BACKGROUND AND OBJECTIVES: Many newborn screening (NBS) programs now perform repeat or serial NBS to detect congenital hypothyroidism. There is wide variation in thyroid-stimulating hormone (TSH) cutoffs used by NBS programs. Data on TSH reference ranges in preterm infants at increasing postnatal age are limited. Our study objective was to determine TSH reference ranges for preterm infants born at <32 weeks’ gestation.

METHODS: We analyzed serial TSH levels on NBS performed on infants born between 22 and 31 weeks’ gestation from 2012 to 2016 in Wisconsin. The study cohort was divided into 2 groups (22–27 and 28–31 weeks), and TSH percentiles were defined from birth to the term equivalent gestational age.

RESULTS: The study cohort consisted of 1022 and 2115 infants born at 22 to 27 and 28 to 31 weeks’ gestation, respectively. The 95th percentile TSH level for the group born at 22 to 27 weeks’ gestation gradually decreased and reached a nadir at ∼10 to 11 weeks. In contrast, for the group born at 28 to 31 weeks’ gestation, the 95th percentile TSH level reached a nadir at ∼5 to 6 weeks. At 3 to 4 weeks after birth, the 95th percentile TSH level ranged from 11 to 11.8 μIU/mL for the group born at 22 to 27 weeks’ gestation and ranged from 8.2 to 9 μIU/mL for the group born at 28 to 31 weeks’ gestation.

CONCLUSIONS: Using a statewide cohort of preterm infants, we constructed TSH reference charts from birth to the term equivalent gestation for preterm infants born at <32 weeks’ gestation. Use of a single cutoff for all preterm infants might lead to misdiagnosis. The differences in TSH levels according to gestational-age categories might explain the increased frequency in congenital hypothyroidism diagnoses among preterm infants. These data are useful for defining age-adjusted NBS TSH cutoffs for preterm infants.

WHAT’S KNOWN ON THIS SUBJECT: There is wide variation in TSH cutoff levels used to identify infants with congenital hypothyroidism. Similar TSH cutoffs are used for preterm and term infants despite differences in thyroid axis physiology.

WHAT THIS STUDY ADDS: TSH percentiles vary with increasing gestational age in preterm infants. Use of a single cutoff might lead to misdiagnosis. Differences in TSH levels according to gestational-age categories and postnatal age may contribute to increased congenital hypothyroidism diagnoses.
Nearly 4 million newborn infants undergo newborn screening (NBS) annually in the United States. Congenital hypothyroidism is 1 of the most commonly diagnosed disease by NBS.1 Congenital hypothyroidism also is a core condition included in the recommended universal screening panel in the United States.2 Approximately 1400 infants in the United States are diagnosed with congenital hypothyroidism each year.3

NBS programs use 3 screening strategies to detect congenital hypothyroidism in neonates: the primary thyroxine (T4)-back-up thyroid-stimulating hormone (TSH) method, the primary TSH-back-up T4 method, and the combined primary TSH plus T4 method.3,4 Primary TSH or combined TSH and T4 methods are used by 60% of NBS programs in the United States.5 There is a wide variation in TSH cutoff levels used by NBS programs to identify infants with congenital hypothyroidism.5,6

Congenital hypothyroidism is diagnosed with increased frequency in infants born preterm.7–10 Many NBS programs now perform repeat or serial NBS to detect congenital hypothyroidism in preterm infants.6,10–12 However, despite differences in gestational age–dependent thyroid hormone physiology, the same TSH cutoffs are used for preterm infants as for the term infants.11,12 TSH cutoffs derived from term infant data could lead to overdiagnosis or underdiagnosis of congenital hypothyroidism among preterm infants. Data on TSH reference ranges in preterm infants at increasing postnatal age are limited.

Our objective for the current study was to determine TSH reference ranges for preterm infants born <32 weeks’ gestation by using a statewide birth cohort of preterm infants in Wisconsin.

METHODS

The study cohort was drawn from the NBS database maintained by the Wisconsin state NBS program. The University of Wisconsin-Madison Institutional Review Board reviewed the study and determined that the study qualifies for exemption. All preterm infants born before 32 weeks’ gestation from 2012 to 2016 who underwent NBS in the Wisconsin state NBS program were included in the study. Infants whose first TSH was done after 96 hours of life and infants with incomplete data were excluded. The information on congenital hypothyroidism screening results of this study cohort was presented in a previous publication.10

The Wisconsin state NBS program uses the primary TSH test strategy to screen for congenital hypothyroidism. The NBS program performs serial NBS for infants with extended hospital stay. After the initial screening at 24 to 48 hours of life, for infants with a birth weight <2200 g, a second specimen is collected at 2 weeks of age, a third specimen at 28 to 30 days of life or discharge, whichever comes first, and the NBS is performed monthly thereafter until discharge. NBS TSH levels were measured by solid phase, time-resolved fluoroimmunoassay from dried newborn blood spots by using PerkinElmer’s AutoDELFIA platform (Waltham, MA). The TSH measurements were then converted to estimated serum levels with the following conversion factor: a blood concentration of 1 µU/mL = a serum concentration of 2.22 µU/mL (assuming 55% hematocrit). The software on the AutoDELFIA platform was used to round the TSH values to a whole number. TSH values recorded as 0 were included as truncated values <0.1 for the analysis.

Infant demographics and TSH values on NBS were obtained from the NBS database. All serial TSH values of the final study cohort were included in the analysis. TSH values recorded after diagnosis of congenital hypothyroidism were excluded. TSH values were recorded in epochs of each postnatal week from birth to term corrected gestational age. TSH values obtained within 24 to 96 hours of life were recorded as TSH values at birth or week 0. Starting from day 5, TSH values were grouped into epochs of 7 days until term corrected gestational age. References values for extremely preterm infants (22–27 weeks) and very preterm infants (28–31 weeks) were presented separately because these 2 groups have distinctly different thyroid hormone physiology, as described before.6,13 We also determined TSH values at 36 (+1) weeks postmenstrual age (PMA) for the cohort and calculated the TSH reference values at this near-term gestational age for the 2 groups.

The data analysis was performed by using SAS version 9.4 (SAS Institute, Inc, Cary, NC). The nonparametric quantile regression approach, on the basis of B-splines, was used to construct the reference charts for TSH.14 Age distributions were assumed for TSH. An estimate of the slope parameter q was performed by using the computationally efficient simplex algorithm. The quantile regression was performed for each gestational-age group separately. TSH median, fifth, 25th, 75th, 95th, and 99th percentiles were defined from birth to the term equivalent gestational age for extremely preterm and very preterm infants.

RESULTS

A total of 3858 infants were born before 32 weeks’ gestational age in the state of Wisconsin from 2012 to 2016. The study cohort included 3137 infants after exclusion of 721 infants on the basis of exclusion criteria. Characteristics of the study cohort are presented in Table 1. The study cohort consisted of 1022 infants.
extremely preterm infants and 2115 very preterm infants. A total of 11,431 TSH values were analyzed. Extremely preterm infants had 4,199 TSH values recorded (~4 TSH levels per infant). There were 7,232 TSH values recorded in very preterm infants (~3.4 TSH levels per infant).

Among extremely preterm infants, the median TSH value changed minimally from 2.1 μIU/mL at birth to 3.1 μIU/mL at the term corrected gestational age. In contrast, the 95th percentile TSH value was highest at birth and gradually declined until the 10th postnatal week of life. Thereafter, the 95th percentile TSH value slightly increased (Fig 1, Table 2).

Among very preterm infants, the median TSH value was 3.4 μIU/mL at birth. The median TSH level gradually declined, reached a nadir at ~4 to 5 weeks of life, and then gradually increased. The 95th percentile TSH level also followed similar trend; it was highest at birth and gradually declined until the fifth to sixth postnatal week of life. Thereafter, the 95th percentile TSH level gradually increased toward that of the term corrected gestational age (Fig 2, Table 3).

At 3 to 4 weeks, when repeat NBS is commonly performed, the median TSH level ranged from 2.5 to 2.6 μIU/mL for both groups. However, the 95th percentile ranged from 11 to 11.8 μIU/mL for extremely preterm infants and from 8.2 to 9 μIU/mL for very preterm infants.

At 36 (±1) weeks PMA, the 50th, 95th, and 99th percentile TSH levels for extremely preterm infants were 3, 9, and 13 μIU/mL, respectively. The equivalent percentile TSH levels for very preterm infants were 3, 8, and 13 μIU/mL, respectively (Table 4).

**DISCUSSION**

In this study, we define the distribution of postnatal TSH values in extremely preterm and very preterm infants using a statewide cohort of preterm infants in Wisconsin. To our knowledge, this is the largest study in which postnatal TSH values in preterm infants are described.
Findings are relatively consistent with previous studies of the Scottish preterm thyroid group, in which the mean TSH concentration among extremely preterm infants at 24 hours of life was 2.3 μIU/mL, compared with a median value of 2.1 μIU/mL in the current study. Similarly, for infants born at 28 to 30 weeks’ gestation, the previously reported mean TSH concentration of 4.5 μIU/mL at 24 hours of life was different only slightly from median TSH level of 3.4 μIU/mL at birth for infants born at 28 to 31 weeks’ gestation in the current study.

The median postnatal TSH values of 2.6 and 2.5 μIU/mL at week 4 in the current study are close to previous reports of a mean TSH value of 3.8 and 3.6 μIU/mL at 28 days of life for infants born at 24 to 27 and 28 to 30 weeks’ gestation, respectively. In contrast, authors of other studies have reported that after the first week of life, a single range for TSH (0.8–12.0 mU/L) appeared appropriate for all premature infants until 40 weeks postconceptional age. However, this study included only a smaller number of extremely preterm infants, and infants who developed significant systemic illness were excluded, which limits the comparability of that study with the current study and limits generalizability to the overall preterm infant population.

In recent decades, the incidence of congenital hypothyroidism has almost doubled. This increase is attributed to increased detection among high-risk groups, including increased detection among preterm infants and methodologic shifts in NBS. A number of NBS programs have elected to lower the TSH screening cutoff, which has led to increased congenital hypothyroidism detection among preterm infants. However, whether mild elevation of TSH in preterm infants is harmful is largely unknown.

Infants with mildly elevated TSH levels in association with normal free thyroxine (FT4) levels are common among the preterm infant population. These infants with mildly elevated TSH levels and normal FT4 levels are defined as infants with subclinical hypothyroidism. American Academy of Pediatrics recommends T4 supplementation for infants with persistently elevated TSH levels >10 mU/L after 1 month of life. The American Academy of Pediatrics acknowledges that the management of infants with elevated TSH levels between 6 and 10 mU/L that persist after the first month of life is controversial. The European Society of Pediatric Endocrinology recommends that providers do further investigations, such as retesting in 2 weeks, or initiate T4 supplementation immediately if the venous TSH concentration is 6 to 20 mU/L beyond 21 days of life in a well infant with an FT4 concentration within the limits for age. The European Thyroid Association’s guideline on management of subclinical hypothyroidism in pregnancy and in children states that a serum TSH concentration >5 mU/L can be considered as abnormal after 1 month of age when modern third-generation assays are used. It also states that there is insufficient evidence to recommend treatment in the majority of children with subclinical hypothyroidism in whom the serum TSH concentration is <10 mU/L.

On the basis of current data, TSH levels of 6 and 10 mU/L at 4 weeks of life are considered as abnormal, which limits the comparability of that study with the current study and limits generalizability to the overall preterm infant population.

TABLE 2 Percentiles of TSH Values (μIU/mL) for Extremely Preterm Infants.

<table>
<thead>
<tr>
<th>Age, wk</th>
<th>No.</th>
<th>TSH Values</th>
<th>Time of Specimen Collection, Mean (SD), h</th>
<th>Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fifth</td>
<td>10th</td>
</tr>
<tr>
<td>0</td>
<td>928</td>
<td>44 (16.9)</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>1</td>
<td>46</td>
<td>200 (48.1)</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>855</td>
<td>332 (24.3)</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>522 (42.3)</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>750</td>
<td>714 (27.4)</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>82</td>
<td>858 (43.3)</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>1033 (41.4)</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>64</td>
<td>1207 (43.1)</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>8</td>
<td>270</td>
<td>1406 (34.9)</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>9</td>
<td>305</td>
<td>1500 (33.3)</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>10</td>
<td>92</td>
<td>1706 (41.0)</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>11</td>
<td>74</td>
<td>1868 (42.6)</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>2046 (45.5)</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>13</td>
<td>370</td>
<td>2188 (30.1)</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>14</td>
<td>71</td>
<td>2375 (42.9)</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>15</td>
<td>62</td>
<td>2544 (40.0)</td>
<td>0.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>
life correspond to those in the 84th and 94th percentiles in extremely preterm infants and correspond to those in the 86th and 97th percentiles in very preterm infants. NBS programs in United States and other countries use TSH levels anywhere from 5 to 40 mU/L as screening cutoffs after the first week of life. However, no specific cutoffs for preterm infants have been used, despite differences in thyroid hormone physiology among preterm infants. This could lead to either overdiagnosis and unnecessary treatment or underdiagnosis and potential compromise in growth and neurodevelopmental outcomes in these infants, depending on the cutoff that is being used.

In the current study, we describe TSH reference ranges for preterm infants from birth to the term corrected gestational age on the basis of their degree of prematurity. We anticipate that these age-adjusted TSH cutoffs will facilitate standardization of screening for congenital hypothyroidism diagnosis among preterm infants and aid neonatologists and endocrinologists in making clinical decisions. We suggest using a predetermined percentile (eg, 95th or 99th percentile) at a given postnatal age to determine if a TSH level is abnormal. However, whether infants with TSH levels above a certain percentile have abnormal growth and neurodevelopmental outcomes should be determined by well-designed outcome research studies.

There are several limitations of the current study. Patients with chromosomal or multiple congenital anomalies could not be excluded.

| Table 3 Percentiles of TSH Values (mU/mL) for Very Preterm Infants |
|---|---|---|---|---|---|---|---|---|---|
| Age, wk | No. TSH Values | Time of Specimen Collection, Mean (SD), h | 5th | 10th | 25th | 50th | 75th | 90th | 95th | 99th |
| 0 | 2003 | 45 (16.7) | 0.1 | 0.1 | 1.3 | 3.4 | 6.8 | 11.5 | 16.3 | 30.6 |
| 1 | 137 | 191 (42.0) | 0.1 | 0.1 | 1.1 | 3.1 | 5.7 | 9.6 | 13.1 | 23.4 |
| 2 | 1913 | 332 (22.8) | 0.1 | 0.2 | 1.1 | 2.8 | 4.9 | 8.1 | 10.6 | 17.7 |
| 3 | 157 | 531 (41.5) | 0.1 | 0.2 | 1.0 | 2.6 | 4.4 | 7.1 | 9.0 | 14.5 |
| 4 | 1588 | 710 (30.2) | 0.1 | 0.2 | 1.1 | 2.5 | 4.1 | 6.6 | 8.2 | 12.7 |
| 5 | 228 | 868 (43.9) | 0.1 | 0.3 | 1.1 | 2.5 | 4.0 | 6.4 | 7.8 | 11.8 |
| 6 | 274 | 1028 (40.0) | 0.1 | 0.4 | 1.2 | 2.6 | 4.1 | 8.5 | 7.8 | 11.5 |
| 7 | 220 | 1202 (42.1) | 0.1 | 0.5 | 1.4 | 2.8 | 4.4 | 6.8 | 8.1 | 11.9 |
| 8 | 286 | 1386 (45.4) | 0.1 | 0.6 | 1.5 | 2.9 | 4.7 | 7.2 | 8.7 | 12.7 |
| 9 | 170 | 1505 (39.5) | 0.1 | 0.7 | 1.8 | 3.2 | 5.2 | 7.9 | 9.5 | 14.0 |
| 10 | 90 | 1700 (37.0) | 0.1 | 0.9 | 2.0 | 3.5 | 5.8 | 8.7 | 10.6 | 15.9 |
Preterm infants born at gestational ages 32 to 36 weeks were not studied; hence, these data cannot be generalized to all preterm infants. TSH values given here are estimated serum values from dried blood-spot TSH values. Of note, dried blood-spot serum values from dried blood-spot TSH values given here are estimated to all preterm infants. These data cannot be generalized to all preterm infants. Hence, these data cannot be generalized to all preterm infants.

CONCLUSIONS
In this study, we define the TSH reference ranges from birth to the term corrected gestational age in extremely and very preterm infants using a statewide cohort of preterm infants in Wisconsin. The current study reveals that NBS TSH percentiles vary with increasing gestational age. This information will be valuable for NBS programs to better define postnatal age–adjusted TSH cutoffs for preterm infants and to standardize diagnosis. Correlation between age-adjusted TSH percentiles and long-term neurodevelopmental outcomes should be determined in future studies.

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: No external funding.

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.

COMPANION PAPER: A companion to this article can be found online at www.pediatrics.org/cgi/doi/10.1542/peds.2019-1706.

REFERENCES

ABBREVIATIONS
FT4: free thyroxine
NBS: newborn screening
PMA: post menstrual age
TSH: thyroid-stimulating hormone
T4: thyroxine

TABLE 4 Percentiles of TSH Values (μIU/mL) at 36 Weeks’ PMA for Extremely Preterm and Very Preterm Infants

<table>
<thead>
<tr>
<th>Gestational Age at Birth, wk</th>
<th>No. TSH Measures</th>
<th>50th Percentile</th>
<th>95th Percentile</th>
<th>99th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>22–27</td>
<td>550</td>
<td>3</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>28–31</td>
<td>1394</td>
<td>3</td>
<td>8</td>
<td>13</td>
</tr>
</tbody>
</table>

because this information was also not available to the NBS program.


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Pediatrics originally published online July 16, 2019;

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