genotoxic and epigenetic mechanisms may lead to better understanding of the effects of dioxin.

ACKNOWLEDGMENTS

We deeply appreciate Mami Morinaga and other clinical staff for their devoted care of patients.

Kiyomi Tsukimori
Department of Obstetrics
Fukuoka Children's Hospital
Fukuoka, Japan
tsukimori.k@fcho.jp

Fumiko Yasukawa
Hiroshi Uchi
Masutaka Furue
Department of Dermatology
Graduate School of Medical Sciences
Kyushu University
Fukuoka, Japan

Seiichi Morokuma
Department of Obstetrics and Gynecology
Graduate School of Medical Sciences
Kyushu University
Fukuoka, Japan

REFERENCES

1. Davis DL, Webster P, Stainthorpe H, et al. Declines in sex ratio at birth and fetal deaths in Japan, and in U.S. whites but not African Americans. *Environ Health Perspect*. 2007; 115:941–946.
2. del Rio Gomez I, Marshall T, Tsai P, Shao YS, Guo YL. Number of boys born to men exposed to polychlorinated byphenyls. *Lancet*. 2002; 360:143–144.
3. Furue M, Uenotsuchi T, Urabe K, Ishikawa T, Kuwabara M. Overview of Yusho. *J Dermatol Sci*. 2005;1:S3–S10.
4. James WH. Evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels at the time of conception. *J Theor Biol*. 1996;180:271–286.
5. Zama AM, Uzumcu M. Epigenetic effects of endocrine-disrupting chemicals on female reproduction: an ovarian perspective. *Front Neuroendocrinol*. 2010;31:420–439.
6. Steinberg RM, Walker DM, Juenger TE, Woller MJ, Gore AC. Effects of perinatal polychlorinated biphenyls on adult female rat reproduction: development, reproductive physiology, and second generational effects. *Biol Reprod*. 2008;78: 1091–1101.
7. Ulbrich B, Stahlmann R. Developmental toxicity of polychlorinated biphenyls (PCBs): a systematic review of experimental data. *Arch Toxicol*. 2004;78:483–487.
8. James WH. Evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels around the time of conception. *J Endocrinol*. 2008;198:3–15.

Negative Control Exposures in Epidemiologic Studies

To the Editor:

In their excellent review on negative controls,[1] Lipsitch and colleagues[1] state they "are not aware of an example of the use of a negative control exposure to detect confounding" in an epidemiologic setting. One such explicit application of this approach relates to the use of paternal exposures as a negative control exposure for maternal exposures considered to have an intrauterine influence on offspring outcomes.[2] Paternal smoking, as a negative control, may show associations with offspring outcomes similar to those of maternal smoking if the associations are generated by shared familial confounding factors or by parental genotypes transmitted to the offspring. If, however, there is an intrauterine influence, then only the maternal exposure would be expected to show an independent association with the outcome.

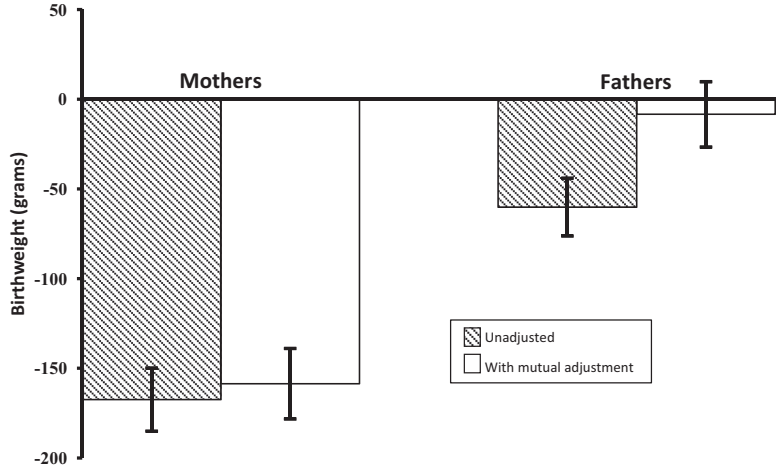

FIGURE 1. Effect of maternal and paternal smoking on offspring birthweight (difference in birthweight between offspring of smokers and nonsmokers in grams).

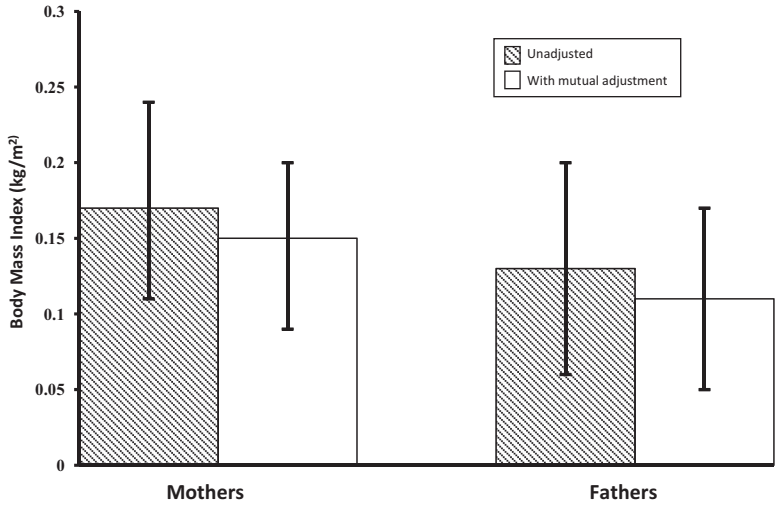

FIGURE 2. Effect of maternal and paternal smoking during pregnancy on offspring body mass index (difference in body mass index between offspring of smokers and nonsmokers in kg/m^2).

Figure 1 demonstrates that the effect of maternal smoking during pregnancy on offspring birthweight is considerably greater than that of paternal smoking during pregnancy, and mutual adjustment (to take account of associative mating by smoking) attenuates the paternal effect to zero. This is in line with the considerable body of evidence that maternal smoking has a causal effect on offspring birthweight. There has been enthusiasm for the proposition that fetal exposure to maternal smoking leads to increased obesity in offspring.[3] Although maternal smoking during pregnancy does indeed demonstrate the expected association, the strength of association with paternal smoking during pregnancy is similar before and after mutual adjustment (Fig. 2).[4] This casts doubt on the causal nature of the association between intrauterine exposure to maternal smoking and offspring adiposity.

As Lipsitch and colleagues[1] argue, the use of negative controls could be usefully expanded in epidemiology. If associations are found with such controls, this does not invalidate the observation under interrogation but does encourage further intense scrutiny of potential biases and confounding that may underlie what is seen.

George Davey Smith
MRC Centre for Causal Analyses in Translational Epidemiology
University of Bristol
Bristol, United Kingdom
Julia.Mackay@bristol.ac.uk

REFERENCES

1. Lipsitch M, Tchetgen Tchetgen E, Cohen T. Negative controls: a tool for detecting confounding and bias in observational studies. *Epidemiology*. 2010;21:383–388.
2. Davey Smith G. Assessing intrauterine influences on offspring health outcomes: can epidemiological findings yield robust results? *Basic Clin Pharmacol Toxicol*. 2008;102:245–256.
3. Power C, Jefferis BJ. Fetal environment and subsequent obesity: a study of maternal smoking. *Int J Epidemiol*. 2002;31:420–421.
4. Leary S, Davey Smith G, Ness A. ALSPAC Study team. Smoking during pregnancy and components of stature in the offspring. *Am J Hum Biol*. 2006;18:502–512.

The authors respond:

We thank George Davey Smith[1] for his thoughtful comments on our paper[2] and for drawing attention to his previous use of a negative control exposure. We agree with his interpretation that the lack of association of paternal smoking during pregnancy (negative control exposure B as shown in Fig. 3 in our original paper,[2] and reproduced here in simplified form as the Figure) with birth weight strengthens the causal interpretation of an observed association of maternal smoking (A) with low birth weight (Y). Comparing Figure 1a and b of Davey Smith, paternal smoking has a univariate association with low birth weight, but this association disappears in a model including maternal smoking. This example emphasizes why the negative control exposure (B) should be evaluated in a model including the exposure of interest (A). The negative control is used to see whether there is evidence for a causal arrow from unobserved confounders (U) to the outcome (Y). Even if no U→Y arrow exists, B will be associated with Y under the alternative hypothesis (A→Y) through the path B←U→A→Y. Conditioning on (A) by including it in the model will close this path.

This example also nicely illustrates the subject-matter knowledge needed to interpret negative controls. A potential problem with paternal smoking as a negative control for maternal smoking is that paternal smoking causes maternal passive smoking; therefore, paternal smoking might be associated with the outcome causally. Observing an association of paternal smoking with birth weight would thus not invalidate in utero effects interpretation of the maternal smoking-birth weight association in this study. Nonetheless, the lack of association with paternal smoking in the birth weight case is reassuring.

The later-life BMI outcome in offspring discussed by Davey Smith is possibly more complicated. Postnatal outcomes could be associated with in utero and postnatal exposures. The finding of an association with paternal smoking during pregnancy (B) after adjustment for in utero exposure to maternal smoking during pregnancy (A) could reflect any combination of three nonmutually exclusive possibilities: (i) uncontrolled confounding of the A→Y association; (ii) an additional causal link B→Y via in utero exposure to passive smoke; or (iii) that some or all of the association A-Y and B-Y is due to effects of postnatal exposure to smoke from either parent. That is, the observed association between parental smoking during pregnancy and adiposity may reflect a causal relationship between postnatal parental smoking and adiposity, combined with a tendency of parents who smoke during pregnancy to continue smoking after the baby is born.

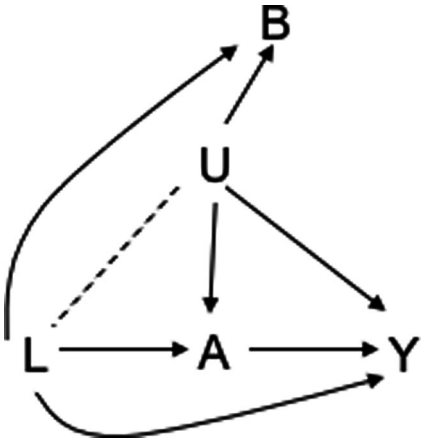

FIGURE. Causal diagram showing an ideal negative control exposure B for use in evaluating studies of the causal relationship between exposure A and outcome Y, with measured confounders L and possible unmeasured confounders U. This figure is simplified from Figure 3 of Reference 2.

Marc Lipsitch
Department of Epidemiology
Harvard School of Public Health
Boston, MA
Department of Immunology and Infectious Diseases
Harvard School of Public Health
Boston, MA
Center for Communicable Disease Dynamics
Harvard School of Public Health
Boston, MA
mlipsitc@hsph.harvard.edu