

Recent studies have focused on possible positive health implications of certain ruminant TFAs.<sup>3</sup> In particular, moderate consumption of ruminant TFAs was not associated with a significant increase in cardiovascular risk, although further studies are needed to understand the implications of various TFA isomers.<sup>3</sup> In addition, certain ruminant TFAs, including vaccenic acid and C9,T11-CLA, have been shown to have cancer-protective factors in animal models, although human studies are limited.<sup>3</sup>

More data are needed to determine if the changes in TFA levels may be attributed primarily to the 2003 legislative changes. It would be particularly interesting to see data regarding plasma content of TFAs before and after regional *trans*-fat bans. Additionally, although the 2003 legislation required labeling for TFAs, there is still a significant amount of TFA content in food products; plasma profiles may be further improved with improved TFA labeling.

Eric Brandt, BS

**Author Affiliation:** Case Western Reserve University School of Medicine, Cleveland, Ohio (ejb90@case.edu).

**Conflict of Interest Disclosures:** The author has completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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**In Reply:** More than 20 different TFAs have been reported in human blood,<sup>1,2</sup> and the selected 4 TFAs measured in plasma in our study are considered to be the major TFAs.<sup>3</sup> The extent to which certain food sources affected blood levels of these 4 TFAs in our population group is not known. Although we agree that such information would be helpful for reasons such as those presented by Mr Brandt, the study did not intend to address this topic.

Assessing the effect of certain food sources on individual TFAs in blood is challenging. The concentrations of individual TFAs from partially hydrogenated vegetable oils may vary considerably depending on the oils used and the methods of processing, both of which vary by brand. Investigations are ongoing to examine (to the extent possible from dietary recall information) the relationship between dietary intake of selected foods and plasma levels of TFA.

Our study on plasma TFA levels provides information on TFAs for US non-Hispanic white adults in 2000 and 2009. Between 2000 and 2009, the US Food and Drug Administration amended its regulations on nutrition labeling to require that TFAs be declared on the nutrition label of conventional foods and dietary supplements.<sup>4</sup> In addition, community and state health departments implemented activities to further limit the use of TFAs, and manufacturers reformulated food to reduce TFAs in supermarket and restaurant food.<sup>5</sup> The study examined

US population-wide changes in TFAs in white adults and did not focus on state or local population changes from regional actions that limited TFA consumption. Because the study was focused on the US population, it is likely that the mean decrease of 58% in 4 TFAs was dominated by population-wide changes, such as actions by the Food and Drug Administration and food manufacturers. Although the 58% decrease shows substantial progress, further reducing levels of TFAs in blood remains an important public health goal.

Hubert W. Vesper, PhD

James L. Pirkle, MD, PhD

**Author Affiliations:** National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia (hvesper@cdc.gov).

**Conflict of Interest Disclosures:** The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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## RESEARCH LETTER

### Cases of Congenital Adrenal Hyperplasia Missed by Newborn Screening in Minnesota

**To the Editor:** The purpose of newborn screening (NBS) for congenital adrenal hyperplasia (CAH) due to 21 $\alpha$ -hydroxylase deficiency is the early identification of newborns with the classic salt-wasting (SW) and simple-virilizing (SV) forms to avoid a potentially life-threatening adrenal or salt-wasting crisis. Cases of classic CAH missed by NBS (false-negatives) are not well documented.<sup>1</sup>

**Methods.** Population-based study of all newborns screened in Minnesota (N=838 241) from January 1999 through December 2010 was performed. As in most NBS programs in the United States, samples were collected 24 to 48 hours after birth and 17 $\alpha$ -hydroxyprogesterone level was measured by a time-resolved fluoroimmunoassay (TABLE 1). Through a partnership between the Minnesota Department of Health and the 3 largest pediatric endocrinology centers in Minnesota, cases of CAH missed by NBS were identified through review of the NBS registry and the medical records of the participating institutions. Institutional review boards at all sites approved the study with waivers of informed consent.

Confirmation of classic CAH of those identified and missed by NBS was based on elevated serum 17 $\alpha$ -hydroxyproges-

terone levels, clinical and biochemical presentation, and in some cases, molecular testing of the *CYP21A2* gene using a common mutation panel or sequencing.<sup>2</sup> Computations were performed using the Binom package in R (R Foundation for Statistical Computing).

**Results.** Of the 838 241 newborns screened during the period, 52 patients with classic CAH were identified and 15 cases were missed (false-negative rate, 22.4%; 95% CI, 14%-34%); 6 males, 9 females; 10 SV, 5 SW) (TABLE 2).

Among the 9 females missed by NBS, 3 had ambiguous genitalia at birth (cases 9, 10, and 15) but were not identified until 3.4 years, 6.5 years, and 3 months of age, respectively. Case 10 had vaginoplasty 6 years before diagnosis. Ambiguous genitalia identified cases 1, 2, 3, 4, and 14. Case 3 was originally assigned the male sex and diagnosed a few days later after workup for penoscrotal hypospadias.

The CAH males missed by NBS (cases 6, 7, 8, 11, and 13) were not diagnosed until ages 2.3 to 5.5 years (except case 5) and had significant bone age advancement (median of 7 years difference between bone age and chronological age). Case 6 presented at age 4 years and was thought to have a left adrenal tumor. He underwent adrenalectomy with histopathology suggestive of CAH.

**Comment.** Over a 12-year period, 22% of diagnosed patients born in Minnesota with classic CAH were not identified by NBS, highlighting that a negative screening result does not definitively rule out classic CAH. We also found that a false-negative result can sometimes delay the diagnosis of CAH even in a newborn female with ambiguous genitalia.

A study limitation is the probability that false-negatives are underestimated due to patients with CAH not yet diagnosed, patients who moved out of the state, or infants who passed away with unidentified CAH.

While other studies have reported some missed cases of SV-CAH,<sup>3</sup> our study is one of the first to document the extent to which both cases of SV-CAH and SW-CAH are missed by NBS. Sensitivity of NBS for SW-CAH is generally reported as 100%,<sup>1</sup> with only 1 report from Germany identifying 2 cases of SW-CAH missed over 7 years at 24 institutions.<sup>4</sup> The lack of a consistent follow-up reporting system for physicians to alert their state's health department when a patient is diagnosed with classic CAH later in infancy or childhood may explain why more missed cases of classic CAH have not been reported.

Similarities in NBS procedures in most US states suggest that false-negative results may be underestimated nationwide. However, the reason for false-negative results is unclear. An unex-

**Table 1.** Congenital Adrenal Hyperplasia Newborn Screening Protocols and Assays in Minnesota

Date	Screening Protocol	First-Tier Assay of 17OHP	Second-Tier Assay	Metabolites and Ratios	Details of Screening Protocol
January 1, 1999-May 30, 2004	One tier with repeat screening for equivocal results	Time-resolved fluoroimmunoassay (manual)	None	None	Results were defined as negative, positive, or equivocal. A first tier positive result was considered presumed positive but not classified as true positive or false-positive until the diagnosis was confirmed by an endocrinologist. For an equivocal (mildly elevated) first tier result, a repeat screening was performed. If negative, the result was classified as negative. If positive, the result was considered presumed positive but was not classified until the diagnosis was confirmed by an endocrinologist.
June 1, 2004-February 17, 2008	Two tier	Time-resolved fluoroimmunoassay (manual and automatic) <sup>a</sup>	LC-MS/MS	17OHP; (17OHP + D4A)/cortisol	Repeat screenings for equivocal results were no longer performed. A first tier positive result was defined as presumed positive and the original dried blood spot on filter paper card was automatically and immediately analyzed at the Mayo Clinic's Biochemical Genetics Laboratory using a second tier method of steroid profiling. A negative second tier result (either normal 17 $\alpha$ -hydroxyprogesterone level or normal ratio) was classified as negative. If second tier result was positive (in which both the 17 $\alpha$ -hydroxyprogesterone level and ratio are elevated), the result was considered presumed positive until the diagnosis was confirmed by an endocrinologist.
February 18, 2008-December 31, 2010	Two tier	Time-resolved fluoroimmunoassay (automatic)	LC-MS/MS	17OHP <sup>b</sup> ; (17OHP + D4A)/cortisol; 11DF; 21DF	Same details as in row above.

Abbreviations: 17OHP, 17 $\alpha$ -hydroxyprogesterone; D4A, androstenedione; 11DF, 11-deoxycortisol; 21DF, 21-deoxycortisol; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

<sup>a</sup>In March 2006, the same 17OHP kit was moved from a manual to an automated platform (AutoDELFLIA, PerkinElmer Life and Analytical Sciences).

<sup>b</sup>Sex-specific 17OHP reference ranges were incorporated.

plained delayed increase in 17 $\alpha$ -hydroxyprogesterone level in certain patients with classic CAH and/or the timing and sensitivity of the assay are possibilities. The false-negative results were not due to procedural changes because 1 to 2 false-negative results were found every year from 1999 through 2010 except 2002, which had none.

Screening programs should educate clinicians about false-negative results so that any patient for whom there is clinical concern for CAH can receive immediate diagnostic testing, particularly females with ambiguous genitalia.

Kyriakie Sarafoglou, MD  
Katie Banks, BS  
Jennifer Kyllö, MD  
Siobhan Pittock, MD  
William Thomas, PhD

**Author Affiliations:** Department of Pediatrics, University of Minnesota Amplatz Children's Hospital, Minneapolis (Dr Sarafoglou; saraf010@umn.edu); Minnesota Department of Health, St Paul (Ms Banks); Department of Pediatrics, Children's Hospitals of Minnesota, Minneapolis (Dr Kyllö); Department of Pediatrics, Mayo Clinic College of Medicine, Rochester, Minnesota (Dr Pittock); and School of Public Health, University of Minnesota, Minneapolis (Dr Thomas).

**Author Contributions:** Dr Sarafoglou had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Table 2.** Clinical Information on Cases of Classic Congenital Adrenal Hyperplasia (CAH) Missed by Newborn Screening<sup>a</sup>

Case No.	Sex	At Diagnosis			Presenting Symptoms	CAH Subtype	Molecular Testing	
		Serum 17 $\alpha$ -Hydroxyprogesterone Level, ng/dL	Age, y	Bone Age			Allele 1	Allele 2
1	F	5514	0.01	NA	Ambiguous genitalia	Salt wasting	g.2108C>T (p.R356W)	g.655C/A>G (In2 splice)
2	F	14 900	0.01	NA	Ambiguous genitalia	Salt wasting	g.999T>A (p.I172N)	g.655C/A>G (In2 splice)
3	F	1112 <sup>b</sup>	0.01	NA	Ambiguous genitalia, initially assigned male sex	Salt wasting	g.655C/A>G (In2 splice)	30kB del
4	F	11 000	0.01	NA	Ambiguous genitalia	Salt wasting	NA	NA
5	M	812 <sup>c</sup>	Prenatal testing	NA	Family history	Salt wasting	g.2108C>T (p.R356W)	30kB del
6	M	10500	4.0	12	Precocious puberty, penile enlargement, growth acceleration	Simple virilizing	NA	NA
7	M	9864	5.0	10	Penile enlargement, growth acceleration	Simple virilizing	g.2494G>A (p.G424S)	30kB del
8	M	8650	5.5	12.5	Pubarche, penile enlargement, growth acceleration	Simple virilizing	NA	NA
9 <sup>d</sup>	F	1130	3.4	6.8	Clitoromegaly, growth acceleration	Simple virilizing	NA	g.1683>T (p.V281L); g.929C>T (p.R149C)
10	F	7210	6.5	11	Ambiguous genitalia, growth acceleration	Simple virilizing	g.1761T>G (p.L307V) <sup>e</sup>	30kB del
11	M	45 100	4.3	12	Penile enlargement, growth acceleration	Simple virilizing	g.655C/A>G (In2 splice)	30kB del
12	F	NA <sup>f</sup>	0.01	NA	None	Simple virilizing	g.999T>A (p.I172N)	g.655C/A>G (In2 splice)
13	M	5920	2.3	6.5	Penile enlargement, growth acceleration	Simple virilizing	g.999T>A (p.I172N)	g.655C/A>G (In2 splice)
14	F	1086 <sup>g</sup>	0.01	NA	Ambiguous genitalia	Simple virilizing	g.999T>A (p.I172N)	g.994C>T (p.Q318X)
15	F	17 500	0.2	NA	Ambiguous genitalia	Simple virilizing	g.999T>A (p.I172N)	30kB del

Abbreviation: NA, data not applicable.

<sup>a</sup>The newborn screening result of the 15 patients in this table was false-negative. None of the mothers received glucocorticoids during pregnancy. An additional patient missed by newborn screening was not added to this table because the mother received prenatal therapy with dexamethasone, which normalizes 17 $\alpha$ -hydroxyprogesterone levels in affected infants.

<sup>b</sup>A second serum 17 $\alpha$ -hydroxyprogesterone level (measured 2 days later) was 11 600 ng/dL with a sodium level of 126 mEq/L.

<sup>c</sup>Patient has older brother with salt wasting CAH and the same genotype. Parental molecular testing also was performed.

<sup>d</sup>Patient has a single expressed *CYP21A2* gene copy with 2 hemizygous nonclass mutations, whose compound effect on protein is expected to result in significantly reduced (<10%) enzyme activity, consistent with the reported phenotype of simple-virilizing CAH.<sup>5</sup>

<sup>e</sup>Patient has a rare mutation [g.1761T>G (p.L307V)] that has been previously reported in trans with the known disease-causing mutation p.Q318X in an individual with a diagnosis of simple-virilizing CAH.<sup>6</sup>

<sup>f</sup>A random serum 17 $\alpha$ -hydroxyprogesterone level measured at 1.5 years of age was 27 300 ng/dL and parental molecular testing was performed.

<sup>g</sup>An ACTH stimulation test at 4 weeks of age showed a baseline and stimulated 17 $\alpha$ -hydroxyprogesterone level of 2970 and 13 300 ng/dL, respectively, as well as a stimulated cortisol level of less than 1  $\mu$ g/dL.

*Study concept and design:* Sarafoglou, Banks, Kylo, Thomas.

*Acquisition of data:* Sarafoglou, Banks, Kylo, Pittock.

*Analysis and interpretation of data:* Sarafoglou, Thomas.

*Drafting of the manuscript:* Sarafoglou.

*Critical revision of the manuscript for important intellectual content:* Sarafoglou, Banks, Kylo, Pittock, Thomas.

*Statistical analysis:* Banks, Thomas.

*Administrative, technical, or material support:* Sarafoglou, Banks, Kylo.

*Study supervision:* Sarafoglou.

**Conflict of Interest Disclosures:** The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

**Funding/Support:** All work performed for this study was done as part of the unique public-private partnership that exists between the Minnesota Department of Health, the Mayo Clinic, Children's Hospitals of Minnesota, and the University of Minnesota Amplatz Children's Hospital.

**Role of the Sponsors:** The funding organizations had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

**Additional Contributions:** We thank Nancy Vanderburg, RN, Amy Gaviglio, MS, CGC, Amy Hietala, MS, and Mark McCann, BA (all with the Minnesota Department of Health, Newborn Screening Program), who provided newborn screening information and reviewed the manuscript. These persons were not compensated for their contributions.

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## CORRECTION

**Errors in Abstract, Text, and Tables:** In the Original Contribution entitled "Effect of Increasing Doses of Saw Palmetto Extract on Lower Urinary Tract Symptoms: A Randomized Trial," published in the September 28, 2011, issue of *JAMA* (2011; 306[12]:1344-1351), errors occurred in the abstract, text, and tables. In the abstract, the 95% CI for mean AUASI score between baseline and 72 weeks for the saw palmetto extract group should have been  $-3.04$  to  $-1.36$ . In the second paragraph of the Results section, the median pill count across attended visits should have been 97.1%. In Table 1, the range of the NIH CPSI pain scale in the Total column should have been (0-3) and in the Placebo column, it should have been (0-3). Also, the superscript "c" in the footnote should have referred to "Fisher exact test." In the third paragraph of the Results section, the proportion of participants achieving a 3-point decrease in AUASI score at 72 weeks should have been 48.9% in the saw palmetto extract group and 52.5% in the placebo group (1-sided Fisher exact test,  $P=.79$ ); and the results of the mixed models repeated measures analysis  $P$  value for saw palmetto extract vs placebo should have been .65. In Table 2, the 95% CI for mean difference in primary AUASI score for the saw palmetto extract group should have been  $-3.04$  to  $-1.36$ . This article was corrected online.