Association between supraclavicular brown adipose tissue composition at birth and adiposity gain from birth to 6 months of age

Sonja Entringer¹, Jerod Rasmussen², Dan M Cooper², Satoru Ikenoue³, Feizal Waffarn², Pathik D Wadhwa⁴ and Claudia Buss¹

BACKGROUND: Brown adipose tissue (BAT) is associated with higher energy expenditure and lower adiposity in adults. However, the relationship between BAT composition and adiposity in early life is unknown. The objective of this study was to test the hypothesis that brown fat composition at birth is prospectively associated with adiposity gain during the first 6 months of postnatal life.

METHODS: \( N = 35 \) healthy infants were followed up prospectively from intrauterine life and birth through 6 months of age. Dixon magnetic resonance imaging (MRI) scans were conducted during the neonatal period to characterize supraclavicular BAT composition. Dual-energy X-ray absorptiometry to assess total body composition was performed within the first and sixth months of life.

RESULTS: After adjusting for potential confounding factors, a more brown-like composition (smaller fat fraction) of the supraclavicular BAT depot was associated with a smaller increase in percent body fat over the first 6 months of postnatal life.

CONCLUSIONS: A more brown-like BAT composition at birth appears to be protective against excess adiposity gain in early life. Newborn BAT tissue may constitute a target for prevention strategies against the subsequent development of obesity.

The severity and magnitude of health and other problems conferred by obesity/adiposity and metabolic dysfunction is well recognized (1). It is also well established that, once an individual becomes obese, it is difficult to lose weight, and even more difficult to sustain weight loss (2,3). For these reasons, it is important to gain a better understanding of the early/developmental origins of individual differences in the propensity for weight and fat mass gain, in order to predict obesity (or, more precisely, excess adiposity) risk, and to develop strategies for primary prevention before an individual becomes overweight or obese, or secondary interventions to increase the likelihood of a more sustained response to weight-loss strategies (3,4).

At the individual level, obesity results when energy intake exceeds energy expenditure. Thus, any effective treatment for obesity must alter total energy balance by either decreasing energy intake and/or increasing energy expenditure. In contrast to white adipose tissue (WAT), which functions to store fat, brown adipose tissue (BAT) is a specialized tissue that expends energy via lipid metabolism and thermogenesis pathways, facilitated by its uncoupling protein UCP-1 (ref. 5). The presence of substantial depots of BAT in newborns and its crucial, life-preserving role in the early postnatal period in thermoregulation has long been recognized (6,7). Several independent studies indicate that BAT persists into older childhood and adult life and remains physiologically relevant, as evidenced by its positive association with resting energy expenditure (8), skeletal muscle volume (9), and glucose regulation (10) as well as its negative association with body mass index (BMI) (8,11,12),...
total body fat (8,11), and visceral fat (11,13), thereby supporting a protective role of BAT against obesity.

Very little is known about the potential protective effects of BAT characteristics in early life on obesity/adiposity in subsequent periods of life, in part likely due to the lack of non-invasive, validated methods to assess BAT mass and BAT activity in newborns and infants. We recently reported on a non-invasive method to segment and quantify BAT composition in infants using magnetic resonance imaging (MRI) (14). In contrast to the unilocular adipocyte composition of WAT, BAT has multilocular adipocytes and is mitochondria- and capillary-dense. Therefore, the ratio of water to fat is greater in BAT compared with WAT, making it ideally suited for chemical shift imaging. Fat protons resonate at a frequency of 3.5 ppm higher than water; therefore, BAT can be differentiated from WAT spectroscopically (15). Chemical shift imaging takes advantage of the frequency difference, or chemical shift, by observing the contributions of differing phase on the overall signal contribution (16,17). This method has now been validated in rodents (18) and through post-mortem histological verification of BAT deposits in a 3-month-old infant (19). Fat signal fraction (FF, the ratio of fat-based signal to the sum of combined water- and fat-based signal) mapping has more recently been used to characterize the depletion of fat stores in BAT depots of hypothermic newborns (20) and to demonstrate BAT composition differences between obese and normal-weight body composition over the first 6 months of life (as measured using Dual X-ray absorptiometry (DXA)). Numerous studies provide evidence that child size and growth velocity particularly during the early period of infancy (first months of postnatal life) are among the strongest predictors of childhood obesity. Increased size or rapid weight or fat gain during this early postnatal growth phase is associated with increased risk for childhood (and adult) obesity (23–26) as well as with other related outcomes including metabolic risk (27), hypertension, and asthma in later life (28,29).

METHODS
Participants
Study participants comprised a cohort of children followed up prospectively from intrauterine life and birth through infancy at the University of California, Irvine, Development, Health and Disease Research Program in Orange, CA. Pregnant mothers were recruited between 2011 and 2015 in early gestation from obstetric care provider clinics. Exclusion criteria for pregnant mothers were multiple pregnancies, uterine or cervical abnormalities, or conditions associated with dysregulated neuroendocrine and immune function such as endocrine, hepatic, or renal disorders or corticosteroid medication use. Exclusion criteria for children (newborns) were preterm delivery at 34 weeks' gestation, perinatal complications associated with neurological consequences (e.g., hypoxia), and congenital, genetic, or neurological disorders (e.g., fetal alcohol syndrome, Down’s syndrome, or other aneuploidies, such as fragile X syndrome). All pregnant mothers who met the inclusion/exclusion criteria at the study clinics were approached consecutively. The final sample for the current analyses consisted of 35 infants. In all, 57% (N = 20) of the children were girls. The characteristics of the study population are presented in Table 1. All babies included in this study were born to mothers with uncomplicated pregnancies (no major medical conditions) and were healthy at the time of birth. A total of N = 3 infants were born between weeks 34 and 37 (late preterm), and N = 4 infants were small-for-gestational age (SGA, birth weight <the 10th percentile for gestational age, N = 1 overlap between late preterm and SGA). All the study procedures were approved by the UC Irvine Institutional Review Board, and all mothers provided a written informed consent.
Magnetic Resonance Imaging (MRI) to Assess BAT Characteristics

MRI was performed on a Siemens 3T Tim Trio system (VB17 software) using a 12-channel head coil and neck coils. Scans were conducted using a previously validated chemical-shift three-dimensional gradient echo Dixon method (Repetition Time = 7.47 ms, TE1/TE2 = 2.45/3.675 ms, NA = 1, Bandwidth/pixel = 977 Hz, Flip Angle = 10, matrix = 512 × 320 × 160, 0.97 × 0.97 × 1 mm, scan time = 3 min 2 s) (14). Regions of interest were defined as the combination of supraclavicular and axillary deposits and segmented using a semi-automatic procedure as previously described in detail (Figure 1) (14). Seed points for active contour segmentation were manually placed in deposits in supraclavicular and axillary regions. Seed bubbles of 3 mm were used to roughly cover these areas while avoiding any subcutaneous WAT partial volume regions. Supraclavicular and axillary ROIs were limited to four seed points per side to ensure consistent definition. The active contour evolution was iterated until the contours were visually covered by label, between 40 and 50 iterations. The supraclavicular/axillary region was chosen as it is a well-documented site for BAT, and the union of supraclavicular and axillary deposits has been shown to be the most repeatable region of BAT measurement in human neonates (14). The FF was averaged across all voxels within the individualized bilateral masks, resulting in a single reported measure for each individual. Higher fat fraction values are believed to reflect a fatter composition (less BAT-like) and have been associated with the obese state in children (21,22). Furthermore, cooling–reheating protocols have demonstrated decreases in BAT FF as a result of lipid consumption during cold exposure (30).
Infants were, on average, about 1-month old at the time of the MRI scan (26 ± 13 mean (± SD) days). Age at the time of MRI scan and gestational age at birth were not associated with the mean BAT FF.

Dual-Energy X-Ray Absorptiometry to Assess Body Composition A whole-body DXA scan was obtained using a Hologic Discovery Scanner (A, QDR 4500 series, Hologic, Bedford, MA) in a pediatric scan mode. Calibration using Hologic’s anthropomorphic Spine QC Phantom was performed before each scan. During the scan, infants lay supine while sleeping, wearing only a disposable diaper and swaddled in a light cotton blanket. If the baby moved during the scan, a single repeat was performed once the baby had been pacified. At the time of the newborn DXA assessment, children were, on average, 25.7 days old (mean (±11.6 (SD) days). The second DXA assessment was conducted, on average, at 6 months of life (6.3 ± 0.46 mean (± SD) months). As older age at the DXA scan of the newborn but not at the DXA scan of the 6-month-old infant was positively associated with total percent body fat (%BF), we used an age-adjusted newborn measure of %BF in the analyses. Adiposity gain was defined as the difference between the age-adjusted newborn %BF and %BF at 6 months of life.

Infant Feeding
The infant feeding method (breast- or formula-feeding) was assessed by maternal interviews at the newborn’s and 6-month-old’s DXA assessment. All babies were exclusively breastfed at the time of the newborn DXA assessment. To control for infant-feeding status over the 6-month postnatal period, a variable was computed indexing whether or not the mother continuously breastfed the child until 6 months of life.

Maternal Anthropometrics, Birth Outcomes, and Sociodemographic Characteristics
Maternal sociodemographic characteristics (age, education, income, parity, and race/ethnicity) were obtained by a standardized structured interview at the first pregnancy visit. Race/ethnicity was determined by self-report using the Office of Management and Budget 1997 Revisions to the Standards for the Classification of Federal Data on Race and Ethnicity (Revision of Statistical Policy Directive No. 15, Race and Ethnic Standards for Federal Statistics and Administrative Reporting). Maternal pre-pregnancy BMI (weight in kg/height in m²) was computed based on pre-pregnancy weight abstracted from the medical record and maternal height measured at the research laboratory during the first pregnancy visit. Maternal weight at delivery was abstracted from the medical record. Total gestational weight gain was calculated by subtracting the pre-pregnancy weight from weight at delivery.

Birth outcomes, including gestational age at birth and newborn’s birth weight, were abstracted from the medical record.

Statistical Analyses
The distributions of continuous variables included in the analyses were tested for normality (Kolmogorov–Smirnov test). All continuous variables were normally distributed (P40.200 for null hypothesis). Pearson product–moment correlations were used to test bivariate associations between the mean BAT FF and other variables (maternal pre-pregnancy BMI, weight gain during pregnancy, gestational age at birth, birth weight adjusted for length of gestation, age at scan, and infant sex). Differences in BAT FF concentrations assumptions were observed, and no observations were removed from the analysis. All statistical analyses were performed using SPSS v21 (IBM Corp., Version 21.0. Armonk, NY, USA), and the statistical significance level was set at α = 0.05.

RESULTS
The mean BAT FF in neonates was 29.4 ±4.06% (SD), and ranged from 22.9 to 36.4%, consistent with previous reports of neonatal supraclavicular BAT FF (14,21). The mean %BF at birth and at 6 months of age was 13.8 ± 4.7 (range: 2.2–27) and 32.6 ± 9.0 (range: 16.8–48.3), respectively. The average change in %BF from 0 to 6 months of age was 18.8 ± 8.5 (range: 5.6–37.6). There was no significant association between BAT FF and newborn %BF ((unstandardized) β (95% confidence interval (CI)) = −0.180 (−0.519 to 0.285), r = 0.154, P = 0.295). There was a significant association between newborn BAT FF and change in %BF from 0 to 6 months of age. In unadjusted analyses, a lower BAT FF (more “brown fat-like” composition) was associated with a smaller change in %BF from 0 to 6 months (β (95% CI) = 0.893 (0.236–1.550), r = 0.428, P = 0.009, Figure 2), and lower %BF at 6 months of age (β (95% CI) = 0.797 (0.048–1.503), r = 0.348, P = 0.037).
These effects persisted after adjusting for maternal pre-pregnancy BMI, maternal gestational weight gain, infant sex, and SGA and infant-feeding status ($\beta$ (95% CI) = 0.951 (0.293–1.610), (partial) $r = 0.492, P = 0.006$) for adjusted effect of BAT FF on change in %BF from 0 to 6 months, and $\beta$ (95% CI) = 0.974 (0.302–1.646; partial) $r = 0.489, P = 0.006$ for adjusted effect of BAT FF on %BF at 6 months). Specifically, in the adjusted model, a one SD unit decrease in newborn BAT FF was associated with an ~ 20% reduction of the average increase in %BF gain from 0 to 6 months of age. Statistical coefficients for between different racial/ethnic groups were tested using a univariate ANOVA. Multiple linear regression was used to quantify the association between BAT FF and change in %BF gain, with and without adjustment for the effects of gestational weight and pre-pregnancy BMI, infant sex, infant-feeding status, and SGA status because these factors have been shown to be associated with gain in infant adiposity in previous studies.

Diagnostics were performed to assess the validity of standard linear regression assumptions. No extreme departures from general the unadjusted and adjusted models are summarized in Table 2.

The mean BAT FF was not significantly associated with birth weight, birth weight percentile, or weight at 6 month (all $r$ values  $\leq 0.132, P$ values 40.444).

Figure 2. Newborn BAT FF is prospectively associated with 0–6-month adiposity gain. Newborn supraventricular brown adipose fat fraction (measured with magnetic resonance imaging) and change in percent body fat (measured by dual-energy X-ray absorptiometry) from 0 to 6 months of age. Higher fat fraction is indicative of a fattier (less BAT-like) composition. BAT, brown adipose tissue.
DISCUSSION

The current study represents, to the best of our knowledge, the first report in humans linking higher brown fat-like composition of newborn supraclavicular fat depot (a primary location of brown fat) with a smaller increase in infant total body fat from birth till 6 months of age (a key indicator of increased childhood and adult obesity risk (23–26)). This effect persisted after adjusting for other potential determinants of newborn and infant %BF and change in %BF. The magnitude of the effect translates to an ~20% reduction of the average increase in %BF gain from 0 to 6 months of age per SD decrease in newborn BAT FF. We suggest that the magnitude of this effect is substantial, particularly for a single factor, considering that in a recent publication (31) a combined set of several factors including race, gestational weight gain, pre-pregnancy BMI, feeding patterns, gestational age at birth, and sex accounted for 19% in the variance of adiposity (% body fat) at birth.

Lower BAT FF values are believed to reflect a more BAT-like composition of fat depots and have been associated with lower obesity risk in children (21,22). The effect observed in the current study may therefore be mediated by the capacity of the more brown-like fat composition to facilitate energy expenditure via lipid metabolism and thermogenesis pathways during the first month of life, thereby reducing white fat deposition from 0 to 6 months of age.

In the current study BAT FF was not associated with newborn %BF. This may be due to the relatively short duration of ex utero thermal exposure at the time of scan (average 26 days after birth) or the relatively limited interindividual variability in newborn fat stores. Whereas BAT and WAT deposition occurs in utero, non-shivering thermogenesis (that is necessary for stimulating BAT) does not begin until the neonate is born and exposed to the relatively cold ex utero environment. Therefore, the effective exposure time to ex utero conditions, and its consequences on BAT lipid content, are minimal in the newborn period.

The limitations of this study include the use of a dual-echo acquisition rather than a multiecho acquisition and the lack of a measure for BAT activity. This acquisition method lacks the multiple echo points needed for full characterization of the complex lipid profile. In theory, this limits the measurement by having an imperfect water–fat separation within the BAT depots. However, the utility of the dual-echo approach has been demonstrated in previous in vivo human BAT MRI (14,32,33), and it has been shown that the use of a dual-echo, vs. a multiecho, acquisition primarily adds a uniform bias relative to MR spectroscopic measurement of hepatic fat fraction (34). Finally, it should be noted that because of the spatial resolution limits of MRI and the interspersed distribution of brown fat cells found in humans, all of the depots measured in this work may contain a partial volume average of brown/beige and white fat cells.

The issue of the physiological relevance of BAT FF within these depots warrants further study. First, while the supraclavicular region has demonstrated functional plasticity through positive UCP1 expression in both BAT-active and -inactive adults (35), our current method is unable to determine whether the neonatal supraclavicular depot exhibits the same physiological properties. Prior evidence suggests a distinct second type of human BAT found in the interscapular region of newborn humans (32). Second, we note that this is a measure of BAT composition and not activity. The FF can then be thought of as either reflective of BAT capacity for future energy expenditure (lower FF is due to greater mitochondrial density) or suggestive of past lipid metabolism (lower FF is due to lipid stores having been utilized) (30). Certainly, these two physiological properties are not uncoupled, and future work is needed to gain a better understanding of the physiological underpinnings of BAT FF. Future studies also are warranted that incorporate measures of BAT characteristics at 6 months of age, as well as concurrent measures of energy expenditure and metabolic function, and also perform longitudinal follow-up to quantify changes in BAT characteristics from birth to infancy, childhood, and beyond, along with the concurrent characterization of other infant and child phenotypes of interest in healthy as well as clinical (e.g., SGA) populations.

Our findings may provide the basis for important clinical applications. BAT may provide a
pharmacological target in the context of the growing problem of obesity (8,11,12,36,37). Recent studies suggest that brown-in-white or beige tissue can be induced from WAT through stimulation (38,39). Thus, potentially altering (optimizing) the initial amount of BAT or BRITE tissue in newborns, and its subsequent rate of attrition during infancy and childhood may serve as a primary prevention strategy against the development of obesity and metabolic dysfunction.

STATEMENT OF FINANCIAL SUPPORT
This study was supported in part by US PHS (NIH) grants R21 DK-098765, R01 HD-065825, R01 HD-060628, R01 MH-091351, and UCI Institute for Clinical and Translational Science (CTSA grant) UL1 TR000153.

Disclosure: The authors declare no conflict of interest.

REFERENCES
1. OECD. Obesity and the Economics of Prevention: Fit not Fat. OECD Publishing: Paris, 2010. Available at http://dx.doi.org/10.1787/9789264084865-en.
2. Butte NF, Christiansen E, Soren sen TIA. Energy imbalance underlying the development of childhood obesity. Obesity 2007;15:3056–66.
3. Muhlhausler B, Smith SR. Early-life origins of metabolic dysfunction: role of the adipocyte. Trends Endocrinol Metab 2009;20:51–7.
4. Öng KK. Early determinants of obesity. Endocr Dev 2010;19:53–61.
5. Hu HH, Gilsanz V. Developments in the imaging of brown adipose tissue and its associations with muscle, puberty, and health in children. Front Endocrinol 2011;2:33.
6. Satterfield MC, Wu G. Brown adipose tissue growth and development: significance and nutritional regulation. Front Biosci 2011;16:1589–608.
7. Dawkins MJ, Scopes JW. Non-shivering thermogenesis and brown adipose tissue in the human new-born infant. Nature 1965;206:201–2.
8. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, et al. Cold-activated brown adipose tissue in healthy men. N Engl J Med 2009;360:1500–8.
9. Gilsanz V, Chung SA, Jackson H, Dorey FJ, Hu HH. Functional brown adipose tissue is related to muscle volume in children and adolescents. J Pediatr 2011;158:722–6.
10. Lee P, Greenfield JR, Ho KK, Fulham MJ. A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 2010;299:E601–6.
11. Saito M, Okamatsu-Ogura Y, Matsushita M, et al. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 2009;58:1526–31.
12. Cypess AM, Lehman S, Williams G, et al. Identification and importance of brown adipose tissue in adult humans. N Engl J Med 2009;360:1509–7.
13. Chalfant JS, Smith ML, Hu HH, et al. Inverse association between brown adipose tissue activation and white adipose tissue accumulation in successfully treated pediatric malignancy. Am J Clin Nutr 2012;95:1144–9.
14. Rasmussen JM, Entringer S, Nguyen A, et al. Brown adipose tissue quantification in human neonates using water-fat separated MRI. PLoS ONE 2013;8:e77907.
15. Hamilton G, Smith DL Jr., Bydder M, Nayak KS, Hu HH. MR properties of brown and white adipose tissues. J Magn Reson Imaging 2011;34:468–73.
16. Glover GH, Schneider E. Three-point Dixon technique for true water/fat decomposition with B0 inhomogeneity correction. Magn Reson Med 1991;18:371–83.
17. Ma J. Dixon techniques for water and fat imaging. J Magn Reson Imaging 2008;28:543–8.
18. Hu HH, Smith DL Jr., Nayak KS, Goran MI, Nagy TR. Identification of brown adipose tissue in mice with fat-water IDEAL-MRI. J Magn Reson Imaging 2010;31:1195–202.
19. Hu HH, Tovar JP, Pavlova Z, Smith ML, Gilsanz V. Unequivocal identification of brown adipose tissue in a human infant. J Magn Reson Imaging 2012;35:938–42.
20. Hu HH, Wu TW, Yin L, et al. MRI detection of brown adipose tissue with low fat content in newborns with hypothermia. Magn Reson Imaging 2014;32:107–7.
21. Hu HH, Yin L, Aggabao PC, Perkins TG, Chia JM, Gilsanz V. Comparison of brown and white adipose tissues in infants and children with chemical-shift-encoded water-fat MRI. J Magn Reson Imaging 2013;38: 885–96.
22. Deng J, Schoeneman SE, Zhang H, et al. MRI characterization of brown adipose tissue in obese and normal-weight children. Pediatr Radiol 2015;45:1682–9.
23. Koontz MB, Gunzler DD, Presley L, Catalano PM. Longitudinal changes in infant body composition: association with childhood obesity. Pediatr Obes 2014;9:e141.
24. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. Br Med J 2005;331:929.
25. Stettler N, Kumanyika SK, Katz SH, Zemel BS, Stallings VA. Rapid weight gain during infancy and obesity in young adulthood in a cohort of African Americans. Am J Clin Nutr 2003;77:1374–8.
26. Druet C, Stettler N, Sharp S, et al. Prediction of childhood obesity by infancy weight gain: an individual-level meta-analysis. Paediatr Perinat Epidemiol 2012;26:19–26.
27. Ekelund U, Ong KK, Linne Y, et al. Association of weight gain in infancy and early childhood with metabolic risk in young adults. J Clin Endocrinol Metab 2007;92:98–103.
28. Huxley RR, Shiell AW, Law CM. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. J Hypertens 2000;18:815–31.
29. Sonnenschein-van der Voort AM, Arends LR, de Jongste JC, et al. Preterm birth, infant weight gain, and childhood asthma risk: a meta-analysis of 147,000 European children. J Allergy Clin Immunol 2014;133: 1317–29.
30. Lundstrom E, Strand R, Johansson L, Bergsten P, Ahlstrom H, Kullberg J. Magnetic resonance imaging cooling-reheating protocol indicates decreased fat fraction via lipid consumption in suspected brown adipose tissue. PLoS ONE 2015;10:e0126705.
31. Sauder KA, Kaar JL, Starling AP, Ringham BM, Glueck DH, Dabelea D. Predictors of infant body composition at 5 months of age: The Healthy Start Study. J Pediatrics 2017;183:94– e1.
32. Lidell ME, Betz MJ, Dahlqvist Leinhard O, et al. Evidence for two types of brown adipose tissue in humans. Nat Med 2013;19:631–4.
33. Romu T, Elander L, Leinhard OD, et al. Characterization of brown adipose tissue by water-fat separated magnetic resonance imaging. J Magn Reson Imaging 2015;42:1639–45.
34. Zand KA, Shah A, Heba E, et al. Accuracy of multiecho magnitude-based MRI (M-MRI) for estimation of hepatic proton density fat fraction (PDFF) in children. J Magn Reson Imaging 2015;42:1223-32.
35. Lee P, Swarbrick MM, Zhao JT, Ho KK. Inducible brown adipogenesis of supraclavicular fat in adult humans. Endocrinology 2011;152:3597–602.
36. Virtanen KA, Lidell ME, Orava J, et al. Functional brown adipose tissue in healthy adults. N Engl J Med 2009;360:1518–25.
37. Yoneshiro T, Aita S, Matsushita M, et al. Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. Obesity 2011;19:1755–60.
38. van der Lans AA, Hoeks J, Brans B, et al. Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J Clin Invest 2013;123:3395–403.
39. Beranger GÉ, Karbiener M, Barquissau V, et al. In vitro brown and "brite"/"beige" adipogenesis: human cellular models and molecular aspects. Biochim Biophys Acta 2013;1831:905–14.

1Institute of Medical Psychology, Charité – Universitätsmedizin Berlin, Berlin, Germany; 2Department of Pediatrics, University of California, Irvine, California; 3Department of Obstetrics and Gynecology, Keio University, Tokyo, Japan; 4Psychiatry & Human Behavior, University of California, Irvine, California. Correspondence: Jerod Rasmussen (rasmussj@uci.edu)