# Reference Intervals for Clinical Chemistry Analytes for Transgender Men and Women on Stable Hormone Therapy

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**Background:** Gender-affirming hormone therapy with either estradiol or testosterone is commonly prescribed for transgender individuals. Masculinizing or feminizing hormone therapy may impact clinical chemistry analytes, but there is currently a lack of published reference intervals for the transgender population.

**Methods:** Healthy transgender and nonbinary individuals who had been prescribed either estradiol ($n = 93$) or testosterone ($n = 82$) for at least 12 months were recruited from primary care and internal medicine clinics specializing in transgender medical care. Electrolytes, creatinine, urea nitrogen, enzymes (alkaline phosphatase, ALK; alanine aminotransferase, ALT; aspartate aminotransferase, AST; gamma-glutamyltransferase, GGT), hemoglobin A1c, lipids [total cholesterol, high-density lipoprotein (HDL), triglycerides], and high-sensitivity C-reactive protein (hsCRP) were measured on 2 clinical chemistry platforms. Reference intervals (central 95%) were calculated according to Clinical Laboratory Standards Institute guidelines.

**Results:** There was minimal impact of gender-affirming hormone therapy on electrolytes, urea nitrogen, hemoglobin A1c, and hsCRP. In general, the enzymes studied shifted toward affirmed gender. Creatinine values for both transgender cohorts overlaid the reference interval for cisgender men, with no shift toward affirmed gender for the estradiol cohort. The effects on lipids were complex, but with a clear shift to lower HDL values in the testosterone cohort relative to cisgender women.

**Conclusions:** Transgender individuals receiving either masculinizing or feminizing hormone therapy showed significant changes in some analytes that have sex-specific variation in the cisgender population. The clearest shifts toward affirmed gender were seen with enzymes for the estradiol and testosterone cohorts and with creatinine and HDL in the testosterone cohort.

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## IMPACT STATEMENT

Gender-affirming hormone therapy with either estradiol or testosterone is the standard of care for the medical transition of transgender and nonbinary people. Here, we established reference intervals for common clinical chemistry analytes in transgender people administered either feminizing or masculinizing hormone therapy. The influence of the androgen blocker spironolactone in the population receiving estradiol is also evaluated. These results help promote evidence-based medical care for the transgender and nonbinary population.

## INTRODUCTION

Transgender people experience incongruence between gender identity (an individual's personal sense of gender) and sex assigned at birth (1). Transgender men were assigned sex as female at birth but identify as men; conversely, transgender women were assigned sex as male at birth but identify as women. There are also people who identify on the gender spectrum as something other than male or female (often identifying with the umbrella term nonbinary with a number of more specific subterms such as gender-queer, gender fluid, or third gender). A cisgender person is someone whose gender identity is congruent with their sex assigned at birth. Transgender people may seek medical interventions that affirm their gender identity and support psychosocial health. Current standard of care for gender-affirming medical interventions includes hormone therapy and/or surgical procedures as necessary to treat gender dysphoria (2–4).

In terms of hormone therapy, testosterone is prescribed to transgender men or nonbinary people that identify as being masculine of center ("transmasculine") (5). Testosterone administration promotes masculinizing secondary sex characteristics such as lower vocal pitch, increased muscle mass, increased facial and body terminal hair development, and cessation of menses. Estradiol is the mainstay of hormonal therapy for transgender women or nonbinary people that identify as being feminine of center ("transfeminine") (6). In addition, a subset of transgender patients prescribed estradiol may also be administered other medications including progesterone (7) or androgen blockers (e.g., bicalutamide, finasteride, spironolactone) (6).

The physiologic and metabolic changes from gender-affirming hormone therapy may impact the concentrations of analytes in commonly ordered laboratory tests (8, 9). One hypothesis is that significant changes in laboratory values due to gender-affirming hormone therapy would be more likely in laboratory tests such as hemoglobin/hematocrit and creatinine that have significant differences between cisgender males and females (8). The impact of gender-affirming hormone therapy on laboratory tests may also be influenced by pharmacokinetic differences in various pharmaceutical formulations for both estradiol (oral, intramuscular, subcutaneous, topical) and testosterone (topical, subcutaneous, intramuscular). An example of this is illustrated by differences in estrone concentrations for different estradiol preparations used by transgender women (10). Progesterone and androgen blockers may also impact laboratory tests. For instance, spironolactone has effect on mineralocorticoid receptors that can lead to changes in sodium and potassium concentrations (6).

The existing literature on the effects of gender-affirming therapy on laboratory tests

has been mostly from retrospective analyses (11–17) or observational studies that followed transgender people for a variety of physiologic and laboratory parameters after initiation of gender-affirming therapies (18–24). Both approaches have been useful in identifying relative changes in laboratory tests; however, these cohorts include some people with pathophysiology that make it difficult to define normative laboratory ranges. More recent prospective studies with defined inclusion and exclusion criteria have provided data for the impact of gender-affirming hormone therapy on hematology (25) and reproductive endocrinology tests (26, 27). The most clear-cut changes with gender-affirming therapy have been observed with hemoglobin/hematocrit and red blood cell count (25). For these parameters, the reference intervals essentially "flip" to those for the opposite sex (i.e., adopt the reference interval for the affirmed gender). For example, the distribution of hemoglobin/hematocrit values of adult transgender men on stable testosterone therapy aligns with adult cisgender men and not adult cisgender women. Similarly, distribution of hemoglobin/hematocrit values of adult transgender women on stable estrogen administration aligns with adult cisgender women and not adult cisgender men.

The objective of this study was to establish reference intervals for common clinical chemistry analytes in transgender people receiving either masculinizing or feminizing hormone therapy. The analytes studied include electrolytes, enzymes (alkaline phosphatase, ALK; alanine aminotransferase, ALT; aspartate aminotransferase, AST; gamma-glutamyltransferase, GGT), hemoglobin A1c, lipids, urea nitrogen, and creatinine. Some of these analytes, such as creatinine, commonly have sex-specific reference intervals in the cisgender population. An additional objective was to evaluate these analytes on 2 different automated clinical chemistry platforms.

## MATERIALS AND METHODS

### Patient Recruitment, Questionnaires, and Sample Collection

Study participants were prospectively recruited from 2 lesbian, gay, bisexual, transgender, and queer (LGBTQ)-oriented primary care and internal medicine clinics in Seattle, Washington, and Iowa City, Iowa, between November 1, 2017, and July 1, 2018. Consent was obtained for venipuncture collection of whole blood (2 × 5 mL into serum separator tube; also an EDTA tube for hemoglobin A1c). Basic demographic information and hormonal therapy (mode of administration, dose, duration of therapy) were collected using a standardized questionnaire. Study numbers were used in place of participant names; no patient identifiers were retained.

Participants were 18 years or older, self-identified as transgender or gender nonbinary, had been prescribed either testosterone or estradiol gender-affirming hormone therapy for at least 1 year, and consented to collection of relevant samples and information. Specific demographics of the study participants have been previously published (25–27). Exclusion criteria included past history of diabetes, severe cardiovascular event (e.g., myocardial infarction or stroke), clotting or blood cell disorders (e.g., sickle cell anemia, deep venous thrombosis), HIV infection, obstructive sleep apnea, active cigarette use, current pregnancy, or current body mass index >30. Eligibility to participate was not influenced by any other criteria. The Western Institutional Review Board (IRB) approved the protocol for samples collected in Seattle (study number 1179338). The University of Iowa IRB approved the protocol for samples collected in Iowa (study number 201710702). As indicated in the study protocol and informed consent documents, the primary purpose of the research study approved by the IRB was to determine reference intervals for clinical chemistry and hematology analytes in healthy transgender persons.

### Sample Analysis

ALK, ALT, AST, creatinine, GGT, high-density lipoprotein (HDL), high-sensitivity C-reactive protein (hsCRP), potassium, sodium, triglycerides (TRIG), and total cholesterol were measured using Beckman Coulter AU680 and Roche cobas 8000 analyzers. The creatinine method on the Beckman was a kinetic modification of the Jaffe procedure (Beckman Coulter AU System Creatinine). The creatinine method on the Roche analyzer was an enzymatic assay (Creatine Plus v.2). Hemoglobin A1c was measured in whole blood on the Roche cobas 8000 (Iowa cohort) and Bio-Rad D-100 Hemoglobin Testing System (Seattle cohort); both assays are standardized according to International Federation of Clinical Chemistry and transferable to Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program (DCCT/NGSP). Chloride and bicarbonate were measured on the Roche cobas only. Calculated low-density lipoprotein (LDL) was calculated using the Friedewald equation [(calculated LDL) = (total cholesterol) − (HDL) − (TRIG)/5; with units in mg/dL]. Measurements were performed either within 8 h of serum collection (Seattle cohort on DxC, Iowa cohort on cobas) or frozen immediately, stored at −80 °C and measured within 3 months of collection (Iowa cohort on DxC, Seattle cohort on the cobas). The participating laboratories followed standard quality practices and are accredited through the College of American Pathologists.

### Statistical Analysis

Data were analyzed following CLSI C28-A3 (28). In brief, distributions were transformed to achieve normality using a Box–Cox transformation. Normality was assessed using the Shapiro–Wilk test. Outliers were evaluated using the Tukey test on the transformed distributions (29). Confidence intervals for reference limits were calculated using bootstrapping. We used the Wilcoxon rank sum test to test for statistical differences between distributions. *P* values were adjusted for multiple comparisons using the method of Holm (30). Statistical calculations were performed using STATA 17 (STATA LLP). Results were considered significant when $P < 0.01$. We did not have a large enough sample size to stratify the estradiol or testosterone cohorts by age or by route of administration or dosage of gender-affirming therapy. A post hoc power calculation estimates a sample size of 80 (slightly smaller than the estradiol and testosterone cohorts) would be sufficient to detect a difference of 0.5 standard deviations 80% of the time.

The cisgender reference ranges included for comparison were institutional reference ranges (including some from package insert or published literature) or target/desirable values (lipids) as described in the Results. These cisgender ranges are included in Tables 1 and 2. We did not undertake a detailed cisgender reference range study as part of our study. As we do not have the underlying raw data for the cisgender reference ranges, we did not compare cisgender ranges statistically to the transgender reference ranges.

## RESULTS

### Sample Participants

A total of 175 transgender people participated in the study. This included 82 transgender men/nonbinary individuals receiving masculinizing hormones ("testosterone cohort") and 93 transgender women/nonbinary individuals receiving feminizing hormones ("estradiol cohort"). The demographics of these cohorts has been previously published (26, 27).

For the testosterone cohort, the median age was 27 years (range 19–55 years; IQR 23–33 years). The most common testosterone formulations were intramuscular or subcutaneous ($n = 76$, 93% of the cohort; median dose 80 mg/week; range and IQR 50–100 mg/week). For the remainder of the cohort, 5 participants (6.1%) used

**Table 1. Reference intervals and confidence limits for chemistry analytes in transgender women across instruments. Intervals were calculated for all participants.**

| | | Reference limits | | | | | | | Cisgender female range | Cisgender male range |
|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Platform | 2.5 | Low CI | High CI | 97.5 | Low CI | High CI | *N* | | |
| ALP (U/L) | Beckman | 25.5 | 14.1 | 36.9 | 92.5 | 80.8 | 104.2 | 89 | 34–104 | 34–104 |
| ALP (U/L) | Roche | 34.6 | 25.5 | 43.8 | 91.0 | 77.7 | 104.3 | 92 | 35–105 | 40–130 |
| ALT (U/L) | Beckman | 5.0 | 3.1 | 6.9 | 48.9 | 334.2 | 63.6 | 90 | 7–52 | 7–52 |
| ALT (U/L) | Roche | 5.6 | 3.8 | 7.4 | 48.9 | 34.1 | 63.6 | 90 | 10–35 | 10–50 |
| AST (U/L) | Beckman | 10.0 | 9.1 | 10.9 | 30.7 | 25.8 | 35.6 | 90 | 13–39 | 13–39 |
| AST (U/L) | Roche | 9.3 | 7.5 | 11.2 | 33.4 | 27.6 | 39.1 | 92 | 5–32 | 10–40 |
| Chloride (mEq/L) | Roche | 94.3 | 93.1 | 95.6 | 105.7 | 103.7 | 107.7 | 92 | 95–107 | 95–107 |
| Creatinine (mg/dL) | Beckman | 0.57 | 0.53 | 0.61 | 1.05 | 1.00 | 1.11 | 90 | 0.6–1.2 | 0.7–1.3 |
| Creatinine (mg/dL) | Roche | 0.70 | 0.60 | 0.80 | 1.10 | 1.08 | 1.12 | 92 | 0.51–0.95 | 0.67–1.17 |
| GGT (U/L) | Beckman | 6.0 | 5.2 | 6.8 | 31.7 | 25.8 | 37.7 | 90 | 9–64 | 9–64 |
| GGT (U/L) | Roche | 5.3 | 4.4 | 6.2 | 32.7 | 26.7 | 38.6 | 92 | 6–42 | 10–71 |
| HbA1C (%) | Roche + Bio-Rad | 4.2 | 3.8 | 4.6 | 5.8 | 5.6 | 5.9 | 91 | 4.8–6.0 | 4.8–6.0 |
| $HCO_3$ (mEq/L) | Roche | 21.0 | 20.3 | 21.7 | 27.7 | 26.3 | 29.0 | 92 | 22–29 | 22–29 |
| HDL (mg/dL) | Beckman | 37.0 | 30.7 | 43.3 | 88.7 | 82.6 | 94.9 | 90 | ≥50 | ≥40 |
| HDL (mg/dL) | Roche | 32.6 | 27.4 | 37.9 | 97.1 | 83.5 | 110.7 | 92 | ≥50 | ≥40 |
| hsCRP (mg/L) | Beckman | <0.2 | NA | NA | 15.1 | 10.8 | 19.4 | 82 | <10 | <10 |
| hsCRP (mg/L) | Roche | <0.2 | NA | NA | 11.8 | 7.8 | 15.8 | 86 | <5 | <5 |
| LDL calculated (mg/dL) | Beckman | 32.6 | 22.3 | 42.8 | 153.0 | 128.6 | 177.3 | 90 | <130 | <130 |
| LDL calculated (mg/dL) | Roche | 28.0 | 17.0 | 39.0 | 147.0 | 128.9 | 165.2 | 92 | <130 | <130 |
| Potassium (mEq/L) | Beckman | 3.5 | 3.42 | 3.58 | 4.60 | 4.39 | 4.81 | 91 | 3.6–5.2 | 3.6–5.2 |
| Potassium (mEq/L) | Roche | 3.60 | 3.51 | 3.69 | 5.07 | 4.81 | 5.32 | 92 | 3.5–5.0 | 3.5–5.0 |
| Sodium (mEq/L) | Beckman | 135.0 | 133.6 | 136.4 | 142.0 | 138.4 | 145.6 | 91 | 135–145 | 135–145 |
| Sodium (mEq/L) | Roche | 134.0 | 132.7 | 135.3 | 143.0 | 142.8 | 143.2 | 92 | 135–145 | 135–145 |
| Triglycerides (mg/dL) | Beckman | 46.5 | 40.3 | 52.8 | 308.4 | 99.9 | 516.9 | 90 | <150 | <150 |
| Triglycerides (mg/dL) | Roche | 46.3 | 37.8 | 54.7 | 313.2 | 121.1 | 505.3 | 92 | <150 | <150 |
| Total cholesterol (mg/dL) | Beckman | 111.8 | 97.0 | 126.7 | 244.8 | 227.2 | 262.4 | 90 | <200 | <200 |
| Total cholesterol (mg/dL) | Roche | 109.6 | 94.7 | 124.6 | 239.4 | 225.2 | 252.6 | 92 | <200 | <200 |

topical testosterone preparations (median dose 50 mg/week; range and IQR 50–100 mg/week) and 1 participant (1.2%) utilized both intramuscular and topical testosterone preparations.

For the estradiol cohort, the median age was 32 years (range 18–69 years; IQR 26–42 years). The most common estradiol formulations were oral ($n = 55$, 59.1% of the cohort). For the remainder of the cohort, 29 participants (31.2%) administered estradiol by intramuscular or subcutaneous routes, and 9 participants (9.7%) administered topically. Other gender-affirming therapies were as follows: 33 participants (35.5%) administered spironolactone, 11 participants (11.8%) administered progesterone, a single participant (1.1%) administered both spironolactone and progesterone. Two of the participants (2.2%) receiving estradiol identified as nonbinary; the

**Table 2. Reference intervals and confidence limits for chemistry analytes in transgender men across instruments. Intervals were calculated for all participants.**

| Analyte | Platform | Reference limits | | | | | | *N* | Cisgender female range | Cisgender male range |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2.5 | Low CI | High CI | 97.5 | Low CI | High CI | | | |
| ALP (U/L) | Beckman | 44.0 | 37.4 | 50.7 | 100.0 | 94.5 | 15.5 | 80 | 34–104 | 34–104 |
| ALP (U/L) | Roche | 40.9 | 35.5 | 46.2 | 113.3 | 101.3 | 125.3 | 75 | 35–105 | 40–130 |
| ALT (U/L) | Beckman | 7.1 | 5.2 | 8.9 | 51.8 | 43.4 | 60.2 | 81 | 7–52 | 7–52 |
| ALT (U/L) | Roche | 7.8 | 4.3 | 11.2 | 69.1 | 35.2 | 103.0 | 74 | 10–35 | 10–50 |
| AST (U/L) | Beckman | 12.0 | 10.3 | 13.7 | 37.0 | 26.9 | 47.0 | 81 | 13–39 | 13–39 |
| AST (U/L) | Roche | 13.8 | 11.2 | 16.3 | 56.4 | 45.9 | 66.8 | 74 | 5–32 | 10–40 |
| Chloride (mEq/L) | Roche | 97.0 | 96.2 | 97.8 | 107.0 | 105.2 | 108.8 | 76 | 95–107 | 95–107 |
| Creatinine (mg/dL) | Beckman | 0.60 | 0.51 | 0.69 | 1.06 | 0.98 | 1.14 | 79 | 0.6–1.2 | 0.7–1.3 |
| Creatinine (mg/dL) | Roche | 0.69 | 0.61 | 0.78 | 1.21 | 1.10 | 1.31 | 76 | 0.51–0.95 | 0.67–1.17 |
| GGT (U/L) | Beckman | 6.0 | 4.3 | 7.7 | 62.0 | 27.9 | 96.0 | 80 | 9–64 | 9–64 |
| GGT (U/L) | Roche | 7.0 | 6.0 | 8.0 | 67.2 | 29.9 | 104.5 | 75 | 6–42 | 10–71 |
| HbA1C (%) | Roche + Bio-Rad | 4.60 | 4.50 | 4.70 | 5.60 | 5.37 | 5.83 | 80 | 4.8–6.0 | 4.8–6.0 |
| $HCO_3^-$ (mEq/L) | Roche | 19.0 | 18.0 | 20.0 | 29.1 | 28.0 | 30.1 | 76 | 22–29 | 22–29 |
| HDL (mg/dL) | Beckman | 32.0 | 29.8 | 34.2 | 66.0 | 62.5 | 69.5 | 79 | ≥ 50 | ≥ 40 |
| HDL (mg/dL) | Roche | 29.6 | 26.3 | 32.9 | 72.1 | 66.8 | 77.4 | 74 | ≥50 | ≥40 |
| hsCRP (mg/L) | Beckman | 0.3 | 0.2 | 0.4 | 31.0 | 13.0 | 49.0 | 75 | <10 | <10 |
| hsCRP (mg/L) | Roche | 0.1 | 0.1 | 0.2 | 21.0 | 13.3 | 28.7 | 73 | <5 | <5 |
| LDL calculated (mg/dL) | AU | 55.8 | 45.3 | 66.3 | 194.1 | 167.9 | 220.4 | 78 | <130 | <130 |
| LDL calculated (mg/dL) | Roche | 58.5 | 45.7 | 71.3 | 171.6 | 145.0 | 198.2 | 74 | <130 | <130 |
| Potassium (mEq/L) | Beckman | 3.6 | 3.48 | 3.71 | 4.50 | 4.40 | 4.60 | 79 | 3.6–5.2 | 3.6–5.2 |
| Potassium (mEq/L) | Roche | 3.70 | 3.48 | 3.89 | 5.11 | 4.92 | 5.30 | 76 | 3.5–5.0 | 3.5–5.0 |
| Sodium (mEq/L) | Beckman | 137.0 | 136.5 | 137.5 | 144.0 | 143.3 | 144.7 | 81 | 135–145 | 135–145 |
| Sodium (mEq/L) | Roche | 137.9 | 137.0 | 138.8 | 145.1 | 144.0 | 146.1 | 76 | 135–145 | 135–145 |
| Triglycerides (mg/dL) | Beckman | 47.9 | 42.3 | 53.6 | 337.1 | 279.9 | 394.3 | 78 | <150 | <150 |
| Triglycerides (mg/dL) | Roche | 44.1 | 36.4 | 51.8 | 348.7 | 293.6 | 403.9 | 73 | <150 | <150 |
| Total cholesterol (mg/dL) | Beckman | 119.0 | 106.0 | 132.0 | 267.0 | 233.7 | 300.2 | 81 | <200 | <200 |
| Total cholesterol (mg/dL) | Roche | 120.0 | 108.5 | 131.5 | 272.1 | 238.9 | 305.3 | 75 | <200 | <200 |

other 91 participants (97.8%) identified as transgender women.

## Reference Intervals

Reference intervals and confidence limits for the estradiol cohort for all analytes on the 2 chemistry platforms were calculated and are listed in Table 1. Similarly, reference intervals and confidence limits for the testosterone cohort are listed in Table 2. The number of samples included in the reference ranges in Tables 1 and 2 corresponds to the number of specimens for which an analyte measurement could be obtained (i.e., measurement not prevented by sample volume exhausted or error), with the exception of the following outliers that were excluded from reference range determination for a particular

instrument/analyte combination: estradiol cohort—ALK (2 samples on Beckman), ALT (1 sample on Roche), hsCRP (4 samples on Roche), and hemoglobin A1c (1 sample); testosterone cohort, HDL (1 sample on Beckman). The reference intervals and confidence limits for the subsets of the estradiol cohort either taking spironolactone or not taking spironolactone are listed in Supplemental Tables 1 and 2, in the online Data Supplement, respectively.

### Comparison of the Testosterone and Estradiol Cohorts

Figure 1 shows the reference intervals for the enzymes ALK, GGT, ALT, and AST for the estradiol and testosterone cohorts using Roche cobas data compared to institutional cisgender reference ranges for these analytes. In general, the values shift toward the affirmed gender, e.g., the estradiol cohort for these 4 analytes is more similar to the cisgender female reference range than to the cisgender male reference range. Supplemental Fig. 1 compares the enzyme data between the Roche cobas and Beckman AU680 instruments.

Figure 2 shows transgender reference intervals for total cholesterol, TRIG, HDL, and calculated LDL for the Roche cobas data compared with cisgender target/desirable values from the 2014 National Lipid Association recommendations

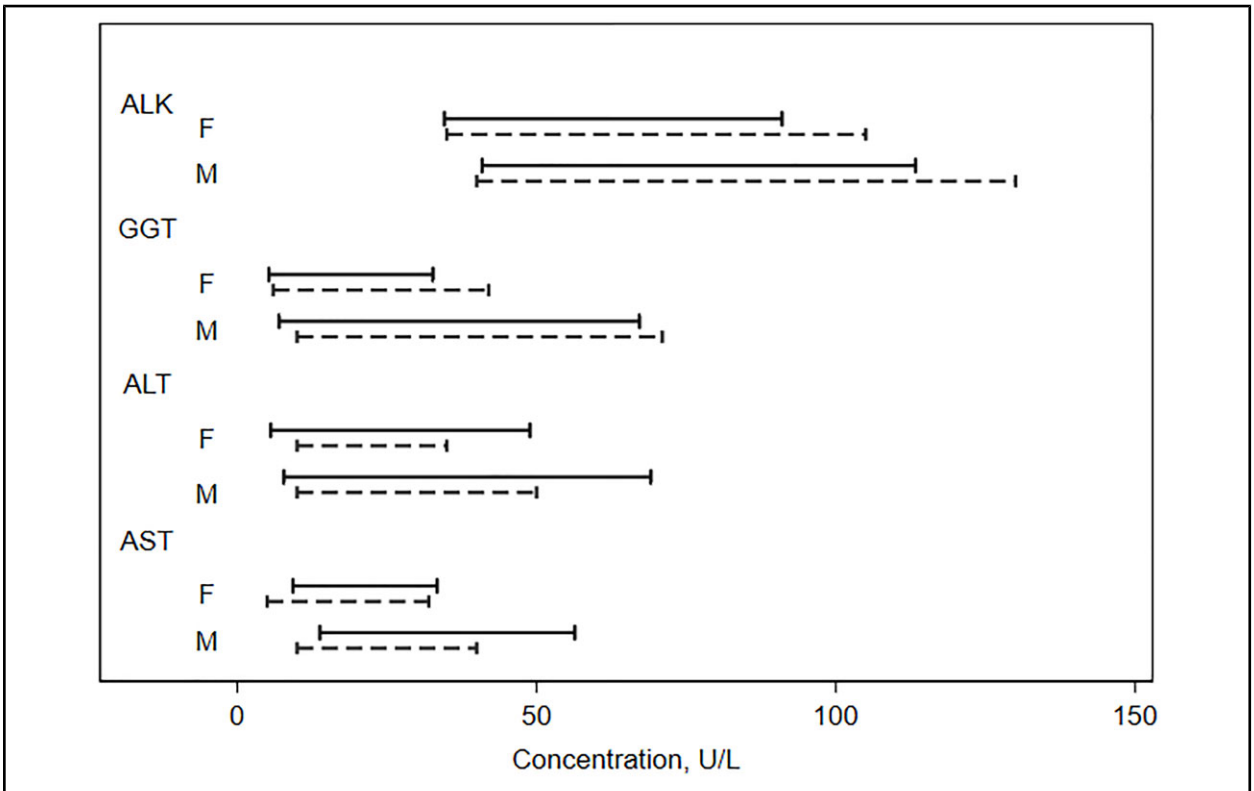


**Fig. 1. Reference ranges for alkaline phosphatase (ALK), gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). F = cisgender female or transgender estradiol cohort, M = cisgender men or transgender testosterone cohort. Solid line = transgender reference ranges from the current study, dashed line = cisgender reference ranges from University of Iowa. Data is for Roche cobas analyzers.**

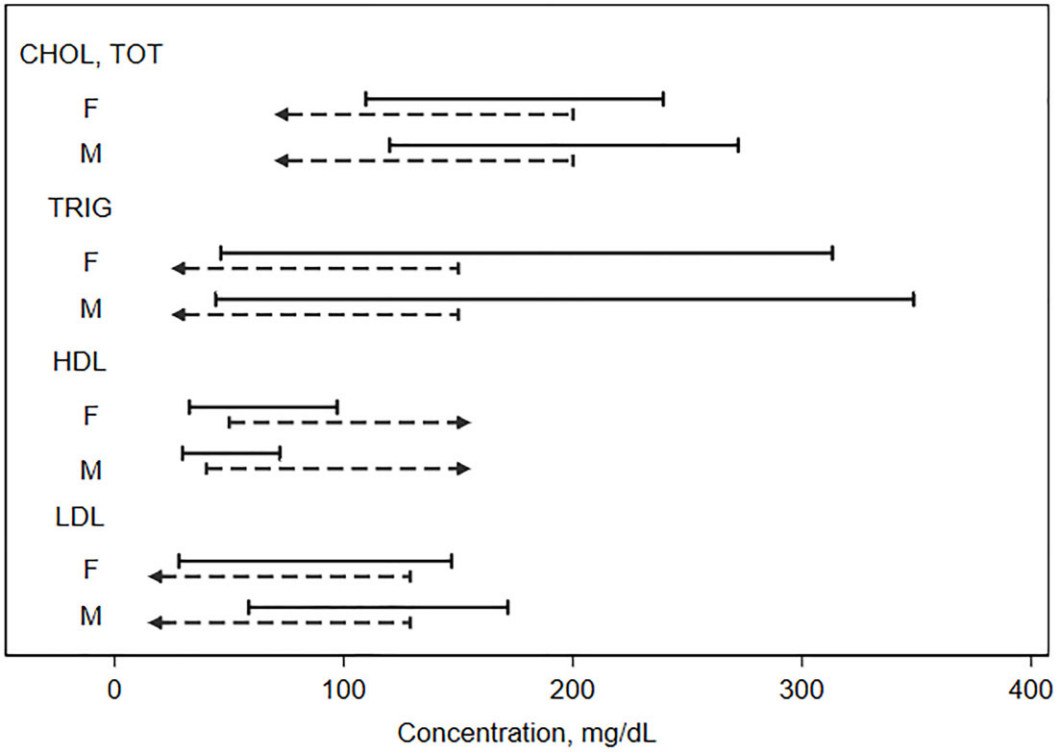


**Fig. 2. Reference ranges for total cholesterol (CHOL, TOT), triglycerides (TRIG), high-density lipoprotein (HDL), and calculated low-density lipoprotein (LDL). F = cisgender female or transgender estradiol cohort, M = cisgender men or transgender testosterone cohort. Solid line = transgender reference ranges (current study), dashed line = target/desirable ranges (see Results; arrows indicate single boundary range). Data are for Roche cobas analyzers.**

(TRIG < 150 mg/dL; HDL ≥ 40 mg/dL for men, ≥ 50 mg/dL for women; LDL < 130 mg/dL) (31) and National Cholesterol Education Program (NCEP) Adult Treatment Plan III (total cholesterol <200 mg/dL) (32). Supplemental Fig. 2 compares the lipid data between the Roche cobas and Beckman AU680 instruments.

Figure 3 shows the reference intervals for creatinine for the estradiol and testosterone cohorts using Roche cobas data compared with the institutional cisgender reference ranges. The creatinine values for the testosterone and estradiol cohorts both overlay with the cisgender male reference range and are distinct from the cisgender female reference range. Supplemental Fig. 3 compares the creatinine data between the Roche cobas and Beckman AU680 instruments.

Figure 4 shows the reference intervals for sodium and potassium for the estradiol cohort either taking spironolactone or not. There is a slight but statistically significant difference (Wilcoxon rank sum test, $P = 0.002$) between the distributions for sodium with and without spironolactone. In contrast, there is no statistically significant difference (Wilcoxon rank sum test, $P = 0.13$) between distributions for potassium with and without spironolactone. Supplemental Fig. 4 plots histograms of the sodium and potassium distributions of the estradiol cohort either taking spironolactone or not taking spironolactone. Reference intervals and

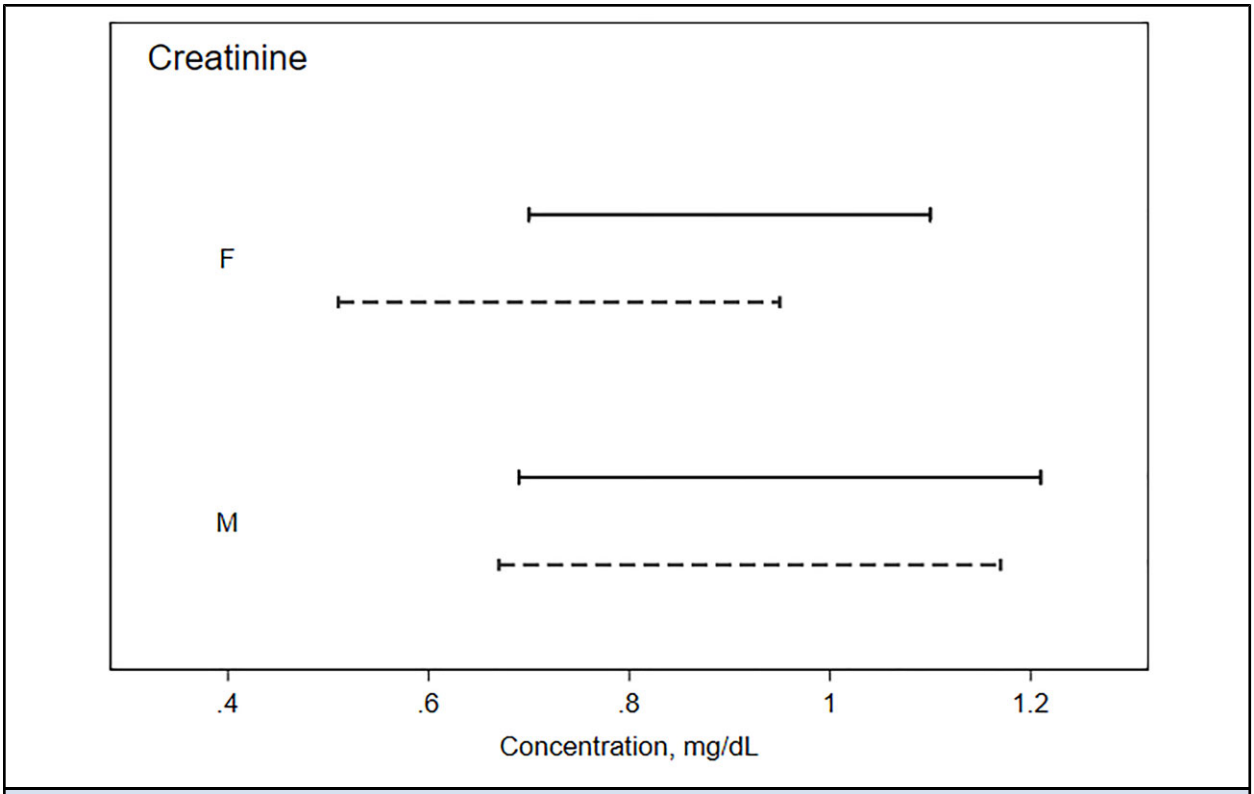


**Fig. 3. Comparison of creatinine levels in cisgender and transgender people. F = cisgender female or transgender estradiol cohort, M = cisgender men or transgender testosterone cohort. Solid line = transgender references ranges from the current study, dashed line = cisgender reference ranges from University of Iowa. Data is for Roche cobas analyzers.**

confidence limits for all analytes for these subsets of the estradiol cohort are listed in Supplemental Tables 1 and 2.

### Comparison of Beckman and Roche Reference Intervals

In general, reference intervals for the analytes on the Beckman AU680 and Roche cobas platforms showed slight or negligible differences from one another (Tables 1 and 2; Supplemental Tables 1 and 2; Supplemental Figs. 1–3). The most notable difference were wider ranges for Roche for the testosterone cohort for ALK, GGT, and ALT compared to Beckman (Supplemental Fig. 1). Creatinine measured on Beckman also trended slightly lower than Roche (Supplemental Fig. 3).

## DISCUSSION

Establishing proper reference ranges is a complicated area of laboratory medicine. In the present study, we determine reference intervals in the adult transgender population for common clinical chemistry analytes including electrolytes, enzymes, lipids, and creatinine. In Supplemental Tables 3 and 4, we compare the results of our study with the findings in prior retrospective and observational (including longitudinal) studies in the transgender population (11–24). In comparing between the present and prior studies, it is important to point out that conditions that were exclusions in our study (e.g., severe cardiovascular disease, diabetes, obesity, cigarette smoking) were

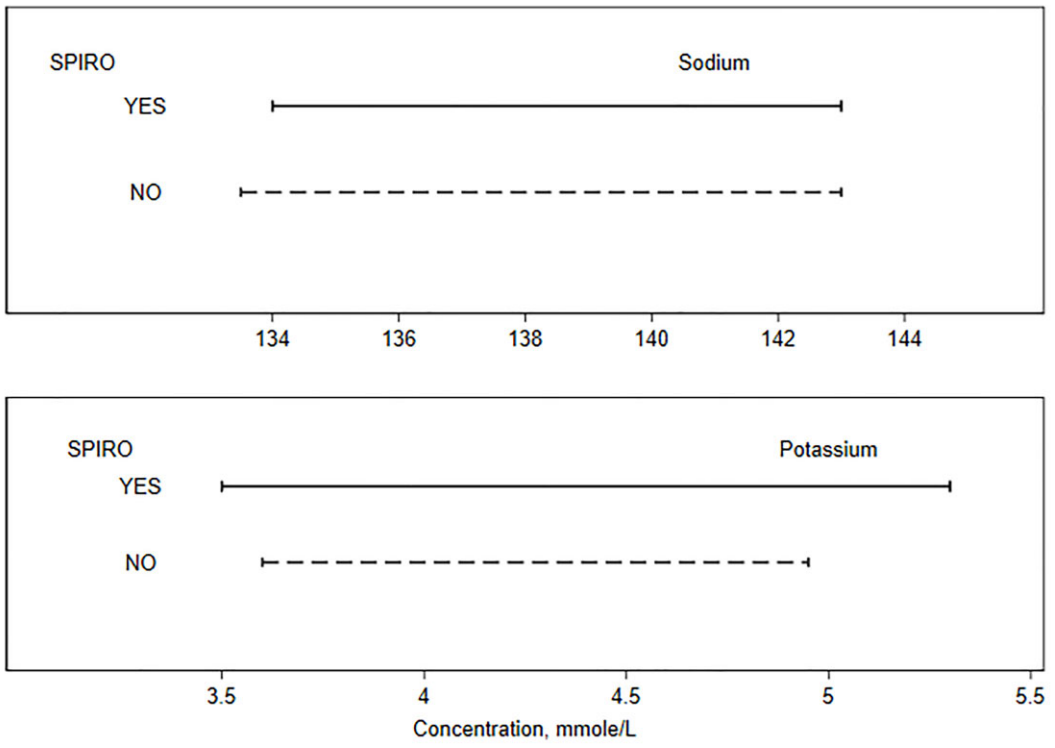


**Fig. 4. Impact of spironolactone (SPIRO) on sodium and potassium levels in the estradiol cohort. Solid line = reference ranges for those in the estradiol cohort taking spironolactone, dashed line = reference ranges for those in the estradiol cohort not taking spironolactone. Data is for Roche cobas analyzers.**

typically not exclusions for the retrospective and observational studies.

The enzymes analyzed in the present study (ALK, ALT, AST, and GGT), often used to assess liver and biliary tract function, showed relative increases in transgender men compared to cisgender women. These findings in transgender men mirror the relative trends reported from retrospective and observational studies (11, 13, 16, 17, 20, 21, 24, 31) (Supplemental Table 4). In contrast, minimal effect or slight decrease on these enzymes was observed in transgender women, consistent with previous studies (11, 13, 15–17, 20, 24). Two recent studies have examined large transgender cohorts and concluded that while there are some slight changes in liver function enzymes in the transgender population, these are unlikely to be clinically meaningful in most cases (17, 20). Nevertheless, gender-affirming hormonal therapy could have implications for clinical laboratories that have sex-specific reference ranges for the liver function enzymes. For example, slight increases in these enzymes in transgender men could move enzyme concentrations slightly outside the cisgender female reference intervals but potentially still within cisgender male reference intervals. In contrast, a slight decrease in these enzymes in transgender women could mask early detection of entities such as nonalcoholic fatty liver disease. It should be noted that the present study did not include any questions or exclusions for recent or chronic ethanol use among the participants, and thus the GGT concentrations should be interpreted with that limitation in mind. Slight

changes in liver mass could be one factor impacting the changes observed in our study and others. Studies in the general population demonstrate relatively increased liver mass in men compared to women, although with significant interindividual variability (33, 34). The impact of gender-affirming hormone therapy on liver mass has not yet been explored in the transgender population.

The impact of gender-affirming hormones on lipids has shown variable results across prior retrospective and observational studies, especially with LDL, TRIG, and total cholesterol (summarized in Supplemental Tables 3 and 4). The most robust trend across studies has been a decrease in HDL in transgender men (11, 13, 16, 18, 19, 21, 22, 24), a finding also observed in the present study. The impact of these lipid concentration changes on overall health in the transgender population has not yet been determined. In addition to gender-affirming therapies, multiple other factors that may occur in the transition process (e.g., changes in diet, weight gain or loss, surgeries) could impact lipids and cardiovascular health. However, awareness of potential changes in lipids following hormone administration can help with interpretation of laboratory studies and decisions on therapies. For example, the National Lipid Association has separate desirable HDL targets for men and women (31), and hormone therapy in transgender men could result in HDL values that go below the target for cisgender women (50 mg/dL) but above the target for cisgender men (40 mg/dL).

We observed that the reference interval for creatinine for transgender men was essentially the same as for cisgender men and increased relative to cisgender women. This finding has also been observed in prior retrospective and observational studies (11–14, 16, 23, 24). In contrast, in our study, creatinine in transgender women was basically unchanged from the reference range for cisgender men. These findings have implications for calculating estimated glomerular filtration rate (eGFR). Equations for eGFR have changed and adapted over time, with recent critical analysis of the inclusion of race-based factors (35). In transgender men, calculation of eGFR using female as the sex in equations could lead to underestimation of kidney function, with implications for assignment of renal failure class and kidney transplantation eligibility or for a decision such as administration of intravenous contrast dye or other agents that may impact the kidney (36, 37). One alternative to creatinine-based eGFR equations in the transgender population would be use of cystatin C-based equations (either using only cystatin C or both cystatin C and creatinine) or more direct GFR determinations in situations where eGFR by creatinine-based equation is near a classification cutpoint (35). However, cystatin C-based equations also often use sex as a variable. To our knowledge, cystatin C has not been studied in the transgender population.

Reference intervals for electrolytes (chloride, bicarbonate, potassium, and sodium), hsCRP, and hemoglobin A1c were relatively unchanged in our study relative to cisgender ranges. Our study criteria excluded known diagnosis of diabetes so the observed results were consistent with those criteria. There was a slight but statistically significant difference in sodium concentration in the subset of transgender women taking spironolactone compared to those not taking spironolactone; in contrast, potassium concentrations were not significantly different between these groups. The impact on sodium concentrations is a known consequence of spironolactone impacting the mineralocorticoid system (38), although the magnitude of changes observed may not be clinically meaningful in many cases. However, the exclusion criteria in our study (e.g., diabetes, prior severe cardiovascular event) and relatively young age of the cohort likely reduced the number of subjects with factors such as administration of angiotensin converting enzyme or angiotensin receptor antagonist medications that may also impact the

mineralocorticoid system. As seen in our study, only a subset of transgender women receiving estradiol are prescribed medication to block androgen effects (e.g., bicalutamide, finasteride, spironolactone), a decision that balances the patient goals for gender-affirming therapy with the risk/benefit and sometimes expense of the various medication options. The clinical and laboratory monitoring of androgen blockers in transgender women is an area of debate (6); the limited data for our study suggest the impact of spironolactone on laboratory parameters is low in healthy transgender individuals.

There are some limitations to our study. First, samples were collected at a single time interval, limiting observations over time. Second, the sample size was limited to 93 participants taking feminizing hormones and 82 participants taking masculinizing hormones. Study recruitment was challenging even at 2 separate sites providing LGBTQ care, and future studies would benefit from larger initiatives, as done through international collaborations for pediatric biochemical reference ranges (39). Larger sizes are also necessary to resolve subgroup differences we could not resolve in our study, including impact of medication dosage and route of administration. Third, 36.6% of the estradiol cohort in our study was prescribed spironolactone as an antiandrogen. There is considerable variability in antiandrogen use in transgender women, with spironolactone more common in the USA and cyproterone more common in some parts of Europe (6). Even within the USA, there can be variable prescribing practices, and other institutions may see higher or lower proportions of spironolactone antiandrogen therapy than in our study. Fourth, we applied the Wilcoxon rank sum, a nonparametric test that makes no assumptions about the underlying distribution, across all tests. This approach increases risk of Type II errors (failure to detect a difference when one exists) but avoids the complex alternative to find best-fit parametric distributions to all data series in our study. Last, some of the exclusion criteria for our prospective study are for conditions that may be more common in the transgender population, including diabetes, obesity, and tobacco use (40–42). These exclusion criteria impacted recruitment. There are significant opportunities for future studies on the interaction of disease and social factors with laboratory testing in the transgender population.

In summary, we have established reference intervals for commonly measured clinical chemistry analytes in the transgender population. These reference intervals can aid laboratories and healthcare providers in providing evidence-based care for the transgender population.

## SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

**Nonstandard Abbreviations:** ALK, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LGBTQ, lesbian, gay, bisexual, transgender, and queer; IRB, Institutional Review Board; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; TRIG, triglycerides; LDL, low-density lipoprotein

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## REFERENCES

1. Winter S, Diamond M, Green J, Karasic D, Reed T, Whittle S, Wylie K. Transgender people: health at the margins of society. Lancet 2016;388:390–400.
2. Coleman E, Bockting W, Botzer M, Cohen-Kettenis P, DeCuypere G, Feldman J, et al. Standards of care for the health of transsexual, transgender, and gender-nonconforming people. Version 7. Int J Transgend 2012;13:165–232.
3. Hembree WC, Cohen-Kettenis PT, Gooren L, Hannema SE, Meyer WJ, Murad MH, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2017;102:3869–903.
4. Irwig MS. Transgender care by endocrinologists in the United States. Endocr Pract 2016;22:832–6.
5. Irwig MS. Testosterone therapy for transgender men. Lancet Diabetes Endocrinol 2017;5:301–11.
6. Tangpricha V, den Heijer M. Oestrogen and anti-androgen therapy for transgender women. Lancet Diabetes Endocrinol 2017;5:291–300.
7. Prior JC. Progesterone is important for transgender women's therapy-applying evidence for the benefits of progesterone in ciswomen. J Clin Endocrinol Metab 2019; 104:1181–6.
8. Goldstein Z, Corneil TA, Greene DN. When gender identity doesn't equal sex recorded at birth: the role of the laboratory in providing effective healthcare to the transgender community. Clin Chem 2017;63: 1342–52.
9. Gupta S, Imborek KL, Krasowski MD. Challenges in transgender healthcare: the pathology perspective. Lab Med 2016;47:180–8.
10. Cirrincione LR, Winston McPherson G, Rongitsch J, Sadilkova K, Drees JC, Krasowski MD, et al. Sublingual estradiol is associated with higher estrone concentrations than transdermal or injectable preparations in transgender women and gender nonbinary adults. LGBT Health 2021;8:125–32.
11. Allen AN, Jiao R, Day P, Pagels P, Gimpel N, SoRelle JA. Dynamic impact of hormone therapy on laboratory values in transgender patients over time. J Appl Lab Med 2021;6: 27–40.
12. Fernandez JD, Tannock LR. Metabolic effects of hormone therapy in transgender patients. Endocr Pract 2016;22: 383–8.
13. Humble RM, Imborek KL, Nisly N, Greene DN, Krasowski MD. Common hormone therapies used to care for transgender patients influence laboratory results. J Appl Lab Med 2019;3:799–814.
14. Maheshwari A, Dines V, Saul D, Nippoldt T, Kattah A, Davidge-Pitts C. The effect of gender-affirming hormone therapy on serum creatinine in transgender individuals. Endocr Pract 2021;28:52–7.
15. Roberts TK, Kraft CS, French D, Ji W, Wu AH, Tangpricha V, Fantz CR. Interpreting laboratory results in transgender patients on hormone therapy. Am J Med 2014;127: 159–62.
16. SoRelle JA, Jiao R, Gao E, Veazey J, Frame I, Quinn AM, et al. Impact of hormone therapy on laboratory values in transgender patients. Clin Chem 2019;65:170–9.
17. Stangl TA, Wiepjes CM, Defreyne J, Conemans E, Fisher AD, Schreiner T, et al. Is there a need for liver enzyme monitoring in people using gender-affirming hormone therapy? Eur J Endocrinol 2021;184:513–20.
18. Colizzi M, Costa R, Scaramuzzi F, Palumbo C, Tyropani M, Pace V, et al. Concomitant psychiatric problems and hormonal treatment induced metabolic syndrome in gender dysphoria individuals: a 2 year follow-up study. J Psychosom Res 2015;78:399–406.
19. Deutsch MB, Bhakri V, Kubicek K. Effects of cross-sex hormone treatment on transgender women and men. Obstet Gynecol 2015;125:605–10.
20. Hashemi L, Zhang Q, Getahun D, Jasuja GK, McCracken C, Pisegna J, et al. Longitudinal changes in liver enzyme levels among transgender people receiving gender affirming hormone therapy. J Sex Med 2021;18:1662–75.
21. Mueller A, Haeberle L, Zollver H, Claassen T, Kronawitter D, Oppelt PG, et al. Effects of intramuscular testosterone undecanoate on body composition and bone mineral density in female-to-male transsexuals. J Sex Med 2010;7: 3190–8.
22. Pelusi C, Costantino A, Martelli V, Lambertini M, Bazzocchi A, Ponti F, et al. Effects of three different testosterone formulations in female-to-male transsexual persons. J Sex Med 2014;11:3002–11.
23. Wierckx K, Mueller S, Weyers S, Van Caenegem E, Roef G, Heylens G, T'Sjoen G. Long-term evaluation of cross-sex hormone treatment in transsexual persons. J Sex Med 2012;9:2641–51.
24. Wierckx K, Van Caenegem E, Schreiner T, Haraldsen I, Fisher AD, Toye K, et al. Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: results from the European network for the investigation of gender incongruence. J Sex Med 2014;11:1999–2011.

25. Greene DN, McPherson GW, Rongitsch J, Imborek KL, Schmidt RL, Humble RM, et al. Hematology reference intervals for transgender adults on stable hormone therapy. Clin Chim Acta 2019;492:84–90.
26. Greene DN, Schmidt RL, Winston McPherson G, Rongitsch J, Imborek KL, Dickerson JA, et al. Reproductive endocrinology reference intervals for transgender women on stable hormone therapy. J Appl Lab Med 2021; 6:15–26.
27. Greene DN, Schmidt RL, Winston-McPherson G, Rongitsch J, Imborek KL, Dickerson JA, et al. Reproductive endocrinology reference intervals for transgender men on stable hormone therapy. J Appl Lab Med 2021;6: 41–50.
28. CLSI. CLSI document C28-A3. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline. 3rd Ed. Wayne (PA):CLSI;2008.
29. Tukey JW. Exploratory data analysis reading. Boston (MA): Addison-Wesley Publishing Company; 1977.
30. Sankoh AJ, Huque MF, Dubey SD. Some comments on frequently used multiple endpoint adjustment methods in clinical trials. Stat Med 1997;16:2529–42.
31. Jacobson TA, Ito MK, Maki KC, Orringer CE, Bays HE, Jones PH, et al. National Lipid Association recommendations for patient-centered management of dyslipidemia: part 1 - executive summary. J Clin Lipidol 2014;8:473–88.
32. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation 2002;106: 3143–421.
33. Chan SC, Liu CL, Lo CM, Lam BK, Lee EW, Wong Y, Fan ST. Estimating liver weight of adults by body weight and gender. World J Gastroenterol 2006;12:2217–22.
34. Chouker A, Martignoni A, Dugas M, Eisenmenger W, Schauer R, Kaufmann I, et al. Estimation of liver size for liver transplantation: the impact of age and gender. Liver Transpl 2004;10:678–85.
35. Delgado C, Baweja M, Crews DC, Eneanya ND, Gadegbeku CA, Inker LA, et al. A unifying approach for GFR estimation: recommendations of the NKF-ASN task force on reassessing the inclusion of race in diagnosing kidney disease. Am J Kidney Dis 2022;79:268–88.e1.
36. Fadich SK, Kalayjian A, Greene DN, Cirrincione LR. A retrospective analysis of creatinine-based kidney function with and without sex assigned at birth among transgender adults. [Epub ahead of print] Ann Pharmacother October 20, 2021, as doi:10.1177/10600280211050120.
37. Whitley CT, Greene DN. Transgender man being evaluated for a kidney transplant. Clin Chem 2017;63:1680–3.
38. Sofer Y, Yaish I, Yaron M, Bach MY, Stern N, Greenman Y. Differential endocrine and metabolic effects of testosterone suppressive agents in transgender women. Endocr Pract 2020;26:883–90.
39. Karbasy K, Ariadne P, Gaglione S, Nieuwesteeg M, Adeli K. Advances in pediatric reference intervals for biochemical markers: establishment of the caliper database in healthy children and adolescents. J Med Biochem 2015;34:23–30.
40. Defreyne J, De Bacquer D, Shadid S, Lapauw B, T'Sjoen G. Is type 1 diabetes mellitus more prevalent than expected in transgender persons? A local observation. Sex Med 2017;5:e215–8.
41. Kyinn M, Banks K, Leemaqz SY, Sarkodie E, Goldstein D, Irwig MS. Weight gain and obesity rates in transgender and gender-diverse adults before and during hormone therapy. Int J Obes (Lond) 2021;45:2562–9.
42. Tami-Maury I, Sharma A, Chen M, Blalock J, Ortiz J, Weaver L, Shete S. Comparing smoking behavior between female-to-male and male-to-female transgender adults. Tob Prev Cessat 2020;6:2.