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Second-to-fourth digit ratio (2D:4D) is unrelated to measures of somatic reproductive effort among young men from Cebu, The Philippines

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ABSTRACT

Objectives: A low second-to-fourth (2D:4D) digit ratio, a retrospective marker of high prenatal androgens, predicts increased investment in costly sexually dimorphic traits in men in some studies, though results are mixed. Here we test the hypothesis that the relationship of low 2D:4D ratios with increased muscularity and decreased adiposity depends on current testosterone (T) levels, such that digit ratio will be a particularly strong predictor of outcomes among men exhibiting a mating-effort-oriented endocrinological profile (high T). We also test the association between 2D:4D and somatic traits independently of T.

Materials and methods: We related 2D:4D digit ratios, and their interaction with T, to handgrip strength, lean mass, arm muscle area, and skinfold thickness in a sample of young, childess men (20-22 y) from Cebu, Philippines (N = 623).

Results: Digit ratio did not significantly predict men's T-dependent somatic traits. Interactions between 2D:4D and morning T, similarly, did not predict male muscularity or adiposity. Although two of the interactions were significant or marginally significant ($P<0.1$), after adjusting for multiple testing the evidence in support of our hypothesis was weak.

Discussion: We found no evidence that 2D:4D predicted measures of somatic reproductive effort in this sample of young men from Cebu, who as a group could be considered mostly mating-oriented. These relationships were also not contingent upon, or stronger, when considering the moderating effect of concurrent T levels. In this sample, 2D:4D was therefore either a poor proxy of prenatal androgen exposure or prenatal androgens had limited influence on adult somatic outcomes.

Introduction

Prenatal exposure to testosterone (T) or other androgens is known to have organizational effects on a range of sexually dimorphic traits in vertebrates (Phoenix et al., 1959; Smith et al., 2013). It can influence neural development, as well as the expression of social and sexual behavior in later life (Forger et al., 1996; Thornton et al., 2009; Hines et al., 2015). Increased prenatal androgen exposure has also been linked to greater fetal and postnatal growth in some study systems, particularly among male offspring (Gill et al., 1998; Helle et al., 2012). Androgens play a role in the processes leading to differentiation of mesenchymal pluripotent stem cells into the myogenic lineage and the inhibition of their differentiation into the adipogenic lineage (Bhasin et al., 2003; Singh et al., 2003; De Gendt and Verhoeven, 2012). Such insights gleaned from *in vitro* studies and knock-out models of mice likely go some ways towards explaining the generally greater muscle mass and lower adiposity of men compared to women – differences that are initiated *in utero*, given known sex differences in weight and body composition at birth (Catalano et al., 1995). Indeed, experimental and comparative data suggest that changes in the role of prenatal testosterone exposure on growth might be an important mechanism underlying the rapid evolution of pronounced sexual size dimorphism in some lineages (Cox et al., 2009). Intra-specifically, therefore, differential exposure to prenatal androgens may lead to inter-individual variation in male growth and physiology with possible downstream effects on reproductive performance and success.

Because of the challenges of studying the effects of prenatal androgen in humans, the ratio of the second to the fourth digit of the hand (2D:4D ratio) has been widely used as a non-invasive, retrospective marker of intra-uterine androgen exposure (Manning et al., 1998; McIntyre, 2006; Voracek and Loibl, 2009; Voracek, 2011). There is both direct and indirect

evidence from animal model experiments and human studies that, under the influence of prenatal androgens, the embryonic fourth digit grows longer than the second digit, thus resulting in a lower 2D:4D ratio (Brown et al., 2002; Okten et al., 2002; Lutchmaya et al., 2004; Berenbaum et al., 2009; Manning, 2011; Zheng and Cohn, 2011; Rivas et al., 2014). Consistent with these effects, men on average tend to have lower 2D:4D ratios than women in most human populations (Peters et al., 2002; Hönekopp and Watson, 2010). Additional support for the role of prenatal androgen exposure as an influence on the 2D:4D ratio comes from studies of opposite-sex dizygotic twins (van Anders et al., 2006), birth order and the sex of older siblings (Saino et al., 2006), and individuals with congenital conditions affecting pre-natal androgen exposure, such as congenital adrenal hyperplasia (Brown et al., 2002; Okten et al., 2002; Rivas et al., 2014) and complete androgen insensitivity syndrome (Berenbaum et al., 2009). Finally, a direct measure of prenatal hormones via amniocentesis showed that infants that experienced higher T to estrogen ratios in utero had lower 2D:4D ratios at 2 years of age (Lutchmaya et al., 2004). Although these results generally align with the premise that 2D:4D reflects prenatal androgen exposure, inconsistencies across studies have fueled continuing skepticism about the ratio and its interpretation (Berenbaum et al., 2009; Constantinescu and Hines, 2012; Hines et al., 2015). For instance, in at least one population women have lower 2D:4D ratios than men (Apicella et al., 2016). Some studies of individuals with congenital conditions and/or direct measures of prenatal or perinatal hormones also do not provide unequivocal support for the prediction that higher prenatal androgen exposure leads to lower 2D:4D ratios (Buck, 2003; Hickey et al., 2010; Ventura et al., 2013; Hollier et al., 2015).

Despite this lack of consensus on the utility of the 2D:4D ratio as a marker of inter-individual differences in prenatal androgen exposure, many studies have documented

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biologically meaningful relationships between 2D:4D ratios and a range of behavioral (Coco, 2013; Butovskaya et al., 2015; Wlodarski et al., 2015; Del Giudice and Angeleri, 2016), endocrinological (Kilduff et al., 2013; Crewther et al., 2015) and reproductive outcomes (Manning et al., 2000; Honekopp et al., 2006; Manning et al., 2009; Klimek et al., 2014), which are interpreted as evidence for effects of prenatal androgen action on longer-term outcomes (but see Putz et al., 2004; Hönekopp et al., 2007).

In terms of male traits related to reproductive effort, lower 2D:4D has been proposed to be a correlate of male competitiveness, fertility and attractiveness, in part through the long-term effects of prenatal androgen exposure on sexually selected male traits that underlie athletic performance, such as strength and endurance (Manning and Taylor, 2001). With some exceptions (Gallup et al., 2007; Voracek et al., 2010; Folland et al., 2012; Muller et al., 2013; Ranson et al., 2015) studies have found that men with lower 2D:4D ratios are stronger, more muscular, and larger (Fink et al., 2006; Hone and McCullough, 2012; Zhao et al., 2012; Halil et al., 2013; Klimek et al., 2014). They also exhibit superior performance in competitive endurance sports and tests of overall physical fitness (Manning and Taylor, 2001; Hönekopp et al., 2006; Bennett et al., 2010; Tamiya et al., 2012; Ranson et al., 2015), and have higher maximal lung oxygen uptake (Hill et al., 2012). A meta-analysis of more than 2500 participants from 24 populations concluded that a lower 2D:4D ratio generally predicted numerous measures of athletic prowess, although findings were relatively modest and also heterogeneous across studies (Hönekopp and Schuster, 2010).

According to the organizational – activational hypothesis, the intrauterine effects of T are presumed to permanently alter (organize) tissues during development, which then experience the activational effects of circulating T, production of which begins at puberty (Phoenix, 2009;

Wallen, 2009). While the scope of the organizational – activational hypothesis was initially restricted specifically to the role of T in organizing tissues related to adult mating behavior (Phoenix et al., 1959), this framework has been extended to account for sex differences in overall phenotypic development (Arnold, 2009). Following from this, one issue that has rarely been considered in the digit ratio literature is the possible contingency of relationships between digit ratios and T-dependent somatic traits on the concurrent activating effect of T. In humans, testosterone production has been shown to decline markedly as adult males shift from mating to pairbonding (Mazur and Michalek, 1998; Burnham et al., 2003) and parenting (Gray et al., 2002; Gettler et al., 2011; Gray et al., 2016). Although functional explanations for these changes in T primarily focus on the hormone's behavioral role, a similar logic could apply to somatic mating effort. Maintaining costly androgen-dependent somatic traits (e.g., muscularity and strength) that may affect male reproductive success, either through providing advantages in male-male competition or via female mate choice (Puts, 2010; Apicella, 2014), would be particularly important while men are engaging in mating (as opposed to paternal) effort. It has been proposed that high T in men of reproductive age is key to maintaining muscle and strength, which is achieved in part by diverting energy away from maintenance functions like immunity and energy reserves (Bribiescas, 2001; Kuzawa et al., 2016; but see Alvarado et al., 2015). If prenatal androgen exposure affects somatic traits that influence mating success, these relationships should be especially salient among men who have high T and are invested in mating effort. Whether this is the case is presently unclear, as analyses of 2D:4D ratios and somatic traits have not considered the role of study participants' T in conjunction with life history stage.

Here we examine the relationship between adult 2D:4D ratios and measures of investment in somatic mating effort (lean mass, grip strength, and arm muscle area) and energy stores (adiposity) in a large, prospectively recruited cohort of men (20-22 years old) living in Cebu, The Philippines. Previous cross-sectional analyses conducted on these men at the same age have shown that T levels were only significantly lower among fathers and that single and pair-bonded age mates had similarly high T (Kuzawa et al., 2009). We thus focus our analyses on a subset of men who have maintained higher T levels throughout their adult lives (i.e. have not become fathers yet). We expect a strong relationship between somatic traits and 2D:4D among men, because their T has likely been consistently high, on average, in support of traits associated with mating effort. To further assess the role of T in affecting the possible relationships between digit ratios and somatic traits, we also tested for interactions between morning T levels and digit ratios, with the prediction that men with high T and low 2D:4D would exhibit greater grip strength, arm muscle area and lean mass, and lower adiposity, in comparison to men with low T and high 2D:4D. We focused in particular on waking T levels, which are higher than measures from the rest of the day (Kuzawa et al., 2016) and predictive of muscularity among the physically active men in this population (Gettler et al., 2010).

Materials and Methods

Study site and subjects

Data come from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), a population-based birth cohort study of mothers and their infants born in 1983-84 from randomly selected urban and rural neighborhoods in Metropolitan Cebu, Philippines (Adair et al., 2011). This research was conducted under conditions of informed consent with human subjects

clearance from the Institutional Review Boards of the University of North Carolina, Chapel Hill and Northwestern University.

Data collection

Adult somatic measures (height, grip strength, arm muscle area, lean mass, and skinfold thickness) were measured in 2005 using standard procedures, as described in detail elsewhere (Gettler et al., 2010). Briefly, grip strength was measured to the nearest kg in triplicate with a dynamometer, with the mean used in analyses. Arm muscle area was estimated from arm circumference (measured with a flexible tape) and triceps skinfolds (measured in triplicate with a skinfold caliper), adjusted for the estimated area of the humerus. Lean mass was calculated from estimated % body composition calculated from triceps, suprailiac, and subscapular skinfold thickness using a body composition predictive formula validated for Asian populations. Information on socioeconomic status, relationship status (co-habitation), parenthood and other factors were collected during in-home interviews (Kuzawa et al., 2009). Digit ratios were measured directly in triplicate with calipers in 2009. For each digit, we calculated the mean of three repeat measures of the 2D:4D ratio for each hand. Intraclass correlation coefficients for the repeated digit measures were high for all digits measured on both hands (>0.99) but we caution that these measures were undertaken in close succession by the same observer and are thus not statistically independent.

Testosterone was measured from saliva samples collected by study participants immediately upon waking and assayed subsequently at the Laboratory for Human Biology at Northwestern University (Kuzawa et al., 2009). T concentrations were determined in duplicate using an enzyme immunoassay protocol developed and validated for use with saliva samples (Salimetrics #1-2402, State College, PA). The inter-assay coefficients of variation were 11.5%

and 13.7 % for high and low controls, respectively (Gettler et al., 2010). T values were adjusted

for time of saliva collection prior to analysis (Kuzawa et al. 2010).

We limit analyses to the sample of men who did not have any children (to rule out those

with reduced T levels due to parenthood), who had a complete set of morphological and hormone

measures collected in 2005, and who were not shift workers due to unusual wake/sleep patterns

that affected T production (Lee et al., 2014). In addition, we excluded 4 and 9 outliers from the

measures of the L and R-hand 2D:4D ratios, respectively (values > 3SD of the mean) retaining a

final analysis sample of 623 men.

Data analysis

To assess the relationship between digit ratios and somatic traits of interest (grip strength

of the stronger hand, arm muscle area, lean mass, and skinfold thickness) we first report results

from simple correlational tests (without controlling for potentially confounding factors), to allow

a direct comparison with previously published studies. Second, for both hands, we constructed

two models for each trait: first, using only 2D:4D as a predictor, and second, including the

interaction between 2D:4D and morning T levels. Previous studies have examined this ratio both

for the left and the right hand, using both a continuous ratio and a binary metric

(low/masculinized v. high/feminized; low being < 1.0; high ≥ 1.0). While we focused our

analyses and interpretation on continuously measured digit ratios, for the purposes of comparison

with the existing literature we also report findings from models that consider a binary measure of

high/low 2D:4D. To address the issue of multiple testing we first report unadjusted P-values for

explorative purposes (because some previous studies only examined one of these 4 alternative

ways of assessing digit masculinization) and then consider the adjusted critical P-value after

correcting for running 32 separate models (P = 0.002). All models controlled for additional

variables that could confound associations between digit ratio and somatic outcomes, including adult household income, age, and height, and binary measures of whether the men engaged regularly in physically demanding work, basketball playing, and weightlifting (see Gettler et al., 2010). All continuous variables were converted to z-scores prior to analysis to allow direct unitless comparison of coefficients across different outcomes. Testosterone values were log-transformed first and adjusted for time of saliva collection, after which the residuals were converted to z-scores. Digit ratio values were modeled as residuals from models regressing measured 2D:4D ratio on observer identity to account for small but systematic differences in measurement across 5 observers. In presenting descriptive statistics and plots, and carrying out simple correlational tests, we use 2D:4D ratios adjusted for observer. Tests are two-tailed throughout with alpha = 0.05 (and p<0.1 considered a statistical trend), unless noted otherwise, and performed in Stata 13.1 (Stata Corporation, College Station, TX). Power analysis was performed in G*Power 3.1 (Faul et al., 2009).

RESULTS

In this sample of young adult men (Table 1), 2D:4D digit ratios were slightly lower on the left hand (L2D:4D: mean ± SE = 0.961 ± 0.001) than on the right (R2D:4D: 0.965 ± 0.001; t = -4.59, N = 623, P < 0.0001) with 56.98% of men exhibiting a lower (relatively more masculinized) 2D:4D ratio on their L hand, compared to the right. The 2D:4D ratio in the sample was categorized as low/masculine (<1.00) for 91.3% of the men based upon the L hand and 90.4% for the R hand.

==== TABLE 1 ======

Men with greater adiposity (sum of skinfolds) tended to have higher R2D:4D (all correlational tests performed on log-transformed values; r = 0.077, N = 623, P = 0.056) but this

relationship was not significant for the left hand (r = 0.004, P = 0.921). No significant

relationships were found between 2D:4D and grip strength (L: r = -0.044, P = 0.270; R: r = -

0.035, P = 0.384), arm muscle area (L: r = -0.023, P = 0.561; R: r = -0.055, P = 0.169), or lean

mass (L: r = -0.010, P = 0.801; R: r = -0.008, P = 0.849) (Fig. 1).

In multiple regression models accounting for potentially confounding factors (height, age,

regular involvement in physically demanding activity and sports, and household income) none of

the four somatic measures we tested (grip strength, arm muscle area, lean mass, and sum of

skinfolds) were significantly associated with 2D:4D on either hand. The only relationship that

approached statistical significance (before correcting for multiple testing) was the positive

association between R2D:4D and adiposity (sum of skinfolds; P = 0.078, Table 2). There was no

evidence for non-linear relationships between 2D:4D ratios and somatic traits (Fig. 1).

===== FIGURE 1=====

In simple correlation tests, waking T levels were not associated with 2D:4D on either

hand (L: r = -0.014, P = 0.721; R: r = -0.045, P = 0.264). To evaluate the potential role of T as a

moderating influence on relationships between 2D:4D ratios and somatic traits we included a

2D:4D x waking T interaction term. One of the multiple regression models showed a significant

positive interaction between R2D:4D and morning T in predicting grip strength (P = 0.043). In a

separate model, R2D:4D and morning T interacted positively and almost significantly (P =

0.077) to predict arm muscle area (Table 2). With the positive slope steepest among men with

the highest T, these interactions suggested that men with higher T and more feminized (higher)

2D:4D tended to have greater grip strength and more arm musculature. Re-running the models

described above with categorical measures of left and right 2D:4D (high vs. low) yielded

consistently non-significant findings (results not shown). Neither the significant interaction (P =

0.043) or the two marginally significant main effects (P < 0.1) noted above, remained significant after adjusting for multiple testing (32 separate models), which lowered the significance threshold to P = 0.002.

==== TABLE 2 ===

DISCUSSION

The aim of this study was to examine the relationship between 2D:4D ratios, a putative marker of prenatal androgen exposure, and somatic traits associated with mating effort in a large sample of young Filipino men. We focused our analyses on young adults who had never been fathers and who were thus likely to have maintained higher average T levels throughout their adult lives (Kuzawa et al., 2009). Although the relationship between 2D:4D and T-dependent somatic traits would be expected to be strongest in these "mating-oriented" men, we found little evidence that digit ratios predicted somatic traits, with or without considering the potentially moderating role of concurrent T levels. These findings suggest that digit ratio is a poor predictor of adult somatic mating effort in this young adult sample of men from Cebu.

Although previous studies examining the relationship between digit ratios and adult somatic traits in humans have produced conflicting results, a meta-analysis of published findings found support for a relationship between low 2D:4D ratios and athletic prowess (including grip strength) in both men and women (Hönekopp and Schuster, 2010). The putative effect of prenatal androgen exposure on athleticism, however, appears inconsistent across studies, and with low to moderate effect sizes (Hönekopp and Schuster, 2010). Studies evaluating the relationship between 2D:4D and adult somatic development (specifically musculature and

adiposity) are less common and have thus far not been the subject of meta-analytical review. In older adults 2D:4D has been shown to be negatively correlated with calf circumference and skeletal muscle mass index in both men and women (Halil et al., 2013). Men and women with higher 2D:4D ratios also have more feminine body fat distribution, although there does not appear to be a relationships between total fatness and digit ratios (Ertuğrul, 2013). A lack of a significant relationship between 2D:4D and either fat free mass or adiposity was also reported in at least one other study (Muller et al., 2013). In the context of these findings, our study contributes to increasing awareness of the low predictive power of adult 2D:4D ratios, as a proxy of prenatal androgen exposure (Berenbaum et al., 2009; Constantinescu and Hines, 2012; Hines et al., 2015).

Given that we focused analyses on a subgroup of young men who were most likely to exhibit peak androgen-dependent somatic trait development, and also examined the potential interaction between concurrent T levels and digit ratios in predicting these traits, our lack of significant findings is particularly striking. Not only were these relationship largely not statistically significant but the single significant effect before accounting for multiple testing (R2D:4D x AMT, Table 2) suggested a relationship between digit ratios and grip strength that was in the opposite direction to the one predicted – i.e. men with higher T levels but with more feminized (higher) digit ratios (rather than with more masculinized/lower ratios) had greater grip strength. Nevertheless, the fact that two of the interactions involving morning T levels were the closest to being significant of all predictors tested here (Table 2) suggests that future work examining the role of prenatal and adult androgen action in concert might provide more insight into male somatic development than studies focusing on either in isolation.

Although, it is possible in theory that the lack of significant findings in our study reflects low power, our sample size suggests otherwise. With 623 men in our dataset, our study was among the larger studies of the adult correlates of digit ratios, and we estimate that we were powered to detect a relationship between 2D:4D and somatic traits as low as $|r| = 0.14$. This value is lower than the average effect size across studies included in a meta-analysis of 2D:4D and athletic prowess (including grip strength) with $r \approx -0.26$ (Hönekopp and Schuster, 2010). For comparison, in our dataset the relationship between digit ratios and grip strength was $r \approx -0.04$.

Set against the larger body of work on this topic our findings are not particularly unexpected. The literature on the putative relationship between 2D:4D measures and a range of adult somatic and behavioral phenotypes is characterized by inconsistent results and frequent null findings. The use of many operational definitions of 2D:4D ratios (left hand, right hand, average of the two hands, continuous or categorical), the frequent lack of multiple-testing corrections (especially when more than one type of operational definition is used), and the inherently noisy nature of this variable (given that most studies measure ratios based on finger length externally rather than the finger bones underneath) contribute to uncertainty in interpreting findings and are likely also compounded by reporting bias (Apicella et al., 2015). Our findings similarly suggest that caution is needed when using digit ratios as proxies of inter-individual differences in prenatal androgen exposure. Alternatively, if 2D:4D ratios do reflect such exposures well, the role of these early influences in shaping investment in somatic reproductive effort in adult men may be limited when placed in the context of other developmental factors, including the early post-natal period when there is evidence for continued organizational effects of T on somatic development (Kuzawa et al., 2010; Kiviranta et al., 2016).

Longitudinal research on pre- and early post-natal organizational effects of androgens on adult reproductive traits should incorporate direct hormonal measures to help distinguish between these two explanations.

Acknowledgements

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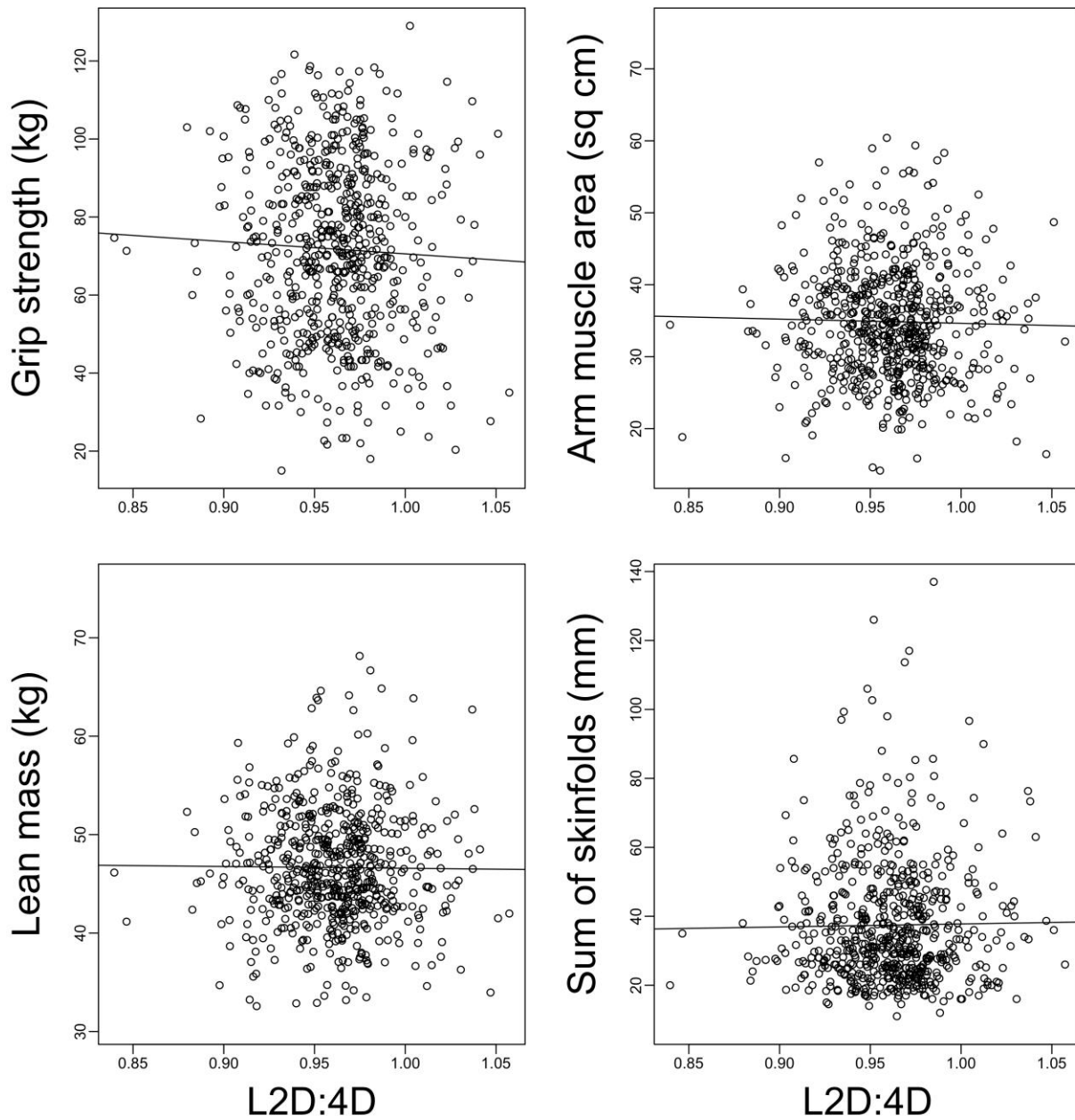
Figure 1. Relationships between 2D:4D ratio and adult somatic traits. Digit ratios for the L hand are shown as they were on average more masculinized (lower) than those for the R hand (plots for the R hand are similar). Raw data shown. See text for statistical details.

Table 1 Characteristics of Cebu men in the analytical sample (N = 623). Digit ratios adjusted for collection of data by multiple observers (see text for details).

Variable	Mean	SD	Min	Max
Age (years)	21.47	0.29	20.79	22.60
L2D:4D (adjusted)	0.961	0.029	0.840	1.057
R2D:4D (adjusted)	0.965	0.027	0.888	1.058
Morning T (pg/ml)	196.90	77.82	26.12	588.43
Grip strength of stronger hand (kg)	72.98	22.03	19.67	129.00
Arm muscle area (cm^2)	34.87	7.81	14.17	60.41
Lean mass (kg)	46.65	5.78	32.58	68.15
Sum of skinfolds (mm)	37.44	17.78	11.00	137.00
Height (cm)	162.78	5.95	144.50	181.20

Table 2. Multiple linear regression models linking adult somatic traits to left and right 2D:4D ratios with and without considering interactions with waking testosterone (N = 623)*.

GRIP STRENGTH

Predictor	Predictor statistics			Full model statistics		
	Estimate	SE	P	F	P	Adj. R2
L2D:4D	-0.038	0.038	0.323	F (7, 615) = 2.42	0.019	0.016
L2D:4D x AMT**	0.071	0.044	0.108	F (9, 613) = 2.40	0.011	0.020
R2D:4D	-0.031	0.040	0.436	F (7, 615) = 2.37	0.022	0.015
R2D:4D x AMT	0.097	0.048	0.043	F (9, 613) = 2.52	0.008	0.022

ARM MUSCLE AREA

Predictor	Predictor statistics			Full model statistics		
	Estimate	SE	P	F	P	Adj. R2
L2D:4D	-0.025	0.037	0.498	F (7, 615) = 7.24	<0.0001	0.065
L2D:4D x AMT	0.050	0.043	0.243	F (9, 613) = 5.91	<0.0001	0.066
R2D:4D	-0.055	0.039	0.155	F (7, 615) = 7.48	<0.0001	0.068
R2D:4D x AMT	0.082	0.046	0.077	F (9, 613) = 6.3	<0.0001	0.071

LEAN MASS

Predictor	Predictor statistics			Full model statistics		
	Estimate	SE	P	F	P	Adj. R2
L2D:4D	-0.019	0.031	0.529	F (7, 615) = 50.34	<0.0001	0.357
L2D:4D x AMT	-0.003	0.035	0.934	F (9, 613) = 39.94	<0.0001	0.355
R2D:4D	-0.021	0.032	0.515	F (7, 615) = 50.35	<0.0001	0.357
R2D:4D x AMT	0.032	0.038	0.405	F (9, 613) = 39.16	<0.0001	0.356

SUM OF SKINFOLDS

Predictor	Predictor statistics			Full model statistics		
	Estimate	SE	P	F	P	Adj. R2
L2D:4D	0.008	0.038	0.828	F (7, 615) = 5.18	<0.0001	0.045
L2D:4D x AMT	-0.025	0.044	0.567	F (9, 613) = 4.05	<0.0001	0.042
R2D:4D	0.070	0.039	0.078	F (7, 615) = 5.64	<0.0001	0.050
R2D:4D x AMT	-0.004	0.047	0.934	F (9, 613) = 4.37	<0.0001	0.047

* All models control for age, height, household income, and engagement in strenuous physical activity, basketball playing and weightlifting (results not shown). 2D:4D entered as residuals of raw 2D:4D on observer ID (See text for details).
** Waking salivary testosterone.