The proposed requirement for approval of coverage is a useful addition to the recommendation made in the Institute of Medicine report that journals require sex-specific analyses for publication.[1] However, if the assertion that a coverage requirement is the "best way" to ensure adequately powered tests of men and women implies that a coverage requirement will be more effective than a publication requirement, I would disagree. In relation to a publication requirement, the *JAMA* Editor's Note accompanying my Commentary questioned whether it would occur too late in the process to be effective. Decisions regarding coverage come even later and are primarily relevant to clinical trials rather than to the full range of scientific studies providing sex-specific analyses.

The impact of either requirement will depend on its salience to researchers as they plan their studies. The publication requirement would need to be phased in since it would be problematic to apply it to studies that are already underway or have completed data collection. Over time, however, its existence should shape the design and conduct of studies. Researchers do not want to undertake studies that cannot be published. If a sex-specific requirement for publication is clearly and consistently applied, they would be highly motivated to ensure adequate enrollment of women for meaningful analysis.

It is not clear that a similar requirement by insurers would have as broad an effect. The approval process for insurance coverage is more distal to research than is publication, and publication requirements are likely to be more salient. Every researcher wants to publish the findings of his or her research, but only a subset will be concerned about insurance coverage decisions.

If it were necessary to choose a single policy, a publication requirement would appear to have greater potential for broad impact and a fundamental shift in research than a coverage requirement. However, it need not be an "either-or" choice. It may well be that both types of requirements are needed to advance women's health. Despite existing regulations by the National Institutes of Health and Food and Drug Administration, female participants are still underrepresented in animal studies, observational research, and clinical trials.[1] The bias toward framing questions based on a male model of disease, and subsequent design and inclusion criteria, may be sufficiently ingrained in the biomedical research culture that requirements are needed at multiple levels to create lasting change.

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1. Institute of Medicine. *Women's Health Research: Progress, Pitfalls and Promise.* Washington, DC: National Academies Press; 2010.

RESEARCH LETTER

Expanded Newborn Screening for Detection of Vitamin B_{12} Deficiency

To the Editor: When undiagnosed, infant vitamin B_{12} deficiency can result in anemia, failure to thrive, developmental regression, and neurological deficits and is most commonly caused by maternal vitamin B_{12} deficiency.[1] Biochemically, vitamin B_{12} deficiency leads to an accumulation of total homocysteine (tHcy), methylmalonic acid (MMA), and propionylcarnitine (C_3). Although vitamin B_{12} deficiency is not a primary target of newborn screening (NBS) programs, measurement of C_3 may incidentally identify vitamin B_{12}–deficient newborns. However, first-tier screening is limited because C_3 levels may not be sufficiently high during the first days of life when most NBS samples are collected.[2] Levels of MMA and tHcy are more sensitive markers of vitamin B_{12} status than serum B_{12} levels in adults,[3] have a strong inverse relationship with serum B_{12} levels,[4] and are usually elevated in infants who are vitamin B_{12} deficient before hematologic or neurologic findings develop.[2] Through the public-private partnership between the Minnesota Department of Health, Mayo Clinic, and University of Minnesota, a lower first-tier cut-off level of C_3 was applied (>9.2 µmol/L to >5.25 µmol/L) and second-tier testing of MMA and tHcy levels in presumptive-positive NBS specimens was introduced in 2005.[5]

Methods. A population-based study of 363 649 infants born in Minnesota was conducted between January 1, 2005, and December 31, 2010. Newborn screening samples with elevated C_3 levels, a C_3 to acetylcarnitine (C_3/C_2) ratio of 0.1 or greater, or a C_3 to palmitoylcarnitine (C_3/C_{16}) ratio of 2 or greater had second-tier testing of tHcy (abnormal, >15 µmol/L) and MMA (abnormal, >5 µmol/L) on the same sample.[5] (To convert tHcy to mg/dL, divide by 7.397.) All newborns with positive NBS results and some of their mothers underwent further laboratory testing. An additional newborn from another state whose first-tier NBS findings were normal presented at age 2 months with clinical complications of vitamin B_{12} deficiency and required prolonged hospitalization. His original NBS sample was retrospectively analyzed to determine if the Minnesota 2-tier protocol would have led to identifying the infant as vitamin B_{12} deficient. The University of Minnesota institutional review board approved review of medical records and waived informed consent.

Results. Of 46 newborns with abnormal C_3 levels or related ratios identified in Minnesota, 11 had vitamin B_{12} deficiency secondary to maternal vitamin B_{12} deficiency (**TABLE 1** and **TABLE 2**) for a detection rate of 3.02 per 100 000 live births; 22 findings were false positives; 1 declined follow-up testing; 1 had unconfirmed vitamin B_{12} deficiency; 3 had cobalamin C defects; and 2 had methylmalonic acidemias. Five were homozygous and 1 a carrier for transcobalamin receptor defects. Newborn screening led to the identification of vitamin

Table 1. Maternal Prenatal History, Serum B_{12} Levels (Pretreatment, Postdelivery), and Newborn Screen Dried Blood Spot Values

Mothers Presumed Vitamin B_{12} Deficient[a]		Newborn Screening Results					
Case	Prenatal History	Serum B_{12} pg/mL[b]	C_3, µmol/L[b]	C_3/C_2 Ratio[b]	C_3/C_{16} Ratio[b]	Second-Tier MMA, µmol/L[c]	Second-Tier tHcy, µmol/L[c]
1[d]	Hyperemesis gravidarum, poor weight gain	294	8.12	0.20	3.40	6.25	7.81
2[d,e]	NA	116	6.57	0.21	3.22	39.00	29.20
3[e,f]	Preeclampsia, preterm delivery	214	6.93	0.27	2.22	15.00	37.70
4[g]	Treatment for syphilis, postterm delivery	207	7.60	0.21	1.80	14.40	18.10
5[d]	B_{12} deficiency, anemia, treatment with hydroxyl-cobalamin, 1 mg/mo	NA	6.61	0.23	2.55	9.69	12.90
6[d,e]	Hypothyroidism, iron-deficient anemia	192	5.10	0.23	1.53	5.10	27.30
7[e,g]	Bariatric surgery; oral cyanocobalamin, 1 mg/d, during third trimester	75[h]	5.26	0.22	1.67	17.00	53.00
8[e,g]	Uncomplicated	197	4.22	0.30	1.95	29.00	65.00
9[e,g]	Hyperemesis gravidarum	201	2.82	0.25	1.20	21.80	22.40
10[d]	Uncomplicated	198	5.16	0.27	2.10	10.90	22.40
11[d]	Bariatric surgery, not compliant with hydroxyl-cobalamin IM treatment	104	2.61	0.29	2.10	15.90	22.40
12[f]	Hyperemesis gravidarum, poor weight gain, pernicious anemia diagnosed postnatally	107	4.31[i]	0.13[i]	1.45[i]	66.80[i]	81.70[i]

Abbreviations: C_3, propionylcarnitine; C_2, acetylcarnitine; C_{16}, palmitoylcarnitine; IM, intramuscular; MMA, methylmalonic acid; NA, not available; tHcy, total homocysteine.
SI conversion factors: To convert serum B_{12} to pmol/L, multiply by 0.7378; to convert tHcy to mg/dL, divide by 7.397.
[a] Maternal vitamin B_{12} deficiency was defined by low serum B_{12} maternal levels or low serum B_{12} levels in the infants on confirmatory testing and if their initial elevated urine MMA or plasma tHcy normalized while receiving vitamin B_{12} supplementation or remained normal after discontinuing vitamin B_{12} therapy. Another cause of low B_{12} levels, cobalamin F deficiency, is unlikely due to positive clinical response to oral vitamin B_{12} therapy or normalization of biochemical markers while not receiving treatment. In case 5, for which serum B_{12} levels were not available in the infant or mother, elevated markers of MMA and tHcy indicated functional vitamin B_{12} deficiency as they normalized after treatment.
[b] Serum B_{12} levels were abnormal if <211 pg/mL. Percentiles (1st-99th) for C_3 based on 469 279 newborn screening samples analyzed.[5] Levels of C_3 were abnormal if >5.25 µmol/L (range, 0.6-5.1 µmol/L). C_3/C_2 ratio was abnormal if ≥0.1, and C_3/C_{16} ratio was abnormal if ≥2.
[c] Based on 200 leftover NBS blood spots for routine newborn screening and yielding normal results.[5] Levels of MMA were abnormal if >5 µmol/L (range, 0.2-2.0 µmol/L) and tHcy abnormal if >15 µmol/L (range, 0.8-10.9 µmol/L).
[d] Prenatal diet information for mother not available.
[e] Mother took prenatal vitamins.
[f] Vegetarian prenatal diet.
[g] Regular prenatal diet.
[h] Value used is reported serum B_{12} during pregnancy.
[i] Retrospective analysis from original dried blood spot specimen.

Table 2. Diet and Pretreatment Biochemical Results of Newborns Presumed Vitamin B_{12} Deficient[a]

Case	Diet	Urine MMA, µg/mg Cr[b]	Plasma C_3, nmol/mL[b]	Plasma tHcy, µmol/L[b]	Serum B_{12}, pg/mL[b]	Age at Start of Therapy, wk	Therapy
1	BM	65	0.48	14.3	126	2	Oral cyanocobalamin
2	BM 1 wk, then IF	S1 = 779; S2 = 370	3.16	S1 = 31.2; S2 = 14.3	459[c]	2	SQ hydroxycobalamin[d]
3	BM and IF	175	2.26	34.3	198	4	Oral cyanocobalamin
4	BM and IF	120	1.06	18.0	311[c]	2	Oral cyanocobalamin
5	BM	S1 = 39; S2 = 118	1.04	13.0	NA	21	Oral cyanocobalamin
6	BM and IF	105	2.83	20.5	337[c]	4	Oral cyanocobalamin
7	BM	180	NA	29.3	NA	1	IM hydroxycobalamin ×1, oral cyanocobalamin
8	BM	195	NA	57.2	NA	<1	Oral cyanocobalamin, then SQ hydroxycobalamin, then multivitamin
9	BM	130	NA	27.0	182	2	Oral cyanocobalamin
10	IF	S1 = 206; S2 = 120	1.25	S1 = 14.0; S2 = 14.9	415[c]	<2	Multivitamin
11	BM and IF	121.6	NA	21.0	665[c]	2	Oral cyanocobalamin
12	BM	7524[e]	NA	S1 = 40.0; S2 = 19.0[e]	196[e]	9	IM hydroxycobalamin, then oral cyanocobalamin

Abbreviations: BM, breast milk; C_3, propionylcarnitine; Cr, creatinine; IF, infant formula; IM, intramuscular; MMA, methylmalonic acid; NA, not available; S, sample; SQ, subcutaneous; tHcy, total homocysteine.
SI conversion factors: To convert tHcy to mg/dL, divide by 7.397; to convert serum B_{12} to pmol/L, multiply by 0.7378.
[a] Maternal vitamin B_{12} deficiency was defined by low serum B_{12} maternal levels or low serum B_{12} levels in the infants on confirmatory testing and if their initial elevated urine MMA or plasma tHcy normalized while receiving vitamin B_{12} supplementation or remained normal after discontinuing vitamin B_{12} therapy. Another cause of low B_{12} levels, cobalamin F deficiency, is unlikely due to positive clinical response to oral vitamin B_{12} therapy or normalization of biochemical markers while not receiving treatment. In case 5, for which serum B_{12} levels were not available in the infant or mother, elevated markers of MMA and tHcy indicated functional vitamin B_{12} deficiency as they normalized after treatment.
[b] Measurements of MMA, plasma C_3, and plasma tHcy came from initial samples obtained during the first month of life unless otherwise noted. MMA level was abnormal if >14 µg/mg Cr. Plasma C_3 level was abnormal if >1.78 nmol/mL. Plasma tHcy level was abnormal if >12 µmol/L. Serum B_{12} level was abnormal if <211 pg/mL.
[c] The elevated markers (urine MMA and plasma tHcy) indicate functional vitamin B_{12} deficiency despite normal vitamin B_{12} levels.
[d] Patient's biochemical and clinical examination findings remained normal 7 months after discontinuing IM hydroxycobalamin therapy.
[e] Samples obtained at age 2 mo after acute clinical presentation.

B_{12} deficiency in 8 of 11 mothers whose status was previously unrecognized. All 11 newborns were asymptomatic when treatment was initiated (Table 2), and subsequently all biochemical markers normalized without clinical signs of vitamin B_{12} deficiency. Retrospective analysis using the 2-tier protocol of the original NBS sample of the symptomatic infant screened by another state had positive results (Table 1, case 12).

Comment. A recent survey of 12 state NBS programs (including Minnesota) identified 32 cases of nutritional vitamin B_{12} deficiency, for a rate of 0.88 per 100 000 newborns.[6] After implementing a lower C_3 first-tier cut-off level and second-tier testing of MMA and tHcy levels in NBS specimens, the detection rate in Minnesota of vitamin B_{12}–deficient infants was considerably higher (3.02/100 000 live births). None of the 11 infants would have been identified by using the previous C_3 cut-off value, potentially explaining the reason no cases of infant vitamin B_{12} deficiency were identified by NBS before 2005 in Minnesota.

Based on the Minnesota experience, vitamin B_{12} deficiency occurs more frequently than is currently recognized. Given the important role vitamin B_{12} plays in DNA synthesis and central nervous system function, and the difficulty in diagnosing vitamin B_{12} deficiency in infancy because of nonspecific clinical findings, early identification and initiation of treatment are crucial to avoid potentially devastating and irreversible neurologic damage.

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CORRECTION

Errors in Tables and Figure: In the Original Contribution entitled "Association of Troponin T Detected With a Highly Sensitive Assay and Cardiac Structure and Mortality Risk in the General Population," published in the December 8, 2010, issue of *JAMA* (2010;304[22]:2503-2512), an error appeared in tables and a figure. The highest cTnT categories in all tables and figures should have been 0.0066-$<$0.014 and $\geq$0.014. This article has been corrected online. A related letter to the editor appears in this issue.