Sponsored Programs at Eastern Kentucky University at 859-622-3636. We will give you a copy of this consent form to take with you.

What else do I need to know?

You will be told if any new information is learned which may affect your condition or influence your willingness to continue taking part in this study.

I have thoroughly read this document, understand its contents, have been given an opportunity to have my questions answered, and agree to participate in this research project.

_____ _____

Signature of person agreeing to take part in the study Date

_____

Printed name of person taking part in the study

_____

Name of person providing information to subject

APPENDIX E:

PAR-Q & YOU

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	**1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	**2. Do you feel pain in your chest when you do physical activity?**
☐	☐	**3. In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	**4. Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	**5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	**6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	**7. Do you know of any other reason why you should not do physical activity?**

If you answered YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

→ **DELAY BECOMING MUCH MORE ACTIVE:**

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

1:12

Supported by: Health Canada Santé Canada

continued on other side...

APPENDIX F:

Pre-Test Questionnaires

Eastern Kentucky University
Department of Exercise and Sports Science
223 Moberly Building
521 Lancaster Avenue, Richmond, KY 40475

VERDICT Study

Pre-Test Questionnaire

Name:_____ **Date:** _____

Please answer the following questions by checking the appropriate box:	**YES**	**NO**
Did you exercise yesterday or today?	☐	☐
Did you exercise a total of 2 hours or more during any given week since you decided to participate in this study?	☐	☐
Have you consumed any nicotine, caffeine, or alcohol within the last 24 hours?	☐	☐
Did you stop taking any medication within the last 7 days that you normally take on a regular basis?	☐	☐
Did you take any new medication within the last 7 days that you had not been taking on a regular basis?	☐	☐
Did you take any ergogenic or performance enhancing drugs?	☐	☐
Have you eaten anything within the last 2½ hours?	☐	☐

Eastern Kentucky University
Department of Exercise and Sports Science
223 Moberly Building
521 Lancaster Avenue, Richmond, KY 40475

Pre-Test Questionnaire

Name:_____ **Date:** _____

Please answer the following questions by checking the appropriate box:	**YES**	**NO**
Did you exercise yesterday or today?	☐	☐
Did you exercise a total of 2 hours or more during any given week since you decided to participate in this study?	☐	☐
Have you consumed any nicotine, caffeine, or alcohol within the last 24 hours?	☐	☐
Did you stop taking any medication within the last 7 days that you normally take on a regular basis?	☐	☐
Did you take any new medication within the last 7 days that you had not been taking on a regular basis?	☐	☐
Did you take any ergogenic or performance enhancing drugs?	☐	☐
Have you eaten anything within the last 2½ hours?	☐	☐
Could you be pregnant?	☐	☐

Eastern Kentucky University
Department of Exercise and Sports Science
223 Moberly Building
521 Lancaster Avenue, Richmond, KY 40475

Pre-Test Questionnaire

Name:_____ **Date:** _____

Please answer the following questions by checking the appropriate box:	**YES**	**NO**
Did you exercise and/or run yesterday or today?	☐	☐
Did you run more than 5 miles two days ago?	☐	☐
Have you consumed any nicotine, caffeine, or alcohol within the last 24 hours?	☐	☐
Did you stop taking any medication within the last 7 days that you normally take on a regular basis?	☐	☐
Did you take any new medication within the last 7 days that you had not been taking on a regular basis?	☐	☐
Did you take any ergogenic or performance enhancing drugs?	☐	☐
Have you eaten anything within the last 2½ hours?	☐	☐

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Department of Exercise and Sports Science
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Pre-Test Questionnaire

Name:_____ **Date:** _____

Please answer the following questions by checking the appropriate box:	**YES**	**NO**
Did you exercise and/or run yesterday or today?	☐	☐
Did you run more than 4 miles two days ago?	☐	☐
Have you consumed any nicotine, caffeine, or alcohol within the last 24 hours?	☐	☐
Did you stop taking any medication within the last 7 days that you normally take on a regular basis?	☐	☐
Did you take any new medication within the last 7 days that you had not been taking on a regular basis?	☐	☐
Did you take any ergogenic or performance enhancing drugs?	☐	☐
Have you eaten anything within the last 2½ hours?	☐	☐
Could you be pregnant?	☐	☐

APPENDIX G:

Demographics Questionnaires

Eastern Kentucky University
Department of Exercise and Sports Science
223 Moberly Building
521 Lancaster Avenue, Richmond, KY 40475

Demographics Questionnaire:

Participant ID: _____

Age: _____ Gender: _____ (M/F)

Nationality: _____

Ethnicity/Race (check most predominant origin):

- ☐ Asian or Pacific Islander: Persons having origins in any of the peoples of the Far East, Southeast Asia, the Indian subcontinent, or the Pacific Islands. This area includes, for example, China, Japan, Korea, the Philippine Islands, Thailand, Vietnam, Indonesia and Samoa.
- ☐ African American (not of Hispanic origin): Person having origins in any of the black ethnic groups of North America.
- ☐ African: Persons having origins in any of the peoples from African countries except North African countries.
- ☐ Hispanic: Persons having origins in any of the Mexican, Puerto Rican, Cuban, Central or South American or other Spanish Cultures.
- ☐ Native American or Alaskan Native: Persons having origins in any of the original peoples of North America.
- ☐ Caucasian (not of Hispanic origin): Persons having origins in any of the original peoples of Europe.
- ☐ Arabic or Middle Eastern: Persons having origins in any of the peoples from Middle Eastern countries, North African countries, Iraq, Iran, Afghanistan, Saudi Arabia, Yemen, and Oman.
- ☐ Other: _____

Eastern Kentucky University
Department of Exercise and Sports Science
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Demographics Questionnaire:

Participant ID: _____ Age: _____ Gender: _____ (M/F)

Nationality: _____

Ethnicity/Race (check most predominant origin):

- ☐ Asian or Pacific Islander: Persons having origins in any of the peoples of the Far East, Southeast Asia, the Indian subcontinent, or the Pacific Islands. This area includes, for example, China, Japan, Korea, the Philippine Islands, Thailand, Vietnam, Indonesia and Samoa.
- ☐ African American (not of Hispanic origin): Person having origins in any of the black ethnic groups of North America.
- ☐ African: Persons having origins in any of the peoples from African countries except North African countries.
- ☐ Hispanic: Persons having origins in any of the Mexican, Puerto Rican, Cuban, Central or South American or other Spanish Cultures.
- ☐ Native American or Alaskan Native: Persons having origins in any of the original peoples of North America.
- ☐ Caucasian (not of Hispanic origin): Persons having origins in any of the original peoples of Europe.
- ☐ Arabic or Middle Eastern: Persons having origins in any of the peoples from Middle Eastern countries, North African countries, Iraq, Iran, Afghanistan, Saudi Arabia, Yemen, and Oman.
- ☐ Other: _____

Personal bests on the track in minutes and seconds (min:sec, e.g. 14:43):

800m: _____ 1,500m: _____ 1,600m: _____

3,000m: _____ 5,000m: _____ 10,000m: _____

For how many years have you participated in track & field, cross country, or road races? _____

How many years have you trained for competitive running? _____

How many miles do you typically run per week? _____

APPENDIX H:

Treadmill Test Instructions

Standardized Instructions for the Treadmill Test

"We want you to do the best that you can on this test by going as long as possible on the treadmill until you are too tired to continue. After a short warm-up where you'll be walking on a level treadmill, the actual test will begin. At this point, the elevation will increase to 10% and the speed will be set to 1.7 mph. It's important that you face forward, and keep your feet as close to the top of the treadmill as you can, and someone will let you know if you are not in correct position. Every three minutes you'll notice the speed and incline of the treadmill has increased. Try to give your best effort by going as long as you can on the treadmill. When you are too tired to continue, or if at any point you experience shortness of breath, chest discomfort, or feel ill, push the stop button on the treadmill (show) and grab the bars in front of you (show). It's important to remember that during this time the speed and incline will decrease right away, but you have to keep moving on the treadmill until it has stopped entirely. You will then walk on the treadmill at no incline for 3 minutes in order to cool down. This is a scale for rating perceived exertion. Perceived exertion is the overall effort or distress of your body during exercise. The number 0 represents no perceived exertion or leg discomfort and the number 10 represents the greatest amount of exertion you have ever experienced. After the first minute of the test you will point to a number, which indicates your rating of perceived exertion at that time. One of the investigators will say the number out loud in order to make sure that we understand your selection. If the number the investigator called out is too low, point up with your index finger. Then the investigator will call out the next highest number. If the number the investigator called out is too high, then point down with your index finger. Show a "thumbs up" when the investigator has called out the correct number. For every subsequent minute, the investigator will ask if you are still at the same number you indicated last or if you have gone up. If you have gone up, point your index finger up until the investigator has called out the correct number at which point you will show a "thumbs up". Do you have any questions?"

APPENDIX I:

OMNI RPE Scale

Rating of Perceived Exertion Scale	
10	Maximum Effort
9	Extremely Hard
8	Very Hard
7	Hard
6	Somewhat Hard
5	
4	Moderate
3	
2	Light
1	Very Light
0	Very, Very Light

APPENDIX J:

Bruce Protocol for the Treadmill

Bruce Exercise Test Protocol for the Treadmill

Stage #	MPH	GRADE
Stage 1	1.7	10 %
Stage 2	2.5	12 %
Stage 3	3.4	14 %
Stage 4	4.2	16 %
Stage 5	5.0	18 %
Stage 6	5.5	20 %
Stage 7	6.0	22 %
Each stage lasts 3 minutes		

American College of Sports Medicine (2000). *ACSM's Guidelines for Exercise Testing and Prescription (6th ed.)*. Philadelphia PA: Lippincott Williams & Wilkins.

APPENDIX K:

Data Collection Form

Eastern Kentucky University
Department of Exercise and Sports Science
223 Moberly Building
521 Lancaster Avenue, Richmond, KY 40475

Data Collection Form

ID:_____	**Date:** _____
Gender: _____ (M/F) Age: _____	

Blood Pressure:	
Systolic: _____ mmHg	Diastolic: _____ mmHg

Digit Length Measurements	Investigator Name: _____	Investigator Name: _____	Average:
Right 2D:			
Right 4D:			
Left 2D:			
Left 4D:			
Right – Left 2D:4D:			

Anthropometric Measures			
Height: _____	Weight: _____	% Body Fat: _____	Bod Mass Index: _____

Age-Adjusted Maximum Heart Rate:
208 - 0.7 x _____ = _____ bpm Age (yrs.)

End of minute:	**Heart Rate (bpm)**	**Rating of Perceived Exertion**	End of minute:	**Heart Rate (bpm)**	**Rating of Perceived Exertion**
1			17		
2			18		
3			19		
4			20		
5			21		
6			22		
7			23		
8			24		
9			25		
10			26		
11			27		
12			28		
13			29		
14			30		
15			Immediate post-test:		
16					
Time on Treadmill: _____ (min:sec)					