NT-proBNP Reference Intervals in Healthy U.S. Children, Adolescents, and Adults

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Background: N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a cardiac biomarker used in the clinical management of heart failure. We sought to create updated reference intervals for NT-proBNP for healthy US children, adolescents, and adults.

Methods: We identified a population of healthy individuals using the 1999 to 2004 cycles of the National Health and Nutrition Examination Survey (NHANES). We measured serum NT-proBNP in 12 346 adults and 15 752 children and adolescents with the Elecsys NT-proBNP assay on the Roche e601 autoanalyzer. We compared 4 methods for reference interval calculation, and presented the final reference intervals using the robust method partitioned by age and sex categories.

Results: NT-proBNP values were available for 1949 healthy adults and 5250 healthy children and adolescents. NT-proBNP concentrations in males and females varied according to age, being higher in early childhood, relatively lower in late adolescence, and highest through middle age and older age. Females tended to have higher NT-proBNP concentrations compared to men from late adolescence until middle age. The upper reference limit, or 97.5th percentile, for 50 to 59 year-old men was 225 ng/L (90% CI: 158 to 236), and for 50 to 59 year-old women, 292 ng/L (90% CI: 242 to 348).

Conclusions: Among healthy individuals, NT-proBNP concentrations varied greatly according age and sex. The reference intervals presented here should inform future clinical decision limits and suggest that age- and sex-specific intervals may be necessary to more precisely characterize risk.

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IMPACT STATEMENT

NT-proBNP is used in the clinical management of heart failure. Individuals at risk for heart failure across the entire life span stand to benefit from accurate and tailored reference intervals that distinguish between normal and abnormally elevated NT-proBNP. We found that the upper limit of NT-proBNP in healthy individuals, the 97.5th percentile, varied tremendously according to age and sex. Our results indicate that the commonly used cutoff of 125 ng/L is not suitable for all population subgroups.

INTRODUCTION

N-terminal pro B-type natriuretic peptide (NT-proBNP) is a cardiac biomarker used in the clinical management of heart failure (1). Though cardiovascular society guidelines endorse NT-proBNP testing, there are no universally accepted cut-points that delineate abnormal from normal (1–5). Establishing a singular cutoff is difficult because natriuretic peptides are used in many settings both to rule in and rule out disease (6). Defining the empirical distribution of NT-proBNP in healthy individuals can aid in distinguishing typical values from extreme values, thus informing optimal cutoffs for clinical practice.

The Clinical and Laboratory Standards Institute (CLSI) defines a reference interval as the range between lower and upper reference limits in a healthy population (7). The lower and upper reference limits are conventionally set to the 2.5th and 97.5th percentiles to capture the central 95% of reference values. Prior NT-proBNP reference intervals have been calculated, but have varied substantially due to differences in laboratory methods, statistical methodology, and characteristics of the underlying populations in which these intervals were derived (8–15).

We measured NT-proBNP concentrations in a large, nationally representative sample of US adults, adolescents, and children, and used demographic, medical questionnaire, laboratory, and physical examination data to rigorously identify a healthy subset of the population without disease or major cardiovascular risk factors. We partitioned the reference population by age and sex, compared multiple methods for constructing reference intervals, and present the calculated upper reference limits.

MATERIALS AND METHODS

Study Population

The National Health and Nutrition Examination Survey (NHANES) is a continuously operating cross-sectional study of community dwelling children and adults in the United States (16). Approximately 5000 individuals are selected each year from multiple counties across the country to obtain a diverse and representative sample. Participants provide demographic, socioeconomic, medical history, and prescription medication information during the interview component and contribute body measurement and clinical data during the examination component. Participants who provided consent and were 12 months or older at the time of examination underwent a blood draw. For the 1999–2004 survey cycles, as part of the NHANES Biospecimen Program, surplus serum was collected and transferred into cryovials for long-term storage at −80°C in the CDC and Agency for Toxic Substances and Disease Registry Specimen Packaging, Inventory, and Repository (17). The measurement of NT-proBNP in stored serum was approved by the ethics review board of the National Center for Health Statistics.

Measurement of NT-proBNP

We obtained aliquots of all available stored serum samples from participants in NHANES 1999–2004. NT-proBNP was measured on the Roche e601 autoanalyzer using the Elecsys NT-proBNP assay at the University of Maryland School of Medicine Clinical Pathology Laboratory from 2018 to 2020. The coefficient of variation was 3.1% for low concentrations of NT-proBNP (mean, 46 ng/L) and 2.7% for high concentrations (mean, 32 805 ng/L). The lower limit of detection for this assay was 5 ng/L and 723 measurements below the lower limit of detection were replaced with 3.54 ng/L ($5/\sqrt{2}$), and 3 measurements above the limit of detection were replaced with 35 000 ng/L. Of the 21 206 serum samples tested, 20 063 (95%) were pristine, meaning they had never undergone a freeze–thaw cycle.

Statistical Analysis

Selection of healthy reference individuals. We used NT-proBNP measured in a healthy subsample of individuals to determine the reference interval. We defined this subsample by excluding individuals if they reported taking any hematologic or cardiovascular medications, were underweight, overweight, obese, were missing information on body mass index (BMI), had a history of hypertension, had a history of diabetes, or had hemoglobin A1c ≥6.5%, were treated for anemia in the past 3 months, or had an estimated glomerular filtration rate of <60 mL/min/1.73 m^2.

Adults 20 years and older were excluded if they reported having a history of congestive heart failure, coronary heart disease, angina, myocardial infarction, stroke, emphysema, bronchitis, liver disease, hypercholesterolemia, or prescription for a cholesterol lowering medication, kidney disease, dialysis, or cancer. BMI was calculated for individuals younger than 24 months using World Health Organization *z*-scores for individuals aged 2 to 19 years, using CDC *z*-scores, and for adults 20 years and older, the weight in kilograms divided by the square of height in meters (18, 19). Children were considered to be in the normal BMI category if their *z*-score was between −2 and +1, and for adults, if their BMI was between 18.5 and 25 kg/m^2 (normal weight). The estimated glomerular filtration rate was calculated for adults aged 20 years and older with the 2021 CKD-EPI creatinine–cystatin C equation, and for individuals younger than 20 years, with the 2021 CKiD U25 equations (20, 21).

The reference population was partitioned by age and sex due to prior evidence of sex and age-based differences in natriuretic peptide concentration among healthy individuals (22–28). We partitioned adults by decade, and used the NHANES analytic guidelines to determine age categories for children and adolescents (29).

Reference interval calculation. We examined the natural distribution of NT-proBNP as a continuous function of age in the healthy subsample using survey weighted quantile regression, with a natural cubic spline of age, stratified by sex, to estimate the 97.5th quantile for males and females separately. We used the age, in months, at the time of the NHANES examination, and age at the time of the interview if examination age was unavailable. Because the distribution of NT-proBNP was right-skewed, reference interval calculations were performed on log-transformed NT-proBNP, and the final estimates with confidence intervals were exponentiated. We assessed the normality assumption of log-transformed NT-proBNP with Kolmogorov–Smirnov and Shapiro–Wilk tests.

We compared quantile regression, parametric, nonparametric, and robust methods to construct the reference intervals (7, 30, 31). Because the sample size for healthy individuals was limited in the oldest age categories, we grouped all adults aged 70 years or older, and used the robust method, which has been shown to have sufficient power even in very small samples (32). We did not remove extreme values within the set of reference values

for healthy individuals under the assumption that these values reflect true variability in the full distribution of NT-proBNP (33). For all 4 methods, we reported the 90% confidence interval for the 2.5th and 97.5th percentile reference limits.

Survey weighting. The sample data in NHANES is weighted to account for survey sampling and nonresponse bias to match as close as possible to the US Census population (16). NHANES survey weights were used for quantile regressions and calculating the proportions of individuals with a NT-proBNP measurement ≥125 and 450 ng/L, age-based and FDA-approved used cutoffs for NT-proBNP in the ambulatory setting to detect heart failure (3, 5). The baseline characteristics tables and the parametric, nonparametric, and robust calculations were created without using survey weights.

Statistical software. All analyses were performed in R (version 4.2.2). The reference intervals were calculated with the referenceIntervals (version 1.3.0) package, and the survey package was used to account for the NHANES sample design (version 4.1–1) (34, 35). Data files used for this analysis are available online from the National Center for Health Statistics and the statistical code to fully reproduce these analyses is available as a Quarto document (Supplemental File 2).

RESULTS

Study Sample

NT-proBNP values were available for 12 346 of the 15 376 adult participants (≥ 20 years) and 8860 of the 15 752 pediatric participants (<20 years of age) in the 1999–2004 NHANES survey cycles. Overall, 15.8% of adult participants were apparently healthy (1949 individuals), and 59.3% of participants between age 1 and 19 were apparently healthy (5250 individuals) (Tables 1 and 2). The number of apparently healthy reference individuals within each sex/age group was >200 for all age groups between 2 and 39 years, but for other age groups, the number of reference individuals ranged between 43 and 157 (Table 3).

Reference Intervals

We regressed the 97.5th percentile of NT-proBNP on the natural cubic spline of age with 3 degrees of freedom, stratified by sex (Fig. 1). The 97.5th percentile upper reference limit for natural distribution of NT-proBNP varied according to age and sex (Fig. 2). The upper reference limit for NT-proBNP was 379 ng/L (90% CI: 303 to 493) in 1-year-old boys, and 367 ng/L (90% CI: 290 to 475) in 1 year-old girls (Table 3). This upper limit was lower through childhood, adolescence, and early adulthood, reaching a nadir of 84 (90% CI: 77 to 91) in 16 to 19 year-old boys, and 149 ng/mL (90% CI: 137 to 162) in 16 to 19 year-old girls. The reference limit was higher with each decade of life after young adulthood and was much higher in older adults. Men aged 70 and older had an upper reference limit of 1286 ng/L (90% CI: 849 to 1930), and women aged 70 and older had an upper reference limit of 976 ng/L (90% CI: 664 to 1510). Females had similar NT-proBNP concentrations compared to males in childhood and late adulthood, but had higher NT-proBNP concentrations than males starting from adolescence until approximately age 50. Reference intervals for children and adolescents partitioned by year are included in Supplemental Table 1 and reference intervals with separate partitions for adults aged 70 to 79 and 80 or older are included in Supplemental Table 2.

The 4 different methods for estimating the upper reference limit produced similar results when the sample size for any particular group was approximately 100 or greater, but there was substantial variability between methods for groups with smaller sample sizes. In particular, the nonparametric method tended to produce estimates that were larger than the other methods when sample size was small. The methods were successful in producing estimates with confidence

Table 1. Baseline characteristics of NHANES adults, 1999–2004.

Characteristic	Healthy participants, n = 1949[a]	Excluded participants, n = 10 397[a]	Overall, N = 12 346[a]
Age at examination (years)	34 (25, 45)	52 (36, 68)	48 (33, 66)
Gender			
Male	944 (48%)	4955 (48%)	5899 (48%)
Female	1005 (52%)	5442 (52%)	6447 (52%)
Race/ethnicity			
Mexican American	426 (22%)	2365 (23%)	2791 (23%)
Other Hispanic	88 (4.5%)	478 (4.6%)	566 (4.6%)
Non-Hispanic White	1012 (52%)	5339 (51%)	6351 (51%)
Non-Hispanic Black	322 (17%)	1881 (18%)	2203 (18%)
Other race—including multiracial	101 (5.2%)	334 (3.2%)	435 (3.5%)
NT-proBNP (ng/L)	38 (19, 71)	55 (25, 135)	51 (24, 119)
BMI category			
Underweight	—	179 (1.7%)	179 (1.4%)
Normal weight	1949 (100%)	1675 (16%)	3624 (29%)
Overweight	—	4332 (42%)	4332 (35%)
Obese	—	3871 (37%)	3871 (31%)
Missing	—	340 (3.3%)	340 (2.8%)
Reported any hematologic or cardiovascular medication	—	3587 (35%)	3587 (29%)
History of hypertension	—	3899 (38%)	3899 (32%)
Prescribed antihypertensive medication	—	3171 (30%)	3171 (26%)
History of hypercholesterolemia	—	3344 (32%)	3344 (27%)
Prescribed hypercholesterolemia medication	—	1789 (17%)	1789 (14%)
History of diabetes	—	1207 (12%)	1207 (9.8%)
Hemoglobin A1c ≥6.5	—	1102 (11%)	1102 (8.9%)
History of kidney disease	—	344 (3.3%)	344 (2.8%)
eGFR <60 mL/min/1.73 m^2	—	757 (7.3%)	757 (6.1%)
Receiving dialysis	—	13 (0.1%)	13 (0.1%)
Receiving anemia treatment	—	423 (4.1%)	423 (3.4%)
History of congestive heart failure	—	389 (3.7%)	389 (3.2%)
History of coronary heart disease	—	555 (5.3%)	555 (4.5%)
History of angina	—	459 (4.4%)	459 (3.7%)
History of myocardial infarction	—	575 (5.5%)	575 (4.7%)
History of stroke	—	419 (4.0%)	419 (3.4%)
History of emphysema or bronchitis	—	913 (8.8%)	913 (7.4%)
History of liver disease	—	390 (3.8%)	390 (3.2%)
History of cancer	—	1060 (10%)	1060 (8.6%)

This table displays the baseline characteristics of 12 346 adults who participated in the combined NHANES cycles from 1999–2004, divided into apparently healthy and excluded participants. The proportion of individuals belonging to each characteristic are given in parentheses. The median age and NT-proBNP concentrations are displayed, along with the interquartile interval in parentheses (25^{th}, 75^{th}).
[a]Median (25^{th}, 75^{th} percentiles); n (%).

Table 2. Baseline characteristics of NHANES children and adolescents, 1999–2004.

Characteristic	Healthy participants, n = 5250[a]	Excluded participants, n = 3610[a]	Overall, N = 8860[a]
Age at examination (years)	13.0 (9.0, 16.0)	13.0 (10.0, 17.0)	13.0 (9.0, 16.0)
Gender			
Male	2639 (50%)	1831 (51%)	4470 (50%)
Female	2611 (50%)	1779 (49%)	4390 (50%)
Race/Ethnicity			
Mexican American	1737 (33%)	1335 (37%)	3072 (35%)
Other Hispanic	219 (4.2%)	154 (4.3%)	373 (4.2%)
Non-Hispanic White	1501 (29%)	831 (23%)	2332 (26%)
Non-Hispanic Black	1575 (30%)	1157 (32%)	2732 (31%)
Other Race—Including Multiracial	218 (4.2%)	133 (3.7%)	351 (4.0%)
NT-proBNP (ng/L)	36 (18, 65)	32 (16, 56)	34 (17, 61)
BMI category			
Underweight	—	273 (7.6%)	273 (3.1%)
Normal weight	5250 (100%)	179 (5.0%)	5429 (61%)
Overweight	—	1439 (40%)	1439 (16%)
Obese	—	1590 (44%)	1590 (18%)
Missing	—	129 (3.6%)	129 (1.5%)
Reported any hematologic or cardiovascular medication	—	55 (1.5%)	55 (0.6%)
History of hypertension	—	120 (3.3%)	120 (1.4%)
Prescribed antihypertensive medication	—	18 (0.5%)	18 (0.2%)
History of diabetes	—	27 (0.7%)	27 (0.3%)
Hemoglobin A1c ≥6.5	—	25 (0.7%)	25 (0.3%)
eGFR <60 mL/min/1.73 m^2	—	2 (<0.1%)	2 (<0.1%)
Receiving anemia treatment	—	136 (3.8%)	136 (1.5%)

This table displays the baseline characteristics of 8860 children and adolescents who participated in the combined NHANES cycles from 1999–2004, divided into healthy and excluded participants. The proportion of individuals belonging to each characteristic are given in parentheses. The median age and NT-proBNP concentrations are displayed, along with the interquartile interval in parentheses (25^{th}, 75^{th}).
[a]Median (25^{th}, 75^{th} percentiles); n (%)

intervals except for the oldest age groups using the weighted quantile regression method because there were too few reference individuals (Supplemental Table 3).

Proportion of Healthy Individuals with Elevated NT-proBNP

Table 4 displays the proportion of healthy individuals within each sex and age category with a NT-proBNP ≥125 ng/L, and in older individuals, ≥ 450 ng/L. In healthy 70–79-year-olds, 49.9% (95% CI: 14.3% to 85.6%) of men and 52.6% (95% CI: 14.0 to 89.4%) of women had a NT-proBNP concentration ≥125 ng/L (Table 4). For adults, the likelihood of an elevated NT-proBNP ≥125 ng/L increased with age, and there was a consistent sex-based difference, as women were more likely to have elevated NT-proBNP compared to men.

Table 3. NT-proBNP reference intervals (robust method).

Age category (years)	Sex	Sample size	2.5th percentile (90% CI)	50th percentile (90% CI)	97.5th percentile (90% CI)
1	Male	60	25 (20, 493)	97 (83, 116)	379 (303, 493)
	Female	57	23 (18, 475)	93 (79, 108)	367 (290, 475)
2 to 5	Male	254	12 (10, 351)	60 (55, 66)	304 (264, 351)
	Female	241	14 (12, 326)	61 (57, 66)	277 (235, 326)
6 to 11	Male	634	11 (10, 296)	54 (51, 57)	267 (241, 296)
	Female	633	12 (11, 279)	55 (52, 58)	254 (233, 279)
12 to 15	Male	810	4 (4, 193)	28 (26, 30)	177 (163, 193)
	Female	883	7 (6, 179)	34 (33, 36)	165 (154, 179)
16 to 19	Male	881	3 (3, 91)	16 (15, 17)	84 (77, 91)
	Female	797	7 (6, 162)	32 (30, 33)	149 (137, 162)
20 to 29	Male	374	3 (3, 103)	16 (15, 18)	91 (80, 103)
	Female	395	10 (9, 223)	44 (41, 47)	199 (179, 223)
30 to 39	Male	229	4 (4, 117)	21 (19, 23)	100 (86, 117)
	Female	272	14 (12, 211)	50 (47, 54)	186 (164, 211)
40 to 49	Male	155	4 (3, 170)	24 (21, 27)	140 (118, 170)
	Female	157	15 (12, 287)	59 (54, 65)	238 (196, 287)
50 to 59	Male	77	7 (5, 326)	41 (35, 46)	225 (158, 326)
	Female	86	17 (13, 348)	71 (61, 81)	292 (242, 348)
60 to 69	Male	48	8 (5, 757)	61 (49, 75)	456 (292, 757)
	Female	52	28 (22, 369)	90 (79, 105)	290 (233, 369)
70+	Male	61	19 (12, 1930)	155 (123, 193)	1286 (849, 1930)
	Female	43	32 (22, 1510)	177 (141, 222)	976 (664, 1510)

The lower reference limit (2.5th percentile), median, and upper reference limit (97.5th percentile) of NT-proBNP in healthy NHANES participants from survey cycles 1999–2004, partitioned by sex and age category, using the robust method are displayed here. This iterative method determines a measure of spread around the median, downweighting outliers in successive iterations.

DISCUSSION

In this nationally representative sample of healthy US children and adults, we demonstrated that the natural distribution of NT-proBNP varied according to age and sex, with females having higher NT-proBNP concentrations from adolescence to the sixth decade of life. Also, many adults older than 50 years without apparent disease had NT-proBNP concentrations greater than the common clinical cutoff of 125 ng/L. Even considering an age-adjusted cutoff of 450 ng/L, in those aged 70 to 79, nearly one-quarter and one-fifth of healthy males and females, respectively, had an elevated NT-proBNP concentration (6).

Our study extends and updates previous reference interval calculations. We applied a consistent methodology and included individuals across the entire life span, beginning at 12 months of age. Our results are consistent with previous studies that showed a decrease in NT-proBNP from birth to early adolescence, with a sex-based difference appearing in adolescence (15, 28). In the Leipzig Research Center for Civilization Diseases cohort, the upper reference limit for 1-year-old children was 362.6 ng/L for males and 379.7 ng/L for

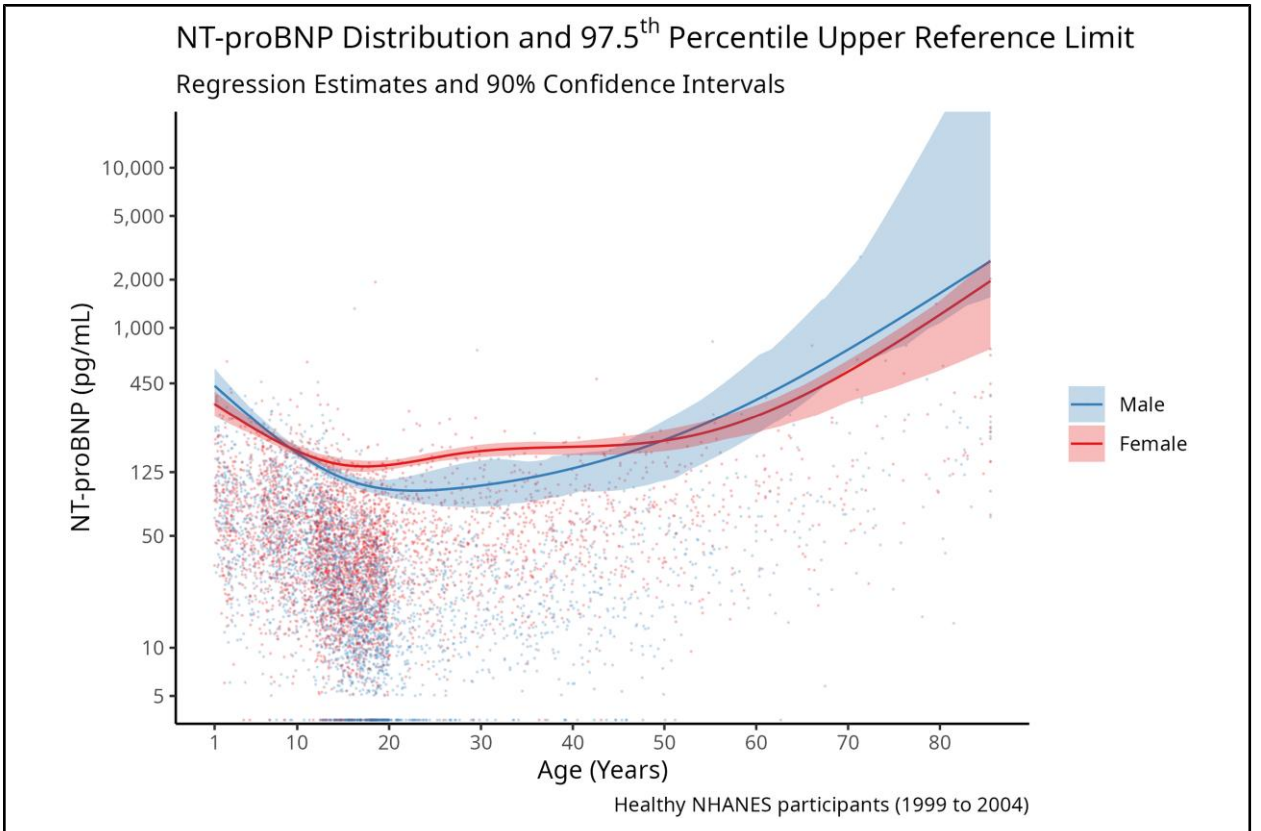

Fig. 1. NT-proBNP distribution and 97.5th percentile upper reference limit. The 97.5th percentile of NT-proBNP was estimated using a natural cubic spline with 3 *df* for healthy males and females. Individual NT-proBNP values are also plotted, with each point representing a single measurement of NT-proBNP. NT-proBNP concentration is on the logarithmic scale, and the regression line gives the 97.5th percentile estimate and corresponding 90% confidence interval. The gap in the distribution of NT-proBNP at low values is an artifact of the limit of detection of the NT-proBNP assay, where all values below 5 ng/L are replaced with 3.54 ng/L.

females, decreasing to 76.2 ng/L and 135.9 ng/L, respectively, by age 17 (28).

In middle-aged adults, previous studies have shown that NT-proBNP increases with age (8, 12). As an example, reference limits calculated from the Framingham Heart Study had an upper limit of 41.8 ng/L in 20–24 year-old men, which increased to 131.2 ng/L by age 50–59. In women, the corresponding reference limits were 103.5 ng/L (20–24 year olds) to 223.8 ng/L (50–54 year olds) (12).

In the oldest adults, upper limits for NT-proBNP reference intervals can often extend beyond the commonly used clinical cut-points of 125, 300, and 450 ng/L used in diagnosis and staging of heart failure. Reference intervals calculated using combined data from 2 German cohorts (Activity and Function in the Elderly Study and the Study of Health in Pomerania) showed that men older than 80 years had an upper reference limit of 697 ng/L, and for women, 1276 ng/L (36). In that analysis, 27.1% and 44.6% of healthy men and

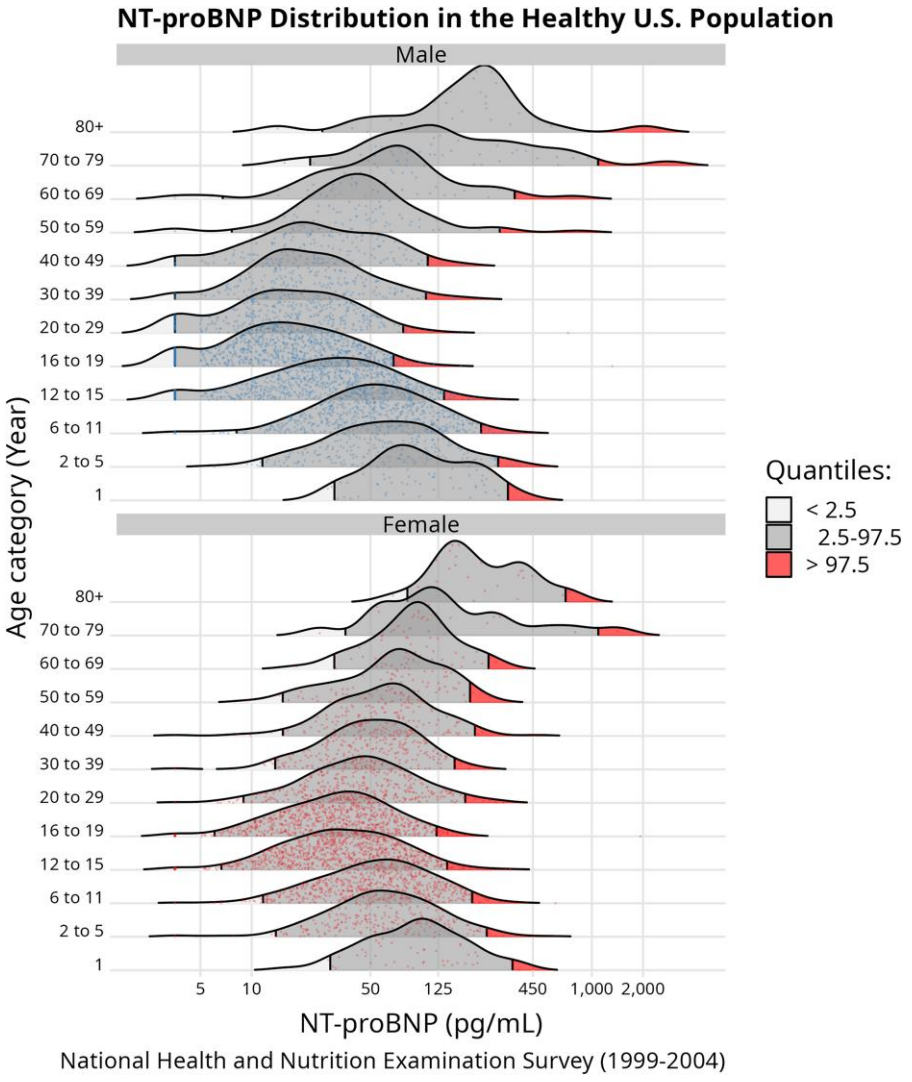

Fig. 2. NT-proBNP distribution in the healthy US population. This ridgeline plot shows the NT-proBNP distribution in healthy NHANES participants from survey cycles 1999–2004, partitioned by age category and sex. Survey weight information was incorporated in the quantile calculation and individual measurements are plotted within the density curve. The portions of the distribution above the 97.5th percentile are shaded in red. The increased densities at the lower portions of the NT-proBNP distributions is an artifact of the limit of detection of the NT-proBNP assay, where all values below 5 ng/L are replaced with 3.54 ng/L.

women aged 65 and above had an NT-proBNP concentration over 125 ng/L. In our study, we discovered that most healthy individuals aged 80 or older had an NT-proBNP concentration above 125 ng/L.

A strength of our study was the nationally representative sampling of NHANES, which included >5000 healthy children and nearly 2000 healthy adults. The standardized physical and clinical measurements allowed rigorous identification of a

Table 4. Weighted proportion of healthy individuals with elevated NT-proBNP.

		Proportion with NT-proBNP at or above 125 or 450 ng/L (95% confidence interval)	
Age category (years)	Sex	125 ng/L	450 ng/L
1	Male	34.2% (17.5, 53.2)	—
	Female	28.8% (12.9, 48.0)	—
2 to 5	Male	15.7% (9.8, 22.7)	—
	Female	17.5% (11.8, 24.2)	—
6 to 11	Male	14.3% (10.3, 18.7)	—
	Female	12.5% (9.2, 16.1)	—
12 to 15	Male	3.3% (2.1, 4.8)	—
	Female	3.5% (2.1, 5.3)	—
16 to 19	Male	0.1% (0.0, 0.3)	—
	Female	2.5% (1.1, 4.3)	—
20 to 29	Male	1.1% (0.1, 3.1)	—
	Female	8.8% (5.9, 12.3)	—
30 to 39	Male	2.0% (0.4, 4.8)	—
	Female	5.9% (2.6, 10.4)	—
40 to 49	Male	3.1% (0.3, 8.6)	—
	Female	14.7% (8.6, 22.1)	—
50 to 59	Male	6.5% (1.0, 16.1)	—
	Female	22.7% (13.4, 33.8)	—
60 to 69	Male	15.1% (4.1, 31.4)	—
	Female	22.5% (7.5, 42.5)	—
70 to 79	Male	49.9% (14.3, 85.6)	23.5% (0.3, 66.5)
	Female	52.6% (14.0, 89.4)	19.5% (0.2, 67.3)
80+	Male	69.3% (34.6, 94.7)	6.4% (0.3, 28.9)
	Female	83.6% (46.4, 100.0)	8.8% (2.2, 46.8)

This table displays the survey weighted proportion of healthy individuals from NHANES survey cycles 1999–2004, partitioned by sex and age category, with an NT-proBNP measurement equal to or above the preselected values of 125 and 450 ng/L. 95% confidence intervals were calculated using the variance-stabilizing arcsine square root transformation for the binomial distribution, subsequently expressed on the probability scale.

healthy subsample within the general population, and our statistical techniques were reproducible. The robust method of reference interval calculation theoretically performs well even if log-transformed NT-proBNP does not follow a normal distribution and also when sample sizes are limited (7, 30).

Our investigation had limitations that should be noted. First, the CLSI working group recommends the direct sampling technique, with clearly defined a priori exclusion criteria, but our reference values were obtained via indirect sampling from a pre-existing cross-sectional study. Direct sampling can alleviate the problem of an inadequate reference sample group size by enrolling a pre-specified number of reference individuals. Second, our definition of the healthy subsample partially relied on self-reported information, which may have resulted in misclassification. Incorrectly classifying unhealthy individuals as healthy

inappropriately inflates the imprecision of the estimates. Third, the reference ranges were derived from a single sample, which discount the potential diurnal and rhythmic patterns of NT-proBNP secretion (37). Fourth, the sample size was limited for the oldest adults which led to imprecise estimates in those groups. The CLSI recommends a 90% confidence interval of "less than 0.2 times the width of the reference interval," which was met or nearly met in the children and middle-aged partitions, but not for the oldest adults (7).

Last, another weakness of this study is that the transport, storage, and measurement of frozen serum is a departure from fresh plasma/blood collection and analysis in clinical practice. However, previous studies have demonstrated that the NT-proBNP assay is stable in long-term storage and robust to freeze–thaw cycles (38, 39). Most prior reference interval studies of NT-proBNP were conducted using stored samples and our results were similar (15, 36). Because concentrations tend to be slightly lower when measured in stored samples, it is possible that the reference intervals presented here may be slight underestimates of the true upper limit of normal had fresh specimens been analyzed. This reinforces our conclusion that the commonly used clinical decision limit of 125 ng/L is actually within the expected range of NT-proBNP, especially for older adults.

Our study has implications for both research and clinical practice. Future studies should extend this study by using contemporary measurements of NT-proBNP, larger sample sizes, especially in older adults, and the direct sampling technique. Our results underscore the need for additional investigation to understand the various factors that modulate the synthesis, secretion, or clearance of NT-proBNP over the lifespan. NT-proBNP reference intervals can inform more accurate clinical decision limits, which can then be prospectively validated. As NT-proBNP testing is indicated for acute diagnosis of heart failure, outpatient management of chronic heart failure, and increasingly being used for cardiovascular risk stratification, the cutoffs could be different for each of these indications. Our results suggest that incorporating age and sex information in future clinical decision limits may increase accuracy and usefulness.

CONCLUSION

We determined that the upper limit of the reference interval for NT-proBNP varied considerably according to age and sex. Clinical decision limits using NT-proBNP for heart failure and other conditions should take into account this heterogeneity and avoid using a single cut-point for more precise and tailored risk estimates.

SUPPLEMENTAL MATERIAL

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

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