Asian Journal of Research and Reports in Hepatology

Volume 5, Issue 1, Page 34-50, 2023; Article no.AJRRHE.100130

The 2D:4D Ratio and Sex Difference in Circulating Liver Enzymes in Adulthood: A Cross-sectional Study in Ghana

Moses Banyeh [a*], Eric Mishio Bawa [b], Thea Kangkpi [a], Simon Bannison Bani [a], Kervin Edinam Zogli [a], Abdul-Rafik Abdulai [a], Hisham Alhassan Osumanu [a], Muniru Mohammed Tanko [a], Lily Gyeng-Yir [a] and Callistus Wedam Bugapeh [a]

[a] Department of Biomedical Laboratory Science, School of Allied Health Sciences, University for Development Studies, Ghana.
[b] Department of Epidemiology, School of Public Health, University of Ghana, Ghana.

Authors' contributions

This work was carried out in collaboration among all authors. Authors MB, SBB and TK did the conceptualization, performed methodology, helped in project administration and wrote the manuscript. Authors EMB, KEZ, LGY and CWB performed the methodology, experimentation, data collection and wrote the manuscript. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/100130

Original Research Article

Received: 17/03/2023
Accepted: 19/05/2023
Published: 31/05/2023

ABSTRACT

Aims: The second-to-fourth digit ratio (2D:4D) and the right-left difference (Dr-l) are the putative markers of prenatal hormone exposure. These digit ratios are sexually dimorphic and are said to be positive and negative correlates, respectively, of circulating testosterone and estrogen in adulthood.

**Corresponding author: E-mail: mosesbanyeh@gmail.com, mbanyeh@uds.edu.gh;*

There is also a sex difference in liver function in adulthood which may be due to sex differences in plasma liver enzyme activity or concentration. The observed sex difference in liver function has been attributed to the sex difference in circulating testosterone and estrogen. The study sought to determine whether prenatal hormone exposure, as indexed by the 2D:4D or Dr-l, may partly account for sex differences in circulating liver enzyme levels in adulthood.

Study Design: The study was cross-sectional.

Place and Duration of Study: The study was conducted from June to December 2021 at the University for Development Studies.

Methodology: There were 190 participants (females=94 and males=96), between the ages of 18 and 32 years. The right-hand (2D:4DR), and the left-hand (2D:4DL) digit ratios were measured using computer-assisted analysis. The right-left hand difference (Dr-l) was then calculated. Venous blood samples were collected and assayed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT).

Results: The adjusted odds ratio (AOR) and 95% confidence interval (CI) showed that males had greater odds of higher plasma GGT than females [AOR=1.035 (95%CI: 1.004-1.067), P=0.025]. However, there were no interactions between sex and digit ratio on adult plasma liver enzymes.

Conclusion: The 2D:4D ratio or Dr-l may not account for sex differences in plasma liver enzyme levels in adulthood. Further studies are however recommended.

Keywords: Liver; digit ratios; testosterone; estrogens; transferases.

1. INTRODUCTION

Sex differences in liver function have been suggested in the literature. The expression of genes that regulate liver enzyme activity is influenced by the brain in both health and disease and has been demonstrated to be sexually dimorphic [1]. Liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) tend to be higher in males than females [2-4]. Moreover, the prevalence and pathophysiology of liver diseases also tend to follow a sexually dimorphic pattern although the findings are varied [5-7]. Previous studies have demonstrated that premenopausal women are more protected from non-alcoholic liver disease (NAFLD), steatosis and hepatocellular carcinoma (HCC) than aged-matched postmenopausal women or males [5,6]. Moreover, there are increased hepatic inflammatory changes and steatosis among ovariectomized women and women with senescence ovaries or polycystic ovarian syndrome (PCOS). Epidemiological studies have demonstrated that the prevalence of NAFLD increases as women age but decreases as men age. It has also been observed that the prevalence of hepatocellular carcinoma ranges from a ratio of 2:1 to 7:1 for male-to-female. However, this has only been observed among premenopausal women and not postmenopausal women or women with PCOS [5,6]. The differences between males and females regarding liver function and enzyme activity have been attributed to sex hormones. Estrogen may play a hepato-protective role due to its hypolipidemic, antioxidant, antisteatotic and antifibrogenic properties [7]. However, studies among persons undergoing hormonal therapy for hypogonadism or sex affirmation have demonstrated both the positive and harmful effects of estrogen and testosterone although some authors have suggested that such observations could be due to differences in hormonal formulations, dosage and the route of administration [8,9].

The Organizational Hypothesis posits that prenatal testosterone (PT) exposure leads to sexual dimorphism in human phenotypic traits including the second-to-fourth digit ratio (2D:4D) [10]. The 2D:4D is the putative marker of PT exposure. However, exposure to PT alone does not fully account for the sexual dimorphism in the 2D:4D ratio, but a balanced exposure to both PT and prenatal estrogen (PE) during a narrow window in prenatal development [11]. Evidence in support of the effect of PT and PE exposure on the 2D:4D ratio has been adduced from persons with congenital adrenal hyperplasia (CAH), a condition characterized by hyperandrogenemia and also persons with Klinefelter's syndrome (KS) or complete androgen insensitivity syndrome (CAIS). Even though there has not been a consensus on them, CAH patients have lower 2D:4D while KS and CAIS patients have higher 2D:4D ratios compared to controls [12-14]. The 2D:4D ratio and the right-left difference (Dr-l) are similar in

the pattern given to the observation that a lower 2D:4D of the right hand may be associated with more masculine traits and higher ratios with feminine traits. The 2D:4D or the Dr-l are negative and positive correlates of PT and PE exposure respectively and are lower in females than males on average [15]. They have also been found to correlate positively with circulating testosterone but negatively with circulating estrogen in adulthood, although this observation has not been universal [16-19]. Also, the use of the 2D:4D ratio or the Dr-l as putative markers of PT and PE exposure has been controversial [20]. However, an overview and a critical review of the available literature have provided pieces of evidence in support of their validity [21,22].

Given the above observations, it may be suggested that hyperandrogenemia or enzyme activity may be correlated with the 2D:4D ratio or the Dr-l. Although previous studies have demonstrated sex differences in liver function and enzyme activity, hitherto, no study has examined the impact of prenatal hormone exposure, as indexed by the 2D:4D ratio or Dr-l on sex differences in liver enzyme activity in adulthood, hence the aim of the study.

2. MATERIALS AND METHODS

2.1 Study Design and Settings

The study was cross-sectional and was carried out on the Tamale campus of the University for Development Studies between June and December 2021. The University for Development Studies is a multi-campus tertiary-level educational institution in the Northern region of Ghana that offers both under- and post-graduate programs in the Medical, Agricultural Sciences and Education.

2.2 Study Population

The study involved 190 healthy participants (females=94, males=96), who were between the ages of 18 years and 32 years. The study population was part of a larger study from which some parts have already been published [23,24]. The participants had no known history of fractures that could markedly affect standing height and finger length measurements. They did not also have any known liver disorders such as hepatitis, steatosis or cirrhosis. Common factors that may influence liver enzyme activity such as pregnancy, childbirth, smoking, oral contraceptive use, coffee or alcohol consumption and exercise were also excluded. The study was not restricted by one's program of study, cultural, religious or political affiliations.

2.3 Variables

The dependent variables were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT). The predictor or independent variables were the 2D:4D ratios and the right-left difference (Dr-l). The variables that were considered confounding variables were age at the time of sampling and body mass index (BMI).

2.4 Anthropometric Measurements

The standing height and body weight were measured to the nearest 0.1 cm and 0.1 Kg using a stadiometer and body weighing scale respectively. The body mass index was calculated in Kg/m^2 as body weight/$(height)^2$. The second and fourth finger lengths of both hands were measured from hand scans using computer-assisted analysis [25]. Each finger length was measured from the most proximal basal crease to the tip of the finger [26]. Each finger was measured twice by the same observer and then averaged (Fig. 1). The right (2D:4DR) and left (2D:4DL) digit ratios were calculated as the ratio of the second-to-fourth digit lengths. The right-left 2D:4D difference (Dr-l) was calculated. The intraclass correlation coefficients (ICC) between the repeated measurements were calculated using the two-way mixed, single measures with absolute agreement technique. The ICC were 0.966 and 0.950 for the 2D:4DL and 2D:4DR respectively.

2.5 Laboratory Analyses

Venous blood samples were collected into K_3EDTA anticoagulated tubes. The blood samples were centrifuged at 1500 rpm for 10 minutes to obtain plasma. The plasma samples were aliquoted and stored at -20^{o}C and were never thawed and refrozen until analysis. The plasma samples were analyzed for AST, ALT, ALP and GGT on the BT 1500 automated biochemistry analyzer (Biotechnica Instruments, SPA, Italy) following the manufacturer's instructions and using the recommended reagents. All measurements and sample collection were performed between 8.00 AM and 12.00 PM local time to reduce diurnal variations.

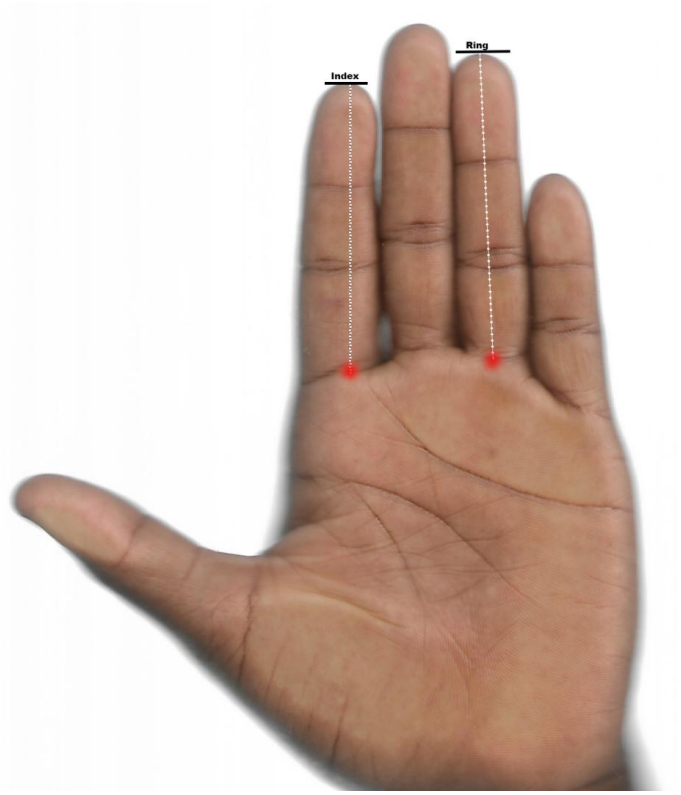


Fig. 1. The scanned image of the palmar surface of the hand. The length of the second and fourth fingers was measured from the most proximal crease to the tip of the finger

2.6 Statistical Analysis

All statistical analyses were performed in SPSS (v23) and GraphPad Prism (v8). The data were for normality and the presence of outliers using the Shapiro-Wilk test. The data were then summarized as mean ± SD, separately for males and females. The differences in male and female mean values were determined using the student t-test (unpaired, 2-tailed). The Dr-l was compared to a reference value of zero for asymmetry, separately for males and females using the one-sample t-test (1-tailed). Moderated linear regression models were then formulated with liver enzymes as the dependent variables and the 2D:4D ratio as the predictor variable. The predictor variables were centered on their means by subtracting the mean value from the variable. Two-way interaction terms were then created between sex and the centered predictor variables (e.g. Sex*2D:4DR-centered). To reduce confounding, the age at the time of sampling and the BMI were added to each model as covariates. To graphically present sex differences in liver enzyme activity, the unstandardized predicted values of the dependent variable were plotted on the y-axis against the mean-centered 2D:4D ratio or Dr-l on the x-axis while sex was made the marking variable. The assumptions of multivariable linear regression were tested using Cooks' distance (Cook's D) for influential multivariable outliers, residual P-P plot for multivariable normality, residual scatter plot for homoscedasticity, the variance inflation factor for multi-collinearity and the Durbin-Watson test for autocorrelation between the independent variables [27]. A P-value <0.050 was considered statistically significant.

3. RESULTS

3.1 Comparison of Male and Female Variables

The male and female variables of the study population are summarized in Table 1. Male participants had a significantly higher ALT and GGT than females (P<0.050). There were no sex differences in the 2D:4D ratio. In the binary logistic regression analysis (Table 2), males had greater odds of increased GGT relative to females [AOR=1.035 (95%CI: 1.004 - 1.067), P=0.025].

Table 1. The comparison of the mean values of male and female variables of the study population

Variable	Female	Male	*t* statistic	P-value
Age (years)	22.3±2.45	23.3±2.60	-1.796	0.076
BMI (Kg/m^2)	23.8±3.58	21.2±4.09	3.331	0.001
AST (IU/L)	21.1±9.95	23.4±14.64	-0.891	0.375
ALT (IU/L)	17.9±11.62	23.5±12.76	-2.229	0.028
ALP (IU/L)	111.6±50.22	116.3±41.42	-0.498	0.620
GGT (IU/L)	24.5±14.52	32.1±16.43	-2.406	0.018
2D:4DR	0.935±0.036	0.935±0.035	-0.020	0.984
2D:4DL	0.937±0.038	0.935±0.033	0.217	0.828
Dr-l	-0.002±0.028	-0.000±0.004	-0.309	0.758

The results are presented as mean ± SD. The mean difference between males and females was compared using the student t-test (unpaired). The Dr-l were compared to the reference value (0) for directional asymmetry using the one-sample t-test (1-tailed). AST= aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase, GGT=gamma-glutamyl transferase

Table 2. The liver enzyme concentration in males relative to females

Variable	*B*	AOR	95%CI of AOR		P-value
			Lower	Upper	
AST (IU/L)	0.008	1.008	0.974	1.044	0.634
ALT (IU/L)	0.027	1.028	0.987	1.070	0.185
ALP (IU/L)	0.003	1.003	0.993	1.013	0.605
GGT (IU/L)	0.035	1.035	1.004	1.067	0.025

Logistic regression analysis with females as the reference sex. The logistic regression models were adjusted for age and body mass index (BMI). AOR=adjusted odds ratio, AST= aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase, GGT=gamma-glutamyl transferase

3.2 Correlation and Linear Relationships between the Dependent and Independent Variables

The correlation and linear relationship between liver enzymes and digit ratios are plotted separately for females (Fig. 2) and males (Fig. 3). In females, the Dr-l was inversely correlated with GGT (r= -0.36, P=0.013). Also, the Dr-l could explain about 13% of the variability of GGT in females (R^2=0.13). However, no significant correlation or relationships were observed in males.

3.3 Interactions between Sex and Digit Ratios on Plasma Liver Enzyme Levels

From Tables 3 and 4, there were no significant interactions between sex and the 2D:4D ratio on adult plasma liver enzyme levels. However, sex differences in the GGT were still significant even after adjusting for age and BMI (Fig. 4). The assumptions of linear regression were tested following recommended guidelines [27,28]. The assumption of homoscedasticity was tested in the univariable regression (Supplementary Figs. S1 and S2). Also, assumptions of multivariable normality and homoscedasticity were tested (Supplementary Fig. S3).

4. DISCUSSION

The study sought to determine whether sex differences in plasma liver enzyme levels are influenced by prenatal hormone exposure as indexed by the 2D:4D ratio. Sex difference in the 2D:4D ratio was not observed and no significant interactions between sex and the 2D:4D ratio on plasma liver enzyme levels were seen. However, males had higher GGT and ALT than females. Moreover, after controlling for age and BMI, only plasma GGT showed a significant sex difference, where males had relatively greater odds of increased GGT than females.

In this study, males had significantly higher plasma ALT and GGT than females. Similar findings have been reported from the Amhara region of Ethiopia, Kenya and Ghana [29-31,4]. However, after controlling for age and BMI, only GGT levels remained significantly higher in males. Gamma-glutamyl transferase is involved in the transfer of amino acids across cell

membranes as well as the metabolism of glutathione and leukotriene [32]. The enzyme, GGT also plays a major role in the maintenance of intracellular homeostasis of oxidative stress [3]. The levels of GGT may rise in response to liver injury and biliary diseases such as fatty liver disease, steatosis, hepatitis and cirrhosis of the liver [33,3,34]. However, among healthy adults, there are sex differences in plasma GGT levels with higher values in males than females.

The 2D:4D ratio could not account for the observed sex differences in plasma GGT levels in adulthood. This may indicate that prenatal exposure to testosterone and estrogen may have no impact on plasma GGT levels among adults. Studies have shown that GGT shows age-specific and female-male variabilities in adulthood, which are not observed in children before adolescence [35]. This may indicate that sex differences in plasma liver enzyme levels may occur postnatally. Developmental changes during puberty and adolescence are probable explanations for sex differences in plasma liver enzyme levels. According to Tahmasebi et al. [36], GGT activity is reduced and without sex differences until adolescence when boys tend to have higher mean values than similarly-aged girls. The genetic factors that influence GGT levels to change over time during adolescence and the impact of these changes may be more pronounced in males than females [35,37]. If sex differences in GGT activity occur in adolescence, it may imply that the activity of sex hormones may partly account for this observation since sex hormones, on average, peak in adolescence [38,39,32].

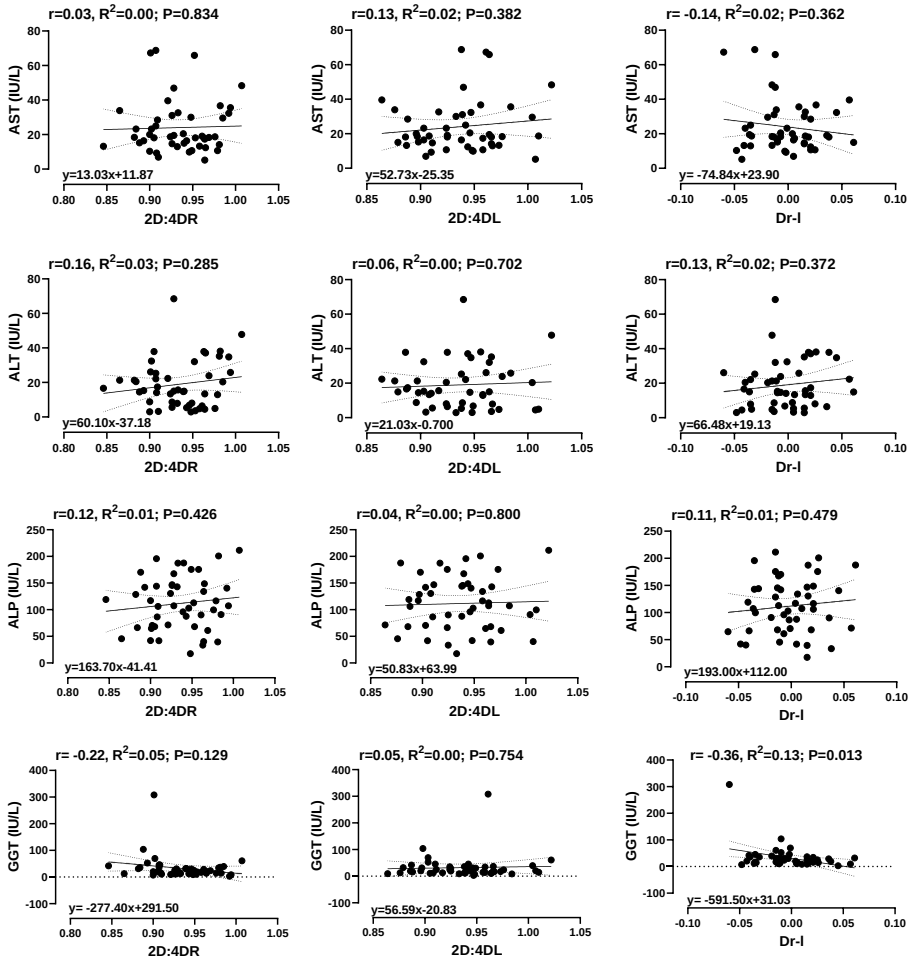


Fig. 2. Correlation and linear relationship between liver enzymes and digit ratios in females. AST= aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase, GGT=gamma-glutamyl transferase

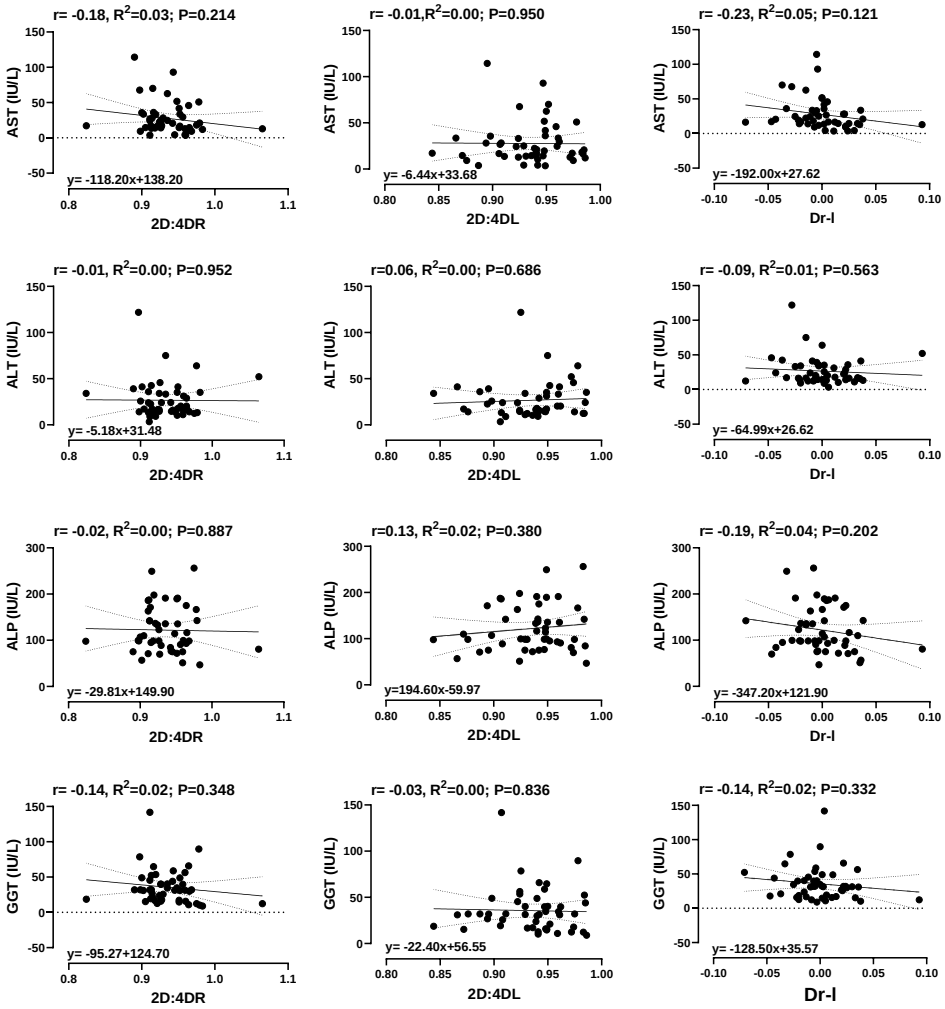


Fig. 3. Correlation and linear relationship between liver enzymes and digit ratios in males. AST= aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase, GGT=gamma-glutamyl transferase

The evidence of the differential effect of testosterone and estrogen on plasma GGT levels has been demonstrated in persons undergoing hormonal therapy although there have been variabilities [8,9,40]. Persons who were undergoing estrogen therapy for gender affirmation have been found to have intrahepatic cholestasis, a condition that may be characterized by elevated plasma GGT. Another source of evidence of the effect of estrogen on plasma GGT levels is the association between pregnancy and cholestasis [41,42]. These observations are, however, contrary to previous studies that have shown that the prevalence of fatty liver disease and other liver disorders are low and their prognosis is better among premenopausal women relative to similarly aged men, postmenopausal women or women with PCOS. These differences in disease prevalence and prognosis have been attributed to the hepato-protective effect of estrogen [7,43,5]. Also, long-term testosterone therapy among men with hypogonadism, complicated by steatosis and metabolic syndrome, tends to have improved liver function with a reduction in GGT levels [8]. This observation may not be consistent as elevated levels of GGT have been found among females on cross-sex hormone therapy using testosterone [44]. The use of plasma GGT as a marker of liver function is however challenged. Some authors argue that GGT cannot replace ALP in the assessment of liver function because GGT is not specific to the liver but may be found in other tissues [8,3].

Table 3. Interactions between sex and the 2D:4D ratio on AST and ALT concentration

LR	Dependent Variable	B	95%CI		P-value
			Lower	Upper	
	AST (IU/L)				
1	(Constant)	25.270	-1.505	52.044	0.064
	Age (years)	0.158	-0.917	1.233	0.771
	BMI (Kg/m2)	-0.323	-1.020	0.373	0.359
	Sex	1.284	-4.351	6.919	0.652
	2D:4DR	51.733	-50.825	154.292	0.319
	Sex*2D:4DR	-85.302	-232.946	62.342	0.254
2	(Constant)	27.630	0.726	54.534	0.044
	Age (years)	0.010	-1.071	1.091	0.985
	BMI (Kg/m2)	-0.285	-0.997	0.427	0.428
	Sex	1.600	-4.060	7.261	0.576
	2D:4DL	24.736	-76.987	126.458	0.630
	Sex*2D:4DL	30.544	-123.265	184.352	0.694
3	(Constant)	25.476	-0.464	51.416	0.054
	Age (years)	0.062	-0.979	1.103	0.906
	BMI (Kg/m2)	-0.240	-0.930	0.449	0.491
	Sex	1.668	-3.869	7.205	0.551
	Dr-l	46.544	-88.512	181.600	0.495
	Sex*Dr-l	-182.466	-372.953	8.021	0.060
	ALT (IU/L)				
1	(Constant)	28.552	3.221	53.882	0.028
	Age (years)	0.240	-0.777	1.257	0.640
	BMI (Kg/m2)	-0.671	-1.330	-0.012	0.046
	Sex	3.585	-1.746	8.916	0.185
	2D:4DR	75.046	-21.981	172.072	0.128
	Sex*2D:4DR	-24.969	-164.649	114.712	0.723
2	(Constant)	27.032	1.287	52.777	0.040
	Age (years)	0.333	-0.701	1.368	0.524
	BMI (Kg/m2)	-0.697	-1.378	-0.016	0.045
	Sex	3.499	-1.917	8.916	0.203
	2D:4DL	40.570	-56.771	137.911	0.410

LR	Dependent Variable	B	95%CI		P-value
			Lower	Upper	
	Sex*2D:4DL	-10.462	-157.645	136.721	0.888
3	(Constant)	24.552	-0.709	49.813	0.057
	Age (years)	0.336	-0.677	1.350	0.511
	BMI (Kg/m2)	-0.592	-1.264	0.080	0.084
	Sex	3.633	-1.760	9.025	0.184
	Dr-l	59.197	-72.325	190.720	0.374
	Sex*Dr-l	-22.489	-207.993	163.015	0.810

Linear regression models with 2-way interaction terms. The 2D:4D were centered on their mean before analysis to reduce multicollinearity. LR=linear regression, AST= aspartate aminotransferase, ALT=alanine aminotransferase

Table 4. Interactions between sex and the 2D:4D ratio on ALP and GGT concentrations

LR	Dependent Variable	B	95%CI		P-value
			Lower	Upper	
	ALP (IU/L)				
1	(Constant)	9.697	-86.624	106.018	0.842
	Age (years)	3.563	-0.304	7.430	0.071
	BMI (Kg/m2)	0.942	-1.564	3.448	0.457
	Sex	3.823	-16.449	24.095	0.709
	2D:4DR	121.478	-247.477	490.433	0.515
	Sex*2D:4DR	-266.620	-797.771	264.531	0.321
2	(Constant)	9.580	-87.597	106.757	0.845
	Age (years)	3.442	-0.463	7.347	0.083
	BMI (Kg/m2)	1.058	-1.512	3.629	0.415
	Sex	4.248	-16.198	24.693	0.681
	2D:4DL	13.155	-354.269	380.579	0.943
	Sex*2D:4DL	-58.175	-613.733	497.384	0.836
3	(Constant)	11.668	-83.174	106.511	0.807
	Age (years)	3.143	-0.663	6.949	0.104
	BMI (Kg/m2)	1.259	-1.263	3.782	0.324
	Sex	5.036	-15.209	25.281	0.622
	Dr-l	196.411	-297.384	690.206	0.431
	Sex*Dr-l	-370.397	-1066.861	326.067	0.293

LR	Dependent Variable	B	95%CI		P-value
			Lower	Upper	
	GGT (IU/L)				
1	(Constant)	21.874	-11.075	54.823	0.191
	Age (years)	0.044	-1.279	1.367	0.947
	BMI (Kg/m2)	0.066	-0.791	0.924	0.878
	Sex	7.803	0.868	14.737	0.028
	2D:4DR	-61.916	-188.126	64.294	0.332
	Sex*2D:4DR	-31.279	-212.972	150.414	0.733
2	(Constant)	26.357	-7.244	59.958	0.123
	Age (years)	-0.168	-1.518	1.182	0.805
	BMI (Kg/m2)	0.078	-0.811	0.967	0.862
	Sex	8.014	0.945	15.084	0.027
	2D:4DL	-4.652	-131.697	122.393	0.942
	Sex*2D:4DL	-1.530	-193.627	190.566	0.987
3	(Constant)	27.386	-4.832	59.604	0.095
	Age (years)	-0.106	-1.399	1.187	0.870
	BMI (Kg/m2)	-0.027	-0.884	0.830	0.950
	Sex	7.897	1.020	14.774	0.025
	Dr-l	-101.470	-269.213	66.272	0.233
	Sex*Dr-l	-41.387	-277.976	195.203	0.729

Linear regression models with 2-way interaction terms. The 2D:4D were centered on their mean before analysis to reduce multicollinearity. LR=linear regression, ALP=alkaline phosphatase, GGT=gamma-glutamyl transferase

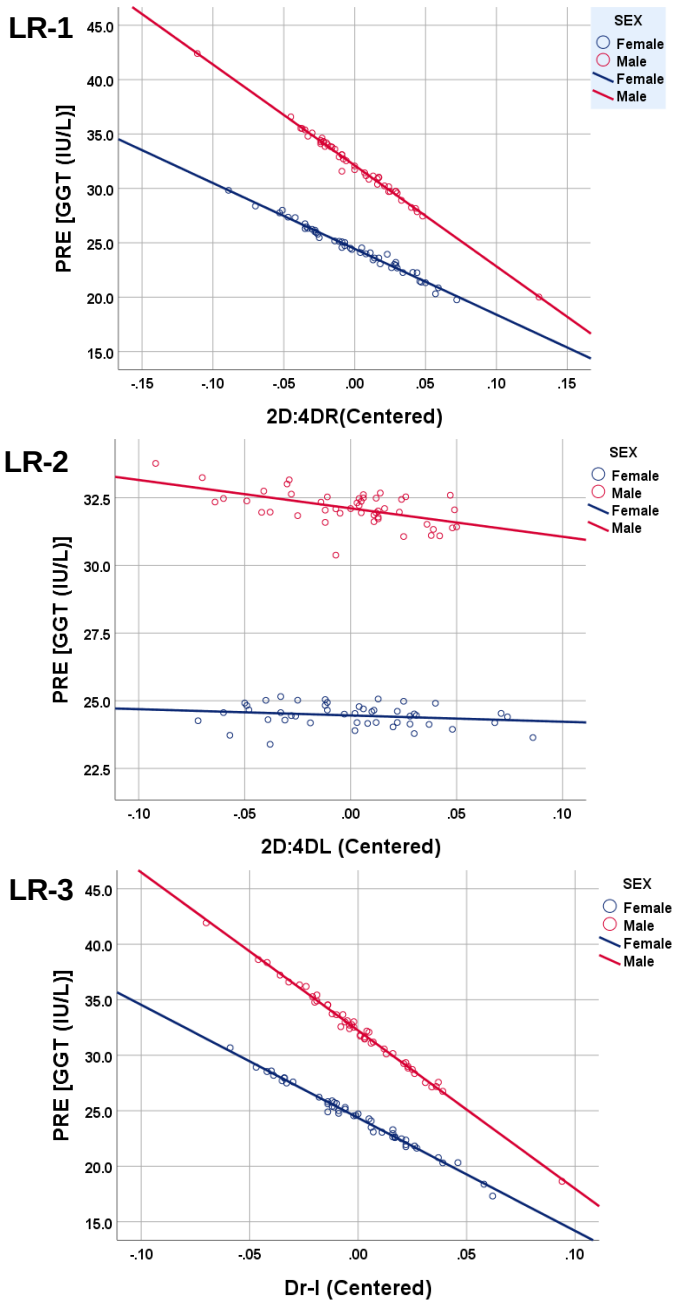


Fig. 4. Moderated linear regression with two-way interaction effects. The unstandardized predicted value (PRE) of the dependent variable (GGT=gamma-glutamyl transferase) was plotted against the centered independent variable (Dr-l). LR=Linear regression

Sex differences in the 2D:4D ratio were not observed and the 2D:4D did not interact with sex on liver enzyme variables. Although previous studies have demonstrated sex differences in digit ratios in human populations, this has not been universal [45,14]. Even though there are critical reviews and overviews in support of the sexual dimorphism in the 2D:4D ratio, some have described it as an allometric artefact since the male hand is usually larger than the female hand. Some have also attributed sexual dimorphism in the 2D:4D ratio to poor methodology in digit length measurements [21,22,14]. Similarly, the suggestion that fetal and adult gonadal activities may be correlated such that the 2D:4D ratio may be a correlate of adult circulating testosterone

and estrogen has not been universal [18,19,15]. Previous studies have sought to show that Leydig cell populations during the fetal period and in adulthood originate from different stem cell populations and that their activities may not, therefore, be correlated [46-48].

The current study has some strengths: Firstly, although sex differences in liver function and plasma liver enzyme levels have been demonstrated [1,3], there has not been any study that has investigated the possible association between plasma liver enzyme levels and the 2D:4D ratio as a marker of prenatal androgen exposure. Secondly, digit lengths were measured by computer-assisted analysis which is a more precise method than direct techniques [25,49]. Thirdly, in the formulation of interaction terms, the predictor variables were centered on their mean to reduce possible multicollinearity and the assumptions of linear regression were also tested [50]. The authors, however, acknowledge that there are population variabilities in digit ratios and liver enzyme activity and will therefore recommend further studies.

5. CONCLUSION

There is sexual dimorphism in gamma-glutamyl transferase activity with higher levels in males than females, independent of age and body mass index. There was no interaction between the marker of prenatal hormone exposure (2D:4D) and sex on liver enzyme activity in adulthood. It may be suggested that sexual dimorphism in adult liver enzyme activity may not be associated with prenatal hormone exposure.

ETHICAL APPROVAL AND CONSENT

The study was approved by the institutional review board of the University for Development Studies (N#: UDS/RB/003/21). Written informed consent was obtained from each participant before the study.

ACKNOWLEDGEMENTS

The authors wish to acknowledge all the participants for consenting to participate in the study. The staff of the Department of Biomedical Laboratory Science are hereby acknowledged for their support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Brie B, Ramirez MC, de winne C, Lopez Vicchi f, Villarruel l, Sorianello e, Catalano P, Ornstein AM, Becu-villalobos D. Brain control of sexually dimorphic liver function and disease: The endocrine connection. Cellular and Molecular Neurobiology. 2019;39:169-180.
2. Belay G, Teklehaymanot G, Gebremariam G, Kaleaye K, Haileslasie H, Gebremichail G, Tesfanchal B, Kahsu G, Berhe B, Tesfay K. Community based reference interval of selected clinical chemistry parameters among apparently healthy adolescents in mekelle city, tigrai, Northern Ethiopia. Plos One. 2020;15:e0231017.
3. Dillon JF, Miller MH. Gamma glutamyl transferase ‘to be or not to be’a liver function test? : Sage Publications Sage UK: London, England; 2016.
4. Dosoo DK, Kayan K, Adu-gyasi D, Kwara E, Ocran J, Osei-kwakye K, Mahama E, Amenga-Etego S, Bilson P, Asante KP. Haematological and biochemical reference values for healthy adults in the middle belt of Ghana. Plos One. 2012;7:e36308.
5. Kurt Z, Barrere-cain R, Laguardia J, Mehrabian M, Pan C, Hui ST, Norheim F, Zhou Z, Hasin Y, Lusis AJ. Tissue-specific pathways and networks underlying sexual dimorphism in non-alcoholic fatty liver disease. Biology of Sex Differences. 2018;9:1-14.
6. Lefebvre P, Staels B. Hepatic sexual dimorphism—Implications for non-alcoholic fatty liver disease. Nature Reviews Endocrinology. 2021;17662-670.
7. Ballestri S, Nascimbeni F, Baldelli E, Marrazzo A, Romagnoli D, Lonardo A. Nafld as a sexual dimorphic disease: Role of gender and reproductive status in the development and progression of nonalcoholic fatty liver disease and inherent cardiovascular risk. Advances in Therapy. 2017;34:1291-1326.
8. Stangl TA, CMW, Defreyne J, Conemans E, ADF, Schreiner T, T'sjoen G, Den Heijer M. Is there a need for liver enzyme monitoring in people using gender-affirming hormone therapy? Eur J Endocrinol. 2021;184:513-520.
9. Yassin AA, alwani M, Talib R, Almehmadi Y, Nettleship JE, Alrumaihi K, Albaba B, Kelly DM, Saad F. Long-term testosterone therapy improves liver parameters and steatosis in hypogonadal men: A

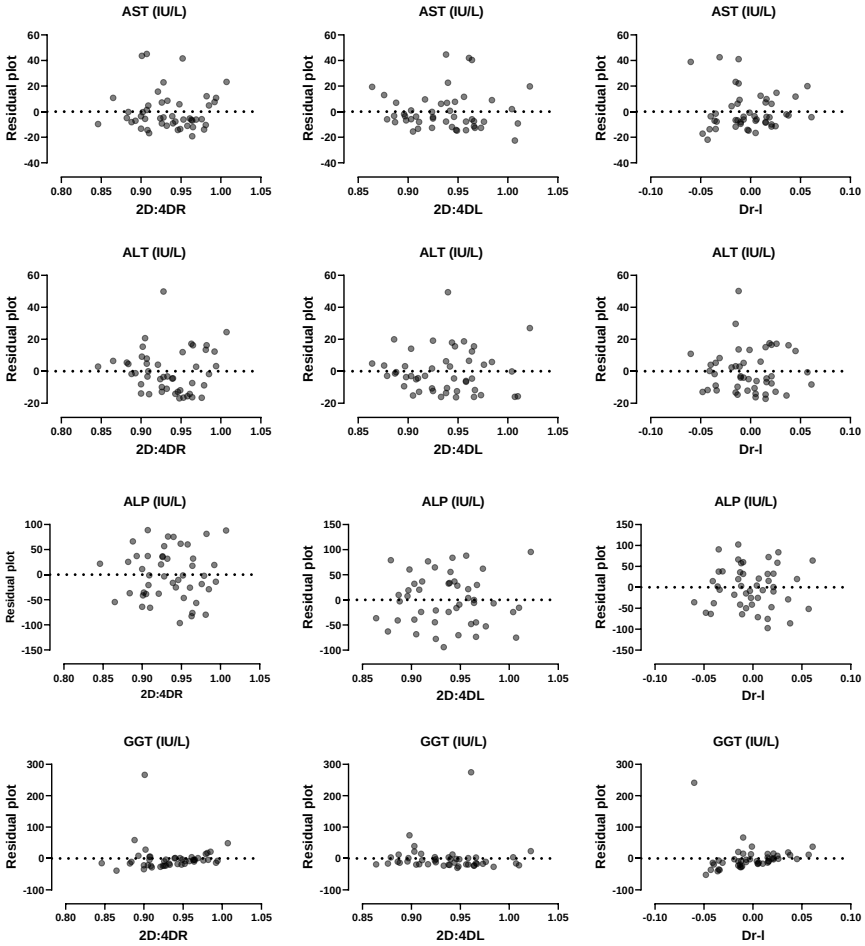
prospective controlled registry study. The Aging Male. 2020;23:1553-1563.
10. Breedlove SM. Minireview: Organizational hypothesis: instances of the fingerpost. Endocrinology. 2010;151:4116-4122.
11. Zheng Z, Cohn MJ. Developmental basis of sexually dimorphic digit ratios. Proceedings of the National Academy of Sciences. 2011;108:16289-16294.
12. Nave G, Koppin CM, Manfredi D, Richards G, Watson SJ, Geffner ME, Yong JE, Kim R, Ross HM, Serrano-Gonzalez M. No evidence for a difference in 2d: 4d ratio between youth with elevated prenatal androgen exposure due to congenital adrenal hyperplasia and controls. Hormones and Behavior. 2021;128: 104908.
13. Richards G, Browne WV, Aydin E, Constantinescu M, Nave G, Kim MS, Watson SJ. Digit ratio (2d: 4d) and congenital adrenal hyperplasia (cah): Systematic literature review and meta-analysis. Hormones and Behavior. 2020;126:104867.
14. Manning Fink B, Mason L, Kasielska-trojan A, Trivers R. The effects of sex, nation, ethnicity, age and self-reported pubertal development on participant-measured right-left 2d: 4d (dr-l) in the bbc internet study. Journal of Biosocial Science. 2022;1-13.
15. Manning Scutt D, Wilson J, Lewis-Jones DI. The ratio of 2nd to 4th digit length: A predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. Hum reprod (oxford, england). 1998;13:3000-3004.
16. Manning JT. Digit ratio: A pointer to fertility, Behavior, and Health, Rutgers University Press; 2002.
17. Richards G, Klimek M, Jasienska G, Marcinkowska UM. Digit ratio (2d: 4d) and circulating testosterone, oestradiol, and progesterone levels across the menstrual cycle. Early Human Development. 2018;117:68-73.
18. Muller DC, Giles GG, Bassett J, Morris HA, Manning JT, Hopper JL, English DR, Severi G. Second to fourth digit ratio (2d: 4d) and concentrations of circulating sex hormones in adulthood. Reproductive Biology and Endocrinology. 2011; 9:1-11.
19. Hönekopp J, Bartholdt L, Beier L, Liebert A. Second to fourth digit length ratio (2d: 4d) and adult sex hormone levels: New data and a meta-analytic review. Psychoneuroendocrinology. 2007;32: 313-321.
20. Leslie M. The mismeasure of hands? Science; 2019.
21. Swift-gallant A, Johnson BA, Di Rita V, Breedlove SM. Through a glass, darkly: Human digit ratios reflect prenatal androgens, imperfectly. Hormones and Behavior. 2020;120:104686.
22. Mccormick CM, Carré JM. Facing off with the phalangeal phenomenon and editorial policies: A commentary on swift-gallant, johnson, di rita and breedlove. Hormones and behavior. 2020;120:104710.
23. Banyeh M, Amidu N, Abdul-wahab D, Zakaria AS. Sexual dimorphism and sex-2d: 4d interactions on fasting lipid variables in an adult ghanaian population. Journal of Lipids; 2022.
24. Banyeh M, Zogli KE, Osumanu HA, Obeng L, Acheampong TK, Dagungong CB, Bayor E, Amidu N. Does sex interact with the 2d: 4d ratio or adult circulating hormones on the estimated glomerular filtration rate? A cross-sectional study in ghana. Physiological Reports. 2022;10:e15516.
25. Fink B, Manning JT. Direct versus indirect measurement of digit ratio: New data from austria and a critical consideration of clarity of report in 2d: 4d studies. Early human development. 2018;127:28-32.
26. Manning JT, Kilduff LP, Trivers R. Digit ratio (2d: 4d) in klinefelter's syndrome. Andrology. 2013;1:94-99.
27. Tranmer M, Elliot M. Multiple linear regression. The Cathie Marsh Centre for Census and Survey Research (ccsr). 2008;5:1-5.
28. Noel J, Kemeza I, Kiaritha EN, Muhwezi D. Parenting styles and self-efficacy among secondary school students in ibanda north, ibanda district, uganda. Studies. 2008;8: 135-148.
29. Mekonnen Z, Amuamuta A, Mulu W, Yimer M, Zenebe Y, Adem Y, Abera B, Gebeyehu W, Gebregziabher Y. Clinical chemistry reference intervals of healthy adult populations in gojjam zones of amhara national regional state, Northwest Ethiopia. Plos one. 2017;12:e0184665.
30. Abebe M, Melku M, Enawgaw B, Birhan W, Deressa T, Terefe B, Baynes HW. Reference intervals of routine clinical chemistry parameters among apparently healthy young adults in amhara national regional state, ethiopia. Plos One. 2018;13:e0201782.

31. Omuse G, Ichihara K, Maina D, Hoffman M, Kagotho E, Kanyua A, Mwangi J, Wambua C, Amayo A, Ojwang P, Premji Z, Erasmus R. Determination of reference intervals for common chemistry and immunoassay tests for kenyan adults based on an internationally harmonized protocol and up-to-date statistical methods. Plos One. 2020;15:e0235234.
32. Kunutsor SK. Gamma-glutamyltransferase—frie%Nd or foe within? Liver International. 2016;36: 1723-1734.
33. Whitfield J. Gamma glutamyl transferase. Critical Reviews in Clinical Laboratory Sciences. 2001;38:263-355.
34. Brennan PN, Dillon JF, Tapper EB. Gamma-glutamyl transferase (γ-gt)–an old dog with new tricks? Liver International. 2022;42:9-15.
35. Middelberg RP, Medland SE, Martin NG, Whitfield JB. A longitudinal genetic study of uric acid and liver enzymes in adolescent twins. Twin Research and Human Genetics. 2007;10:757-764.
36. Tahmasebi H, Higgins V, Woroch A, Asgari S, Adeli K. Pediatric reference intervals for clinical chemistry assays on siemens advia xpt/1800 and dimension exl in the caliper cohort of healthy children and adolescents. Clinica Chimica Acta. 2019;490:88-97.
37. Van beek JH, de Moor MH, de Geus EJ, Lubke GH, Vink JM, Willemsen G, Boomsma DI. The genetic architecture of liver enzyme levels: ggt, alt and ast. Behavior Genetics. 2013;43:329-339.
38. Ansah EO, Abaidoo CS, Diby T, Tetteh J, Appiah AK, Atuahene O, Darko ND. A preliminary anthropometric study of height and sex determination using percutaneous ulnar and femoral lengths. Int J Anat Res. 2017;5:3638-3643.
39. Rissech C, Schaefer M, Malgosa A. Development of the femur—Implications for age and sex determination. Forensic Science International. 2008;180:1-9.
40. Al-qudimat A, Al-zoubi RM, Yassin AA, Alwani M, Aboumarzouk OM, Alrumaihi K, Talib R, al Ansari A. Testosterone treatment improves liver function and reduces cardiovascular risk: a long-term prospective study. Arab J Urol. 2021; 19:376-386.
41. Kremer A, Wolf K, Ständer S. Intrahepatic cholestasis of pregnancy: Rare but important. Der hautarzt; Zeitschrift fur Dermatologie, Venerologie, Und Verwandte Gebiete. 2017;68:95-102.
42. Piechota J, Jelski W. Intrahepatic cholestasis in pregnancy: Review of the literature. Journal of clinical Medicine. 2020;9:1361.
43. Vandel J, Dubois-chevalier J, Gheeraert C, Derudas B, Raverdy V, Thuillier D, Gaal l, Francque S, Pattou F, Staels B. Hepatic molecular signatures highlight the sexual dimorphism of nonalcoholic steatohepatitis (nash). Hepatology. 2021;73:920-936.
44. Vita R, Settineri S, Liotta M, Benvenga S, Trimarchi F. Changes in hormonal and metabolic parameters in transgender subjects on cross-sex hormone therapy: A cohort study. Maturitas. 2018;107:92-96.
45. Marczak M, Misiak M, Sorokowska A, Sorokowski P. No sex difference in digit ratios (2d: 4d) in the traditional yali of papua and its meaning for the previous hypotheses on the inter-populational variability in 2d: 4d. American Journal of Human Biology. 2018;30:e23078.
46. Wu X, Wan S, Lee MM. Key factors in the regulation of fetal and postnatal leydig cell development. Journal of Cellular Physiology. 2007;213:429-433.
47. Dong l, Jelinsky SA, Finger JN, Johnston DS, Kopf GS, Sottas CM, Hardy MP, Ge RS. Gene expression during development of fetal and adult leydig cells. Annals of the New York Academy of Sciences. 2007;1120:16-35.
48. Zirkin BR, Papadopoulos V. Leydig cells: Formation, function, and regulation. Biology of Reproduction. 2018;99:101-111.
49. Ribeiro E, Neave N, Morais RN, Manning JT. Direct versus indirect measurement of digit ratio (2d: 4d) a critical review of the literature and new data. Evolutionary Psychology. 2016;14:1474704916632536.
50. Robinson C, Schumacker RE. Interaction effects: Centering, variance inflation factor and interpretation issues. Multiple Linear Regression Viewpoints. 2009;35:6-11.

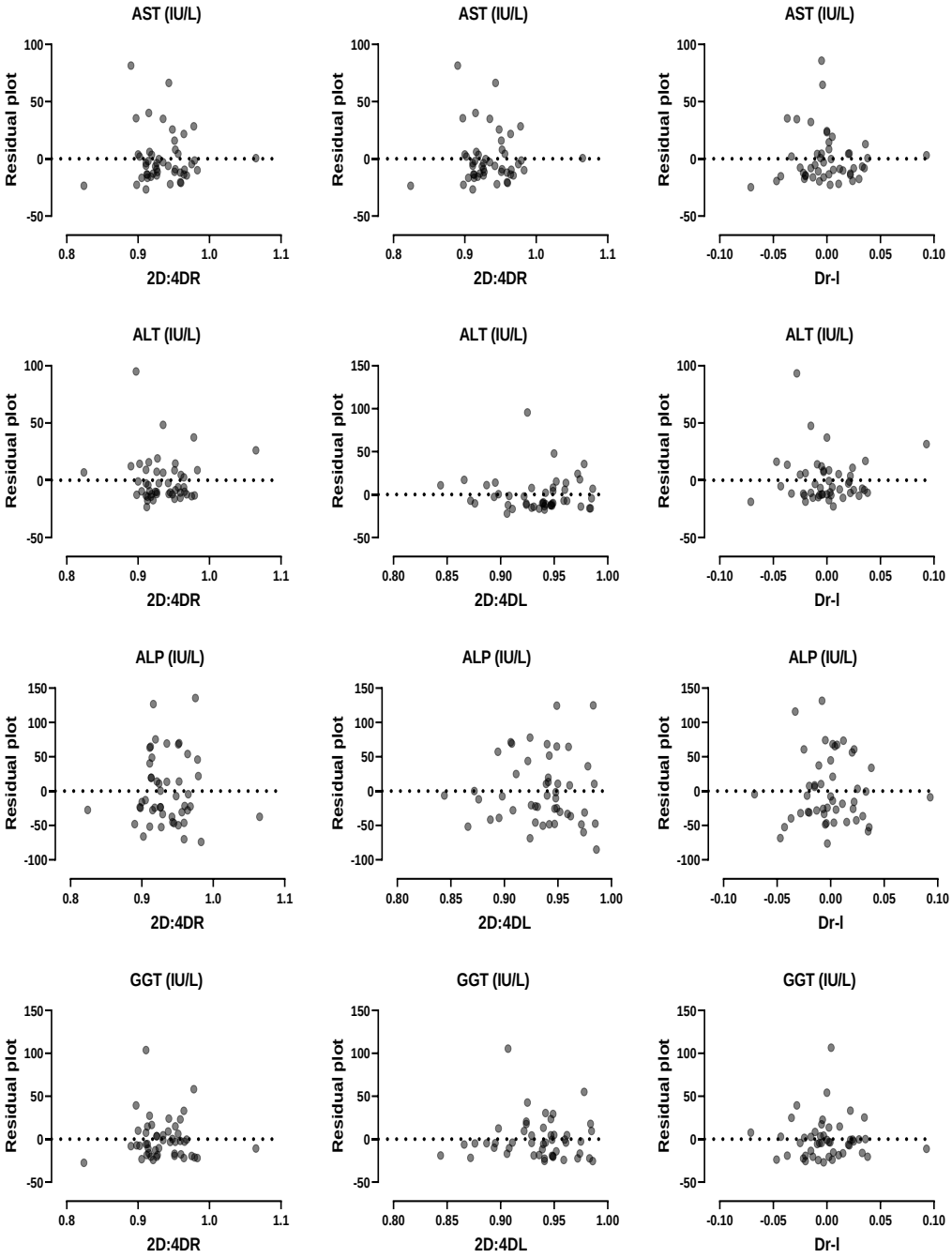
SUPPLEMENTARY

1. The 2D:4D ratio and sex difference in circulating liver enzymes in adulthood? A cross-sectional study in Ghana

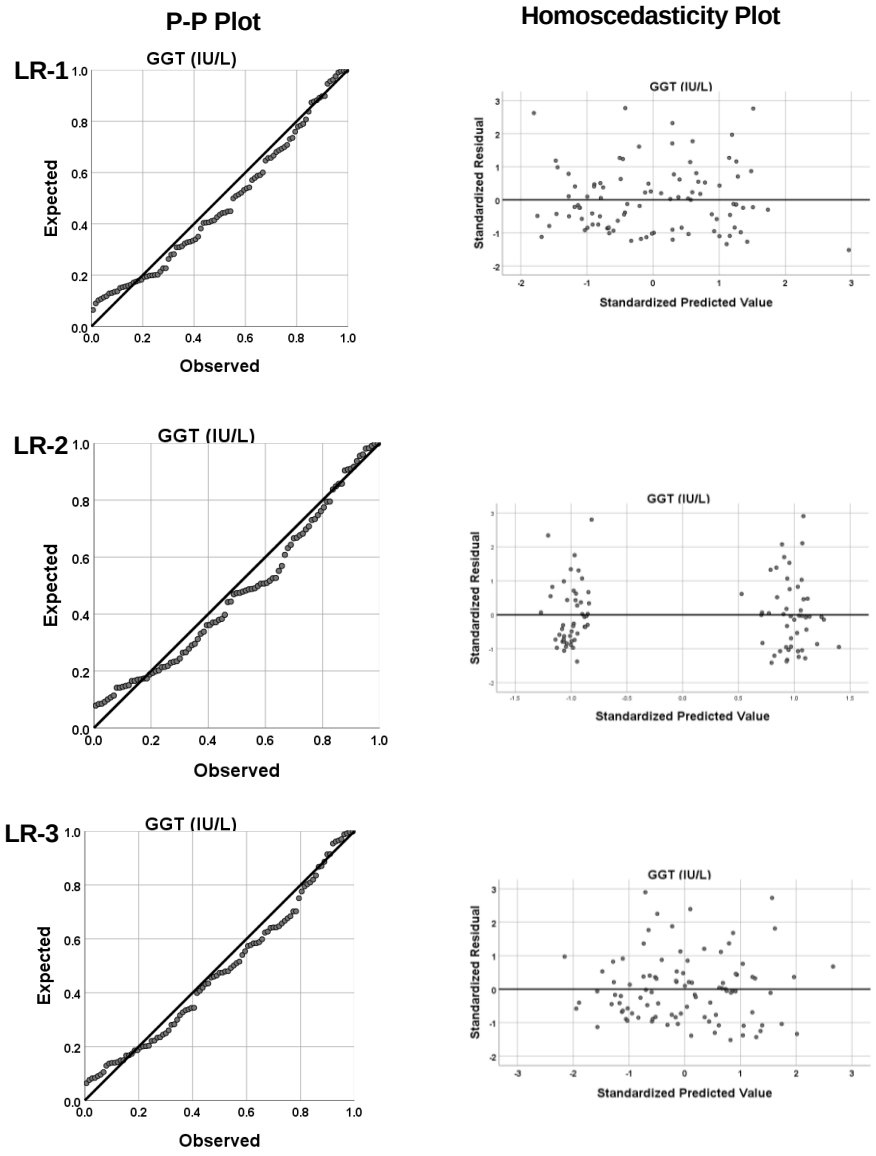
Test of assumptions of linear regression: The assumptions of multivariable linear regression were tested for models in which GGT was the dependent variable (LR1, LR2 and LR3). The following were the outcomes for Durbin-Watson (LR1: 1.80, LR2: 1.75 and LR3: 1.80), VIF (LR1: 1.18-1.99, LR2: 1.22-1.92 and LR3: 1.19-2.01) and the Cook's D (LR1: 0.00-0.19, LR2: 0.00-0.23 and LR3: 0.00-0.07). The assumption of homoscedasticity was tested in the univariable regression (Supplementary Figs. S1 and S2). Also, assumptions of multivariable normality and homoscedasticity were tested (Supplementary Fig. S3). The Dr-l (LR-3) was more reliable in explaining the sex differences in GGT given its better multivariable normality and homoscedasticity. The testing of the assumptions of linear regression is recommended to see the fitness of the models. For a good model fitness in a multivariable analysis, it is recommended that the Durbin-Watson should be within 1.50-2.50, the VIF should be <10, and the Cook's D should be <1.00 [27,28]. These assumptions were met by all the models based on GGT. However, multivariable normality and homoscedasticity varied from model to model. The fitness of the regression residuals onto the diagonal line in the P-P plot was best in LR3 (Dr-l). Similarly, the distribution of regression residuals was more random in LR3 (Dr-l), indicating more homoscedasticity in that model. Although digit ratios had no impact on adult GGT activity, the Dr-l has explained more of the variance in GGT between males and females than the right or left 2D:4D ratio [27].



Supplementary Fig. S1. Residual scatter plots of the univariate linear regression analysis between liver enzymes and digit ratios in females. AST= aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase, GGT=gamma-glutamyl transferase



Supplementary Fig. S2. Residual scatter plots of the univariate linear regression analysis between liver enzymes and digit ratios in males.
AST= aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase, GGT=gamma-glutamyl transferase



Supplementary Fig. S3. Residual scatter plots of the multivariable moderated linear regression analysis between GGT and digit ratios. AST= aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase, GGT=gamma-glutamyl transferase, LR=Linear regression, P-P=probability-probability

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/100130