TITLE

Fractional exhaled nitric oxide (FeNO) values in Indigenous Australians aged 3-16 years

AUTHORS

Tamara L Blake (MClSc)^{1,2}, Anne B Chang (PhD)^{3,4}, Mark D Chatfield (MSc)^{4,5}, Julie M Marchant (PhD)^{1,3}, Helen L Petsky (PhD)⁶, Margaret S McElrea (PhD)^{1,2,3}

1: Centre for Children's Health Research, Queensland University of Technology, Brisbane, QLD, Australia

2: Indigenous Respiratory Outreach Care Program, The Prince Charles Hospital, Brisbane, QLD, Australia

3: Department of Respiratory and Sleep Medicine, Queensland Children's Hospital, Brisbane, QLD, Australia

4: Child Health Division, Menzies School of Health Research, Darwin, NT, Australia

5: The University of Queensland, Brisbane, QLD, Australia

6: School of Nursing and Midwifery, Griffith University, Nathan, QLD, Australia

CORRESPONDING AUTHOR DETAILS

Tamara Blake MCISc BBSoc Hons

Respiratory Scientist/Researcher, PhD Candidate

Queensland University of Technology

Level 7, Centre for Children's Health Research (CCHR)

Cnr Graham St and Raymond Tce

South Brisbane, QLD, Australia 4101

Ph: 3069 7275

E-mail: tamara.blake@hdr.qut.edu.au

CONFLICT OF INTEREST

No conflicts of interest for any of the authors.

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ACCEPTED MANUSCRIPT

ABSTRACT

Background: Fractional exhaled nitric oxide (FeNO) levels can identify eosinophilic asthma phenotypes. We aimed to determine FeNO values of 'healthy' Aboriginal and/or Torres Strait Islander (Indigenous) Australians, differences between these Indigenous ethnic groups and appropriateness of published cut-off values.

Methods: We measured FeNO in 1036 Indigenous Australians (3-16 years). Participants were classified into 'healthy' (i.e. no asthma or atopy history) or asthmatic and/or atopic groups.

Results: Median FeNO values and distribution did not differ between Indigenous ethnicities. For healthy participants <12 years (n=390), 7.2% of our cohort fell into the 'inflammatory' zone of the American Thoracic Society (ATS), National Institute for Health and Care Excellence (NICE) and British Thoracic Society/Scottish Intercollegiate Guidelines Network (BTS/SIGN) guidelines (cut-off 35 ppb) but only 3.8% when using the Global Initiative for Asthma (GINA) guidelines (50 ppb). Similarly, when using the NICE and BTS/SIGN guidelines (40 ppb) for 12-16 years (n=213), more healthy participants fell into the 'inflammatory' zone compared to the ATS and GINA guidelines (50 ppb) (9.9% vs. 4.7%).

Conclusion: FeNO values for healthy Indigenous Australians children (aged 3-16 years) are likely higher than published Caucasians-based values. The GINA recommended cut-off value (>50 ppb) appears the most appropriate for identifying healthy Indigenous children but requires confirmation from a larger study.

1 ABBREVIATIONS LIST

- 2 ATS American Thoracic Society
- 3 BTS British Thoracic Society
- 4 ERS European Respiratory Society
- 5 FeNO Fractional exhaled nitric oxide
- 6 GINA Global Initiative for Asthma
- 7 NICE National Institute for Health and Care Excellence
- 8 ppb Parts per billion
- 9 SIGN Scottish Intercollegiate Guidelines Network

INTRODUCTION

Fractional exhaled nitric oxide (FeNO) is increasingly used to assist in the diagnosis and monitoring of asthma, predicting exacerbations and identifying possible corticosteroid responsive individuals.¹⁻⁴ Current published guidelines for clinical interpretation of FeNO recommend the use of cut-off values which are dependent only on age.¹⁻⁴ The use of age-based cut-off values remain controversial in clinical practice due to the known influence of other factors such as atopy, smoking status and respiratory infections⁵⁻⁷ on FeNO values. Furthermore, FeNO cut-off values are predominantly based on Caucasian data despite findings from several studies that show ethnicity can also influence FeNO values.⁸⁻¹⁰ Our systematic review¹¹ found that current cut-off values recommended by the American Thoracic Society (ATS)¹ may not be suitable for use in some non-Caucasian populations, given that >5% of healthy non-Caucasian children and adults tested had FeNO values above the age-dependent inflammatory cut-off value which could lead to potentially unnecessary treatment interventions. Thus, understanding the range of healthy FeNO values in ethnic groups with a high burden of respiratory diseases including asthma is clinically important.

Incorporating FeNO into routine clinical practice is further complicated by variations in current published guidelines¹² (Table 1) with respect to different definitions of child vs. adult, the age defined cut-off value that would suggest the presence of inflammation, and how a positive test should be interpreted. Depending on which guideline is followed these differences can not only influence whether a patient is diagnosed as having asthma, but also the treatment pathway.

There are currently no studies that have examined FeNO in healthy Aboriginal and Torres Strait Islander (hence forth referred to as Indigenous) Australians. Among Indigenous Australians, asthma is the most common self-reported respiratory illness¹³ with a higher burden (exacerbations and hospitalisations) compared to their non-Indigenous counterparts.¹³⁻¹⁶ Therefore, there is a need to determine FeNO values in healthy Indigenous Australians. Our overall aim was to describe the distribution of FeNO values in 'healthy' (i.e. no asthma or atopy history) participants (3-16 years) who identify as Aboriginal, Torres Strait Islander or Both (Aboriginal and Torres Strait Islander). We also further aimed to (i) identify if there is any difference in FeNO between these three Indigenous ethnic groups, (ii) report the percentage with FeNO results above current recommended cut-off values described in Table 1 and (iii) report on preliminary FeNO results for Indigenous participants who have asthma and/or atopy history.

Table 1: Current FeNO guidelines

	Age	FeNO value suggestive of inflammation	Interpretation
ATS	Child <12 yrs. or Adult	>35 ppb >50 ppb	Suggest inflammation, consider history
NICE	Child <17 yrs. or Adult	≥35 ppb ≥40 ppb	Diagnose & treat
BTS/SIGN	Child <17 yrs. or Adult	≥35 ppb ≥40 ppb	Suggest inflammation, consider history
GINA	All ages	>50 ppb	Suggest inflammation, consider history

ATS: American Thoracic Society; **BTS/SIGN:** British Thoracic Society/Scottish Intercollegiate Guidelines Network; **GINA:** Global Initiative for Asthma; **NICE:** National Institute for Health and Care Excellence; **ppb:** parts per billion

MATERIALS AND METHODS

Study participants and design

We recruited 1036 participants aged between 3-16 years who identified as Aboriginal, Torres Strait Islander or Both from eight communities in Queensland, Australia. In the Australian context an Aboriginal or Torres Strait Islander is a person who identifies (self-reports) as such and is accepted by the community in which he (or she) lives. Participants were recruited from local child care centres, schools and community events. Ethics approval was given by the Human Research Ethics Committees of Children's Health Queensland (HREC/14/QRCH/111), Far North Queensland (HREC/14/QCH/96-929), Darling Downs (HREC/15/QTDD/27) and the Menzies School of Health Research/Northern Territory Health (HREC-2017-2833). Written informed consent was obtained from parents/guardians at the time of recruitment.

FeNO measurement

The NIOX MINO (Aerocrine AB, Solna, Sweden) hand held device was used to measure FeNO levels in parts per billion (ppb). As per current recommended guidelines,¹⁷ the participant exhaled directly into the device through a filter mouthpiece ('online' technique) at a controlled flow rate (50 mL/s with an exhalation pressure between 10-20cm H₂O) until the allocated exhalation time was reached (6 seconds if <10 years and 10 seconds if >12 years).¹⁸ The measurement was performed at least twice to achieve results within 10% of each other as per current guidelines¹⁷ and the mean value was recorded for analysis.

Questionnaires and medical record information

Two brief questionnaires completed by parents/guardians were used for this study. The first questionnaire collected information relating to the participant's self-identification of ethnicity (Aboriginal, Torres Strait Islander or Both), current health and medical history,

smoking status and exposure, and gestational age at birth. The second, a modified ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire,¹⁹ was used to determine the history of asthma, allergic rhinitis and/or eczema for each participant. Questionnaires were self-administered with the help of local Indigenous research assistants if required.

Paper and electronic medical records (inpatient and outpatient) from relevant health centres and hospitals were examined. We documented information relating to any respiratory disease (with or without hospitalisation) including asthma, bronchitis, bronchiolitis, pneumonia, bronchiectasis and croup. Evidence of allergic rhinitis, eczema, scabies, impetigo, otitis media and tonsillitis was also noted. Gestational age and birth weight data were collected when available.

Data management and clinical groups

All participant data (questionnaire responses, medical record information and test results) were entered into a secure database (FileMaker Pro 15, CA, USA). Exclusion criteria for the final dataset (Figure 1) were inability to meet FeNO acceptability/repeatability criteria (n=136), chronic respiratory disease (e.g. bronchiectasis) that would impact FeNO results (n=3), and current smokers (n=41) incomplete medical history (n=11).

Participants were separated into two age groups; participants <12 years or participants 12-16 years to facilitate comparison with the guidelines.¹⁻⁴ Participants were then classified into one of four groups according to their respiratory and atopic history; (1) healthy i.e. no asthma or atopy, (2) no asthma but with atopy, (3) with asthma but no atopy or (4) with

both asthma and atopy (Figure 1). Asthma history was considered present if there was documented evidence in medical records, or a positive response was recorded on the ISAAC questionnaire for *'ever had asthma'*. Atopy history was considered present if there was any medical record evidence of allergic rhinitis or eczema or, positive responses to ISAAC questions for *'ever had hayfever'* and *'ever had a problem with sneezing, or a runny, or blocked nose when not sick'* and/or *'ever had eczema'* were recorded. When there was a discrepancy between information sources, medical record evidence overrode negative questionnaire responses.

Statistical analyses

FeNO results are reported as medians since data were not normally distributed even after log-transformation. The Mann-Whitney test was used to assess the difference in FeNO results between healthy Aboriginal and Torres Strait Islander participants. We then calculated the percentage of our healthy cohort with FeNO values suggestive of eosinophilic inflammation as recommended by the various guidelines (see table 1). All analyses were performed using Stata15 software (StataCorp, TX, USA).

RESULTS

Between June 2015 and October 2017, 1039 participants attempted FeNO measurements. After exclusions, a total of 848 participants (81.7%) were included in the final analysis. Cohort demographics and health categories are described in Table 2. The cohort spanned an age range of 3.2 to 16.9 years and consisted predominantly of participants <12 years of age (63.0%). In both age groups the majority of participants had no asthma or atopy (<12 years

73.0% and 12-16 years 67.8%). Participants with both asthma and atopy were the least recruited for both age groups (5.4% and 5.7% respectively).

Table 2: Cohort demographics

	Participants <12 years n=534	Participants 12-16 years n=314	119 120 121 122 Total (n=848)
Male n (%)	259 (48.5)	155 (49.4)	414 (48.8)
Age years (median, IQR)	8.6 (7.1-10.4)	14.0 (13.0-15.3)	10.5 (8.0-13.3)
Height cm (median, IQR)	132.1 (122.4-142.9)	162.5 (157.3-169.0)	143.5 (128.5-159.3)
Indigenous Ethnicity n (%)			
Aboriginal	170 (31.8)	95 (30.3)	265 (31.3)
Torres Strait Islander	159 (29.8)	130 (41.4)	289 (34.1)
Aboriginal and Torres Strait Islander (Both)	205 (38.4)	89 (28.3)	294 (34.6)
Group categories & FeNO results (ppb)			
No asthma No atopy (healthy) n (%)	390 (73.0)	213 (67.8)	603 (71.1)
Median (IQR)	10 (7-14)	11 (7-19)	
With atopy # n (%)	50 (9.4)	43 (13.7)	93 (11.0)
Median (IQR)	11 (7-27)	23 (11-46)	
With asthma ^{##} No atopy n (%)	65 (12.2)	40 (12.7)	105 (12.4)
Median (IQR)	11 (8-23)	19 (10-34)	
With atopy # n (%)	29 (5.4)	18 (5.7)	47 (5.5)
Median (IQR)	19 (13-33)	24 (9-54)	

ppb: parts per billion; **#:** any evidence of allergic rhinitis or eczema from medical records or questionnaires; **##:** any evidence of asthma history from medical records or questionnaires

FeNO distribution in healthy participants with no asthma or atopy

The distribution of FeNO results for healthy Aboriginal, Torres Strait Islander and Both participants is shown in Figures 2A-B. For participants <12 years (Figure 2A), there was little

difference in the median FeNO values between the three ethnic groups (Aboriginal=11 ppb, Torres Strait Islander=10 ppb, Both=9 ppb). There was no statistically significant difference in FeNO distributions between Aboriginal and Torres Strait Islander participants ($p=0.73$). For participants 12-16 years (Figure 2B), again no difference was seen between the ethnic groups with respect to median FeNO values (Aboriginal=9 ppb, Torres Strait Islander=12 ppb, Both=11 ppb) and there was no statistically significant difference in distributions between Aboriginal and Torres Strait Islander participants ($p=0.17$). Results did not differ when data was analysed with the definition of 'asthma in the last 12 months' (Supplement file).

Interpretation according to current guidelines based on age

As there was no difference in FeNO results between the ethnic groups, we combined all data and examined the percent of the healthy participants (with no asthma or atopy) that would be considered positive for inflammation according to the current guidelines. When using ATS, NICE or BTS/SIGN guidelines for participants <12 years of age (all use the same cut-off value of 35 ppb), 7.2% of healthy participants ($n=28$) in our cohort would be considered in the 'inflammatory' zone (Figure 2A). In comparison, the GINA guidelines with a higher cut-off value (>50 ppb), identified only 3.8% of healthy participants <12 years ($n=15$) in the 'inflammatory' zone. For healthy participants aged between 12-16 years, the NICE and BTS/SIGN guidelines again identified more participants ($n=21$, 9.9%) to be positive for inflammation compared to the ATS and GINA ($n=10$, 4.7%) guidelines due to the differing cut-off values recommended (35 ppb vs. >50 ppb) (Figure 2B).

FeNO values in participants with asthma and/or atopy history

For both age groups, FeNO values were higher for those with asthma and/or atopy history compared to those without. Participants aged <12 years (Figure 3A) with both asthma and atopy had the highest median FeNO values (19 ppb), followed by participants with asthma but no atopy and no asthma but with atopy (both 11 ppb). For participants between 12-16 years (Figure 3B) median FeNO values were highest in participants with both asthma and atopy (24 ppb), followed by participants with no asthma but with atopy (23 ppb) and with asthma but no atopy (19 ppb).

DISCUSSION

To our knowledge this is the first study to report FeNO values for Aboriginal and Torres Strait Islander Australians aged between 3-16 years. We found no difference in the distribution of FeNO results of healthy participants (i.e. those with no asthma or atopy) who were <12 years or 12-16 years when separated according to their Australian Indigenous ethnicity. For those <12 years, 7.2% of our healthy participants had FeNO values in the 'inflammatory' zone when using the ATS, NICE or BTS/SIGN cut-off value (>35 ppb), however only 3.8% when using the GINA guidelines (cut-off value >50 ppb). Respective values for those aged 12-16 years, were 9.9% when using the NICE or BTS/SIGN cut-off value and (4.7%) when using the ATS or GINA cut-off value (>35 ppb vs. >50 ppb)

Our data is important in the context of the high asthma burden among Indigenous Australians,¹³ and our recent systematic review¹¹ that identified the healthy FeNO levels in several ethnic groups (e.g. African-American, Chinese, Korean) were not well characterised within the ATS cut-off values. In these groups, between 5 and 11% of healthy participants had FeNO values in the ATS defined 'inflammatory' zone.¹¹ Using the ATS,¹ NICE² and

BTS/SIGN³ recommended cut-off value (35 ppb), 7.2% of our healthy Australian Indigenous participants <12 years had results in the inflammatory zone. In comparison, the GINA⁴ guidelines (cut-off value >50 ppb) only identified 3.8% of healthy participants. Similarly for participants 12-16 years, the NICE² and BTS/SIGN³ recommended cut-off value (≥ 35 ppb) identified 9.9% of healthy participants in the inflammatory zone with a smaller proportion (4.7%) identified when using the ATS¹ and GINA⁴ recommended cut-off value (>50 ppb). Thus, care is still needed when selecting which guidelines and cut-off values to apply when interpreting FeNO values among Australian Indigenous children as inappropriate cut-off values have the potential to over diagnose asthma and result in unnecessary treatment interventions.

Two studies have reported FeNO values in healthy Australian children^{20,21} but to our knowledge, neither contained data collected from Indigenous Australians.¹¹ The geometric mean in one study²⁰ was lower than the median FeNO value in our study (7.2 vs. 9-12 ppb) but the second study²¹ had a similar geometric mean (11 ppb). We reported medians as even with log transformation, our data did not have a normal distribution. The FeNO testing methods in both these studies^{20,21} differed from ours in that both used higher expiratory flows and mouth pressures than our study. The higher expiratory flows may result in lower FeNO values compared to when using the current ATS standard pressure and flow parameters.^{20,22,23} Furthermore, we are unable to comment on differences in upper limits of normal (95th percentile) as neither study reported them.

There is still uncertainty regarding the use and definition of clinically meaningful cut-off values for interpreting FeNO values. This is due to the influence of confounding factors (such

as diet, exercise and atopy) and the overlap in results between certain disease profiles. Individuals can have high FeNO values despite no asthma or atopy history^{24,25} and conversely, well controlled asthmatic individuals can have FeNO results within the normal range.²⁶ Several studies have found an association between atopy²⁷⁻³⁰ or the number of positive skin prick tests^{21,31,32} with high FeNO results suggesting that the relationship between asthma and FeNO may in fact be largely influenced by atopy itself.³³ For both age groups in our cohort, we noted median FeNO results were highest for participants who had a history of both asthma and atopy, followed by participants with no asthma but with atopy and participants with asthma but no atopy (participants <12 years; 19 vs 11 vs 11 ppb and participants 12-16 years; 24 vs 23 vs 19 ppb). However as seen in Figure 3, there is considerable overlap in the distribution of FeNO between the asthma and/or atopy groups for both age groups.

Current international guidelines are based predominantly on data collected from Caucasian populations. The reason FeNO levels of healthy children and adults differ between different ethnic groups is still largely unknown. Differences in the activity of NO synthase (the enzyme essential for NO production) and in allele frequencies of the NO synthase genes has been shown between some ethnic groups.³⁴⁻³⁶ Ethnicity refers to a group of people who identify with each other based on common ancestral, social, cultural, or national experiences³⁷ and as such, complex interactions between genetic and biological, and/or environmental factors i.e. diet, second hand smoking, air pollution, and socioeconomic status are likely to result in differences in FeNO readings between ethnic groups.³⁸⁻⁴⁰

Taking into consideration evidence that shows FeNO to be a sensitive tool for monitoring adherence to asthma medications and predicting exacerbations following withdrawal of inhaled corticosteroid therapy,^{1,41} it is possible that until cut-off values (or reference equations) are inclusive of the known influencing factors on FeNO, the clinical utility of FeNO may lie more in the monitoring of asthma in an individual rather than as a diagnostic tool. Nevertheless, future studies in this population should examine the distribution of FeNO results in individuals with asthma and atopy phenotypes preferably with skin prick testing and direct sputum eosinophil counts to determine how sensitive current cut-off values are in accurately identifying eosinophilic inflammation and asthma in Indigenous Australians.

Limitations

There are some important limitations to our study. The primary limitation is that we were unable to perform any direct measurements of atopy (skin prick test) or sputum eosinophil counts to positively identify participants with eosinophilic inflammation. Rather, we relied solely on self-reported and medical record information to identify participants with a history of these conditions. We acknowledge that some participants who were classified as having asthma historically would not have had current eosinophilic asthma. As we were unable to verify asthma phenotype or medication use we grouped any evidence of asthma together to ensure robustness of the healthy group. There is a possibility that some participants may have been atopic at the time of testing but relevant information was missing or incorrectly reported on questionnaires and in medical charts. We attempted to minimise this risk by using medical chart history to augment self-reported (questionnaire) responses. During data collection, we were also unable to account for other known influencing factors which increase FeNO values such as nitrate intake and strenuous exercise, and we acknowledge

that this may influence some of our results. Further, our results are only applicable for Aboriginal and Torres Strait Islanders up to the age of 17 living in Queensland. Continued work is needed in adults and in other communities around Australia. With respect to FeNO results from individuals with asthma and/or atopy history, continued studies that specifically target these groups are needed to better understand how to incorporate FeNO effectively into asthma management pathways for Aboriginal and Torres Strait Islander Australians.

CONCLUSION

This is this first study to report FeNO values of Aboriginal and Torres Strait Islander Australians. We found no difference in the distribution or median FeNO results between healthy Aboriginal and/or Torres Strait Islander participants. For our healthy cohort of Indigenous Australians between 3 and 16 years of age, the GINA recommended cut-off value of 50 ppb appears to be the most appropriate for identifying healthy individuals and considering abnormality. Continued work is still needed to confirm the most robust and clinically meaningful cut-off value to identify eosinophilic inflammation and to determine how best to incorporate FeNO testing into asthma management pathways for Indigenous Australians.

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TB had full access to the data and takes responsibility for the integrity of data and accuracy of analysis. TB, AC and MM contributed to study design and data collection. TB performed statistical analysis with guidance provided by MC. All authors were involved in interpretation of results. TB contributed to preparation of the manuscript as well as the design of figures and tables. AC, MC, HP, JM and MM edited manuscript. All authors agreed on the final version before submission.

FIGURE LEGENDS

Figure 1: Flow diagram depicting exclusion criteria used to identify participants for analysis and separation according to asthma and/or atopy history.

Figure 2A: Box and whisker plots of FeNO for 'healthy' (no asthma or atopy) Australian Indigenous participants <12 years (n=390) separated according to ethnicity. Red horizontal line marks the ATS/NICE/BTS/SIGN recommended cut-off value of 35ppb. Purple horizontal line marks the GINA recommended cut-off value of 50ppb. **Both:** Aboriginal and Torres Strait Islander; **ppb:** parts per billion

Figure 2B: Box and whisker plots of FeNO for 'healthy' (no asthma or atopy) Australian Indigenous participants 12-16 years (n=213) separated according to ethnicity. Red horizontal line marks the NICE/BTS/SIGN recommended cut-off value of 40ppb. Purple horizontal line marks the ATS/GINA recommended cut-off value of 50ppb. **Both:** Aboriginal and Torres Strait Islander; **ppb:** parts per billion

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297 **Figure 3A:** Box and whisker plots of FeNO for Australian Indigenous participants <12 years
298 (n=534) separated according to asthma and/or atopy history. Red and purple horizontal
299 lines as per Figure 2A. **ppb:** parts per billion

300

301 **Figure 3B:** Box and whisker plots of FeNO for Australian Indigenous participants 12-16 years
302 (n=314) separated according to asthma and/or atopy history. Red and purple horizontal
303 lines as per Figure 2B. **ppb:** parts per billion

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e-Appendix 1.

We reanalysed data with the definition of 'asthma in the last 12 months' (c.f. definition of 'ever asthma' in the main text). Using the aforementioned definition, an additional 55 participants from our cohort were included into the 'healthy' groups. However, no difference was seen in the median FeNO values for the two age groups (participants <12 years = 10ppb or 12-16 years = 11ppb) with the inclusion of these participants (Table S1). An additional four participants <12 years and three participants 12-16 years were identified by the guidelines as being positive for eosinophilic inflammation. This also made little difference to overall results; 7.6% identified using ATS/NICE/BTS/SIGN (originally 7.2%) vs. 4.5% using GINA (originally 3.8%) for participants <12 years and 10.2% identified using NICE/BTS/SIGN (originally 9.9%) vs. 4.2% using ATS/GINA (originally 4.7%) for participants 12-16 years.

e-Table 1. FeNO results for healthy Indigenous Australian participants

	FeNO levels (ppb)		n (%) of cohort above recommended cut-off values	
	Geometric mean (x/GSD)	Median (IQR)		
Participants <12 years (n=422)	11 (x/1.9)	10 (7-15)	ATS/NICE/BTS/SIGN 32 (7.6)	GINA 19 (4.5)
Participants 12-16 years (n=236)	13 (x/2.0)	11 (8-19)	NICE/BTS/SIGN 24 (10.2)	ATS/GINA 10 (4.2)

GSD: geometric standard deviation; **ppb:** parts per billion

For participants <12 years: ATS/NICE/BTS/SIGN recommended cut-off value of 35ppb. GINA recommended cut-off value of 50ppb.

For participants 12-16 years: NICE/BTS/SIGN recommended cut-off value of 40ppb. ATS/GINA recommended cut-off value of 50ppb.

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Tamara L. Blake, MCISc, Anne B. Chang, PhD, Mark D. Chatfield, MSc, Julie M. Marchant, PhD, Helen L. Petsky, PhD, Margaret S. McElrea, PhD

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