



**NEUROMetrix**

NC-stat<sup>®</sup> DPNCheck<sup>®</sup> Normative Data:  
Collection, Analysis and Recommended Normal Limits

## Introduction

A normal limit is a value below (or above) which a diagnostic test result is deemed abnormal. Nerve conduction normal limits depend on measurement variables including instrument specifications, electrode placement, and temperature compensation.<sup>1</sup> Best practices dictate use of normal limits developed from data obtained with identical methods to those used in clinical practice.<sup>1</sup>

A study meeting recommended quality standards<sup>1-3</sup> was conducted to develop normal limit recommendations for clinicians using the NC-stat DPNCheck device. The first objective of this study was to obtain a large data base of sural nerve conduction responses, using the NC-stat DPNCheck device, in a population of normal non-neuropathic subjects. The second objective was to derive normal limits for the sural response amplitude and conduction velocity (CV).

## Methods

This was a prospective study of a broad spectrum population. Potential study subjects were recruited, between August and October 2012, from communities in two US states (Massachusetts and Iowa) through advertising, referrals, testing days at technology and light manufacturing companies, and testing days at senior centers. Recruited subjects completed written informed consent and a clinical questionnaire. The questionnaire included demographics (gender, age, height, and weight), a medical history suggestive of peripheral neuropathy risk (e.g., previously diagnosed neuropathy, diabetes, cancer), and the Diabetic Neuropathy Symptom (DNS) score.<sup>4</sup> The DNS score consists of 4 questions (see Table 1) and is a validated predictor of peripheral neuropathy. Inclusion criteria were age over 18 and no physical impediment to testing the sural nerve bilaterally. Exclusion criteria were any of the following (i) BMI > 35 kg/m<sup>2</sup>, (ii) medical history positive for peripheral neuropathy, diabetes, renal failure, cancer, hypothyroidism, B12 deficiency, or alcoholism, and (iii) DNS score > 0.

Table 1. Diabetic Neuropathy Symptom (DNS) Questionnaire

1. Are you unsteady when you walk?
2. Do you have a burning, aching pain or tenderness at your legs or feet?
3. Do you have prickling sensations in your legs or feet?
4. Do you have places of numbness on your legs or feet?

Each question answered *yes* or *no*.

Score is the number of *yes* answers.

All subjects underwent bilateral sural nerve conduction testing. All nerve conduction tests were performed using the NC-stat DPNCheck device (Software Version 2.0). Two tests were performed on each limb for a total of 4 sural responses per subject. The sural nerve was stimulated, using stainless steel probes, just posterior to the lateral malleolus. The sural response was recorded 92.2 mm proximally at the calf with a pair of electrodes (25 mm length) in a bipolar configuration (20 mm center to center spacing). The nerve was stimulated supramaximally with averaging of 4-7 responses. The amplitude was measured peak to peak. An undetectable sural response was defined as amplitude less than 1.5  $\mu$ V and was displayed as 0  $\mu$ V. The CV was measured to the onset of the initial negative deflection. If the onset could not be reliably determined then CV was not reported. Skin temperature was monitored by an infra-red digital thermometer with CVs normalized to 28°C using a temperature correction factor of 1 m/s per °C. The NC-stat DPNCheck device displays amplitude and CV as rounded whole numbers (e.g., 6  $\mu$ V, 52 m/s) per recommended reporting precision.<sup>1</sup>

The study data set was created by taking a single sural response (first test on left limb) from each subject in the study population. Amplitude and CV normal limits were defined as the lower 5<sup>th</sup> percentile. The use of the 5<sup>th</sup> percentile is based on the accepted practice of a 5% false-positive rate in statistical testing. This level has been adopted in several published nerve conduction normal

limit studies.<sup>1,6,7</sup> Because the 5<sup>th</sup> percentile represents a specificity of 95%, the positive predictive value of an abnormal test will be high in patients with moderate pre-test probability of peripheral neuropathy. For example, in a typical diabetic population the pre-test probability is at least 50%<sup>8</sup> and therefore the positive predictive value is greater than 90%, even if test sensitivity is relatively low.

The dependence of the normal limits on demographic variables (e.g., subject age, height) was evaluated using quantile regression.<sup>3</sup> Using this method, the normal limit is expressed as a linear function of demographic variables:

$$\text{Normal Limit} = K + C_1V_1 + C_2V_2 + \dots + C_nV_n$$

where  $K$  is a constant,  $V_i$  is the  $i^{\text{th}}$  demographic variable, and  $C_i$  is the coefficient for the  $i^{\text{th}}$  demographic variable. Demographic variables that were statistically significant predictors ( $p < 0.05$ ) of the normal limit were retained. The precision of the resulting normal limits was assessed by calculating both point estimates and 95% confidence intervals (95% CI) for representative demographics using the bootstrap method with 100,000 random samples. A precise normal limit has a narrow confidence interval, indicating the appropriateness of the nerve conduction and statistical methods.<sup>9</sup>

## Results

A total of 856 subjects were recruited. Of these, 329 (38.4%) met the exclusion criteria leaving a study population of 527. Study subject characteristics are summarized in Table 2. The mean age was 48.3 years, with 21.8% of the subjects 65 years or older.

The amplitude normal limit was statistically dependent on subject age, decreasing by 0.99 (95% CI, 0.60 – 1.3)  $\mu\text{V}$  per decade. Figure 1 shows the relationship between age and amplitude. The green line is the age dependent normal limit. The CV normal limit was statistically dependent on subject age and height, decreasing 1.3 (95% CI, 1.0 – 1.7) m/s per decade and 2.0 (95% CI, 1.4 – 2.6) m/s per 10 cm, respectively. Figure 2 shows the relationship between height and CV. The two green lines

show the height dependent normal limits for subjects 45 (upper line) and 65 (lower line) years of age. Table 3 shows estimates of normal limits and confidence intervals for subjects aged 25, 45, and 65 years and height 172 cm.

Table 2. Study subject characteristics.

Characteristic	Mean (Stdev) or %
Gender (%Female)	51.2
Age (years)	48.3 (18.5)
Height (cm)	167 (11.5)
Weight (kg)	73.1 (16.8)
BMI (m/kg <sup>2</sup> )	26.0 (4.09)
Amplitude ( $\mu\text{V}$ )	16.9 (8.62)
Conduction Velocity (m/s)*	53.0 (5.17)

\*Conduction velocity available for 523 subjects

Table 3. Point estimates and confidence intervals for ages 25, 45 and 65 years.

Parameter	5 <sup>th</sup> Percentile Normal Limit (95% CI)		
	25 yrs	45 yrs	65 yrs
Amplitude ( $\mu\text{V}$ )	9 (8–10)	7 (6–8)	5 (4–6)
CV (m/s)	48 (47–49)	45 (45–46)	43 (42–44)

Two of the 527 (0.38%) study subjects had an undetectable sural response among their 4 tests. On this basis, an undetectable response was estimated to have specificity of over 99.5%. The two subjects had ages 58 and 67 years. Although they did not report any prospective exclusion criteria, one of the subjects had a partial foot amputation.

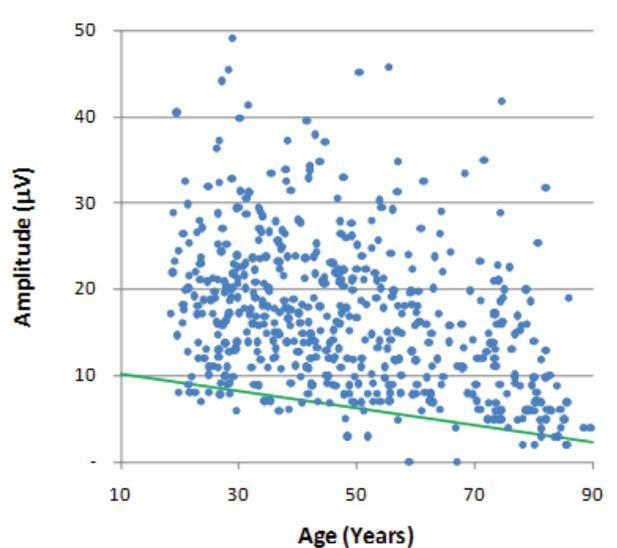


Figure 1. Relationship between subject age and sural nerve amplitude. Green line indicates recommended age dependent normal limit.

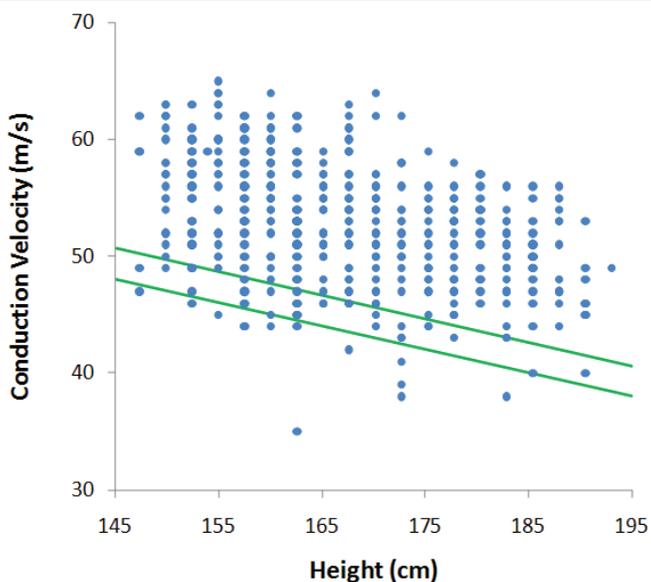


Figure 2. Relationship between subject height and sural nerve CV. Upper and lower green lines indicate recommended height dependent normal limit for subjects 45 and 65 years of age, respectively.

## Discussion

The purpose of this study was to develop a large data base of NC-stat DPNCheck data in subjects without evidence of neuropathy and to use the data set to establish statistically robust normal limits. Sural amplitude was found to depend on subject age, declining about 1  $\mu\text{V}$  for every decade. This clinically significant amplitude reduction represents a 153 fibers/ $\text{mm}^2$  reduction in sural nerve myelinated fiber density.<sup>10</sup> Sural conduction velocity was dependent on both age and height, decreasing by 1.3 m/s for every decade and 2.0 m/s for every 10 cm of height.

The normal limit confidence intervals for both sural amplitude and CV were narrow, at about 1  $\mu\text{V}$  and 1 m/s respectively. These values support the validity of the nerve conduction and analysis methods used in the present study. These confidence intervals are narrower than prior reports of sural normal limits,<sup>6,7</sup> reflecting the large sample size. Over 20% of the subjects in the present study were at least 65 years of age. The narrow confidence intervals in this age group and the low rate of undetectable responses suggest the recommended normal limits are reliable in elderly subjects.

Despite studies suggesting that nerve conduction normal limits vary with demographic variables,<sup>1</sup> it remains common practice to use fixed thresholds. The disadvantage of this approach is that diagnostic specificity will vary with the patient's age and potentially other demographic characteristics. The specificity may be very high in younger and short patients, but only modest in elderly and the very tall. For example, at a fixed normal limit of 5  $\mu\text{V}$  (i.e., amplitudes 4  $\mu\text{V}$  or less are labeled abnormal), the overall specificity in this study population is 96.6%, which is similar to the target specificity of 95%. However, the specificity varies with age. It is 100% for subjects < 45 years, 98.1% for subjects 45–64 years, and 87.0% for subjects  $\geq$  65 years. By contrast, when using the recommended age dependent normal limits, the specificity is 94.6%, 96.1% and 93.0% for the three age groups.

A number of studies have reported sural normal limits.<sup>6,7,11-17</sup> The sample size in the present study (N=527) is the largest among prospective studies meeting recommended quality standards for collection and analysis of normative data.<sup>1,2</sup> Although comparisons of specific normal limit values must account for differences in methodology, relevant conclusions can be drawn from the general findings. In a widely referenced study, Stetson and colleagues<sup>13</sup> reported sural normal limits in 105 subjects using ordinary linear regression and Gaussian transformation of nerve conduction parameters. They found that sural amplitude was related to gender, age and height and conduction velocity to height. The authors suggested calculation of sural normal limits as 2 standard deviations below the demographic adjusted mean. Although this parametric approach is common,<sup>1,11,12,17</sup> it makes the generally incorrect assumption that amplitude and conduction velocity variance are independent of demographics.<sup>3,14</sup> Benatar and colleagues<sup>7</sup> reported sural normal limits, including the 5<sup>th</sup> percentile, in 190 subjects using quantile regression. Similar to the present study, they found that sural amplitude was related to age. In contrast to the present and other studies,<sup>11,13,14</sup> they did not find a statistically and clinically significant relationship between conduction velocity and age or height. Esper and colleagues<sup>6</sup> evaluated the relationship between the sural amplitude normal limit at the 5<sup>th</sup> percentile and age in 92 subjects using conventional percentiles<sup>1</sup> and the bootstrap method. They reported a dramatic drop in the normal limit with age, decreasing from 14.0 (95% CI, 10.4–19.0)  $\mu$ V in subjects < 40 years to 3.2  $\mu$ V (95% CI, 2.1–5.6) in subjects  $\geq$  60 years. Although the broad confidence intervals obscured the exact normal limits, the conclusion of a strong dependence of the amplitude normal limit on age was robust.

## Recommendations

The values in Table 4 may be entered into the NC-stat DPNCheck Communicator™ software to implement the normal limits described above. These recommendations are provided for information purposes only and do not constitute medical advice. A decision to utilize this information must be made by an appropriately trained medical professional.

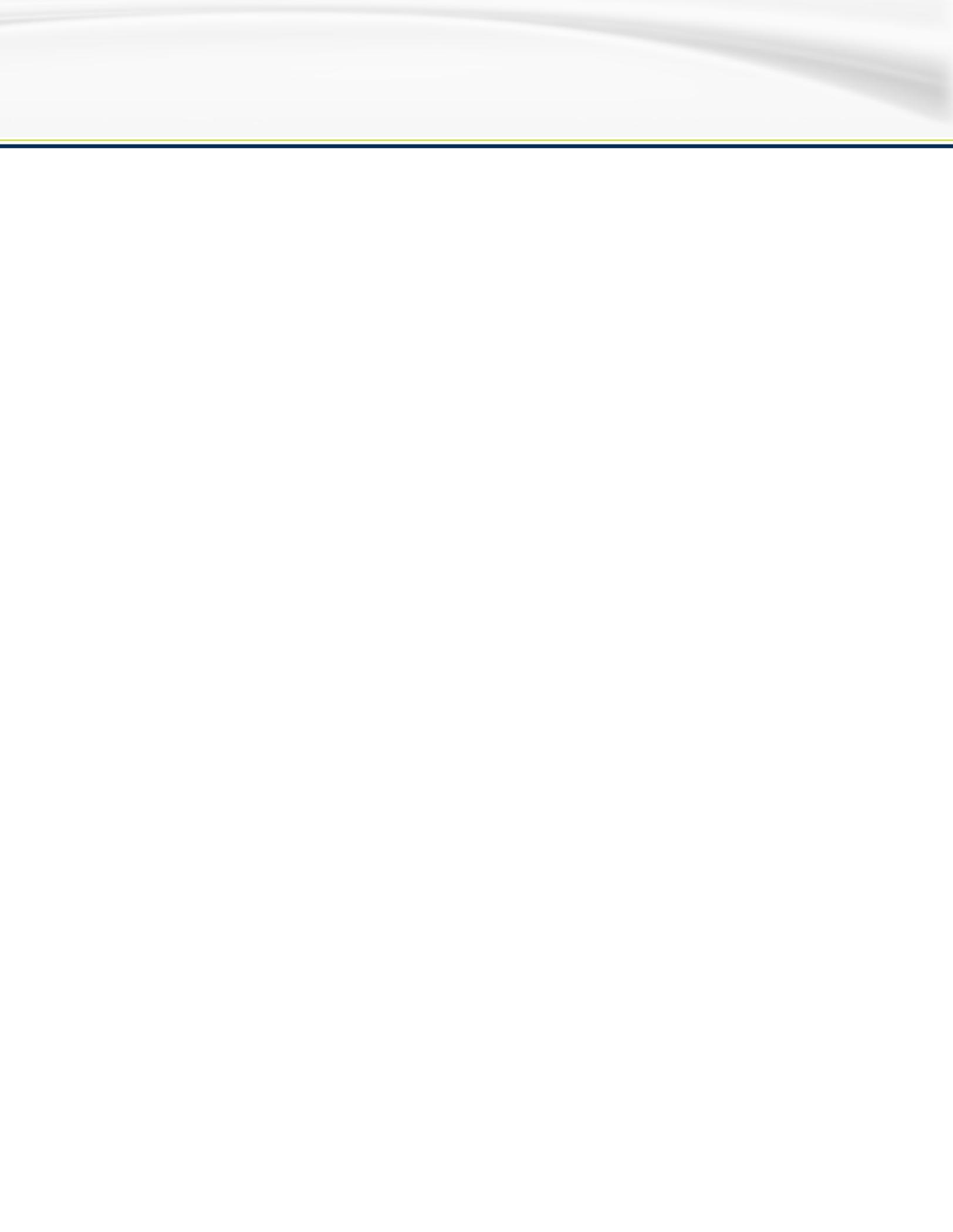
Table 4. Recommended Normal Limits.

Parameter	Constant	Age Coefficient	Height Coefficient
Amplitude	11.2	-0.099	0
CV	85.5	-0.13	-0.20

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