that allows simultaneous analysis of these 2 anions and the use of
$^{15}$N-labeled internal standards (4). Using this method, Tsikas (4)
reported endogenous plasma nitrite concentrations in the order of
12 $\mu$M compared with our value of 4 $\mu$M. Apart from dietary intake
of nitrate/nitrite, there are likely to be other factors that could affect
the accurate measurement of plasma nitrite, eg, its short half-life of
110 s in human blood (5). Standardization of dietary sources of
nitrate and nitrite will be an important factor to consider in future
work in this area.

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Breastfeeding and reduced risk of childhood
obesity: will randomized trials on breastfeeding
promotion give the definite answer?

Dear Sir:

Whether breastfeeding might reduce the risk of later overweight
and obesity in childhood or not has been subject to numerous pub-
lcations over the past 30 y. These publications reflect the results of
observational studies, most of them using a cross-sectional design,
and a few cohort studies. Like all observational research, these stud-
ies are fraught with possible bias or confounding. Several meta-
analyses evaluated these studies critically and yielded conflicting
conclusions (1–4). Irrespective of potential publication bias and
potential residual confounding (3, 4), the reported magnitudes of
effects of breastfeeding may provide a benchmark for the potential
size of effects of breastfeeding on overweight, obesity, or BMI to be
confirmed or refuted in randomized trials.

Breastfeeding cannot be randomized. A large cluster-randomized
trial on the promotion of breastfeeding (5), therefore, might provide
an alternative tool to solve the controversy. However, no significant
differences in mean BMI (cluster-adjusted mean difference: 0.1;
95% CI: −0.2, 0.3) and prevalence of obesity [odds ratio (OR): 1.2;
95% CI: 0.8, 1.6] were found between the intervention group
and the control group in the Promotion of Breastfeeding Interven-
tion Trial (PROBIT) (6). Could this be due to lack of power with
respect to the potential size of the effect and the increment in
breastfeeding achieved by promotion of breastfeeding?

To address these questions, we performed power calculations for
effects of breastfeeding on mean BMI of children and on the prev-
ance of overweight and obesity in the PROBIT. For mean BMI,
calculations were based on the $t$ test for 2 independent means.
For the prevalence of obesity, calculations were based on the chi-
square test. We allowed a maximum probability for type 1 error ($\alpha$) of 5%.
For all calculations, we assumed a sample size of 8000
children per arm as in the PROBIT. Expected effect sizes were
derived from pooled estimates in meta-analyses.

In the setting of the PROBIT, in which access to breastfeeding
promotion—but not breastfeeding—was randomly assigned, both
the control group and the intervention group contained breastfed
and nonbreastfed children. Intention-to-treat analysis was applied
in the PROBIT, ie, all children from the intervention group were
compared with all children in the control group, which has influence
on the power of the study.

The overall effect size of breastfeeding that is possibly detectable
with the PROBIT setting and sample size is clearly dependent on the
difference in proportion of breastfed children between the 2 groups.
Based on a 5% prevalence of obesity among formula-fed children
aged 6.5 y, the prevalence of obesity would be expected to be $\approx$
OR × 5% among breastfed children. If the difference in the propor-
tion of breastfed children was 100%, ie, if all children in the in-
tervention group were breastfed and all children in the control
group were formula fed, this would correspond to a true randomization
of breastfeeding: for a prevalence of obesity of 0.05 (5%) in the con-
trol group and a given OR of 0.67, the prevalence in the intervention
group can be calculated as 0.67 × 0.05 = 0.034.

In PROBIT, 49.8% of mothers were still breastfeeding when the infant
was 6 mo in the intervention group compared with 36.1% in the control
group. The hypothesized difference in the prevalence of obesity of 5%
compared with 3.4% would therefore be diluted to a much smaller dif-
ference. The prevalence of obesity in the control group would be
$\pi_c = 0.361 \cdot 0.67 \cdot 0.05 + (1 − 0.361) \cdot 0.05 = 0.044$ and $\pi_i =
0.498 \cdot 0.67 \cdot 0.05 + (1 − 0.498) \cdot 0.05 = 0.042$ in the intervention
group.

When comparing the difference in mean BMI between interven-
tion and control groups, the power for the $t$ test is dependent on
effect size of breastfeeding on mean BMI (difference in mean BMI
between breastfed and nonbreastfed children). Based on a difference
in mean BMI of −0.19, as reported by Owen et al (3), the expected
mean difference between the 2 groups in the PROBIT setting would be
$(0.497−0.361) \times (−0.19) = −0.03$.

All power calculations were carried out according to the PROBIT
setting: 8000 mother-child pairs in each study arm, a prevalence of

LETTERS TO THE EDITOR 653

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...
The impact of different proportions in breastfeeding and different obesity in controls of 5%, and a prevalence of overweight of 13%.

TABLE 1

<table>
<thead>
<tr>
<th>Effect size and type</th>
<th>OR for obesity</th>
<th>PROBIT setting</th>
<th>Randomization of breastfeeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arenz et al (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>0.67</td>
<td>10.9</td>
<td>99.9</td>
</tr>
<tr>
<td>Adjusted</td>
<td>0.78</td>
<td>7.4</td>
<td>92.1</td>
</tr>
<tr>
<td>Owen et al (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>0.87</td>
<td>5.8</td>
<td>49.5</td>
</tr>
<tr>
<td>Adjusted</td>
<td>0.93</td>
<td>5.2</td>
<td>17.8</td>
</tr>
<tr>
<td>Mean difference in BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Owen et al (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted</td>
<td>-0.01a</td>
<td>2.8</td>
<td>5.6</td>
</tr>
<tr>
<td>Crude</td>
<td>-0.04a</td>
<td>4.0</td>
<td>31.9</td>
</tr>
<tr>
<td>Small-scale studies</td>
<td>-0.19a</td>
<td>16.1</td>
<td>99.9</td>
</tr>
</tbody>
</table>

1 Calculations are based on a sample size of 8000 children per arm and a prevalence of obesity among formula-fed children of 5% and allowed for a probability of type I error of 5%. Power is shown for different estimates of effect from meta-analyses. OR, odds ratio; PROBIT, Promotion of Breastfeeding Intervention Trial.
2 49.8% compared with 36.1% of children who were breastfed at 6 mo as observed in PROBIT.
3 100% compared with 0% of children who were breastfed at 6 mo.
4 Mean values.

The main advantage of a randomized trial is its power to avoid bias and confounding, common causes of erroneous effect estimates in observational epidemiology. The absence of a protective effect of breastfeeding on childhood obesity or mean BMI as observed in the PROBIT therefore casts considerable doubt in the assumption of a protective effect of breastfeeding on childhood obesity. The failure to reject the null hypothesis (equal risk of later obesity among breastfed and nonbreastfed children) does not confirm the null hypothesis, however. The absence of evidence does not imply evidence of absence, particularly not if the power of the randomized trial was low.

In conclusion, this cluster-randomized trial on the promotion of breastfeeding had the greatest effect on the proportion of women who exclusively breastfed. In the intervention group, 43.3% compared with 6.4% in the control group were exclusively breastfeeding at 3 mo. The OR for breastfeeding at 1–3 mo according to the meta-analysis of Harder et al was 0.81 (95% CI: 0.74, 0.88). The power of a corresponding chi-square test would be 41.7%. At 6 mo, 7.9% of the children were exclusively breastfed in the intervention group compared with 0.6% in the control group. The OR for overweight according to Harder et al was 0.76 (0.67, 0.86) for children who were breastfed for 4–6 mo. The power of a corresponding chi-square test would be 7.2%.

We did not consider a cluster-randomized design in our power calculations. Power for cluster-randomized trials is dependent on the intra-cluster correlation. However, we do not feel that this is a serious shortcoming because the intracluster correlation found in the PROBIT was low. Furthermore, the power would be even lower when considering the cluster-randomized approach.

In conclusion, this cluster-randomized trial on the promotion of breastfeeding, which is the largest such trial thus far, was underpowered to assess the association between breastfeeding and childhood obesity. The failure to detect significant protective effects of breastfeeding on childhood obesity in the PROBIT may therefore not necessarily reflect better adjustment for confounding than in observational studies and avoidance of bias but may instead reflect lack of power.

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Reply to S Rückinger and R von Kries

Dear Sir:

We thank Rückinger and von Kries for their reflections on statistical power in studies of breastfeeding and subsequent childhood obesity and, more particularly, on the evidence from the Promotion of Breastfeeding Intervention Trial (PROBIT), our cluster-randomized trial of a breastfeeding promotion intervention in the Republic of Belarus. Rückinger and von Kries argue that because of the overlap in breastfeeding behavior between our experimental and control groups, even a study as large as ours (13,889 children followed up at age 6.5 y), statistical power is insufficient to detect protective effects of breastfeeding against subsequent obesity.

Unfortunately, Rückinger and von Kries have ignored 2 key issues and seem unaware of many of our other publications from PROBIT. First, breastfeeding is not an all-or-none behavior. Rückinger’s and von Kries’s analysis compares breastfed with nonbreastfed children, whereas the PROBIT intervention was designed to increase both the duration and exclusivity of breastfeeding among children who were initially breastfed. Thus, none of the children in PROBIT were formula-fed from birth. If breastfeeding is protective, more exclusive breastfeeding should be more protective. Second, breastfeeding trajectories (i.e., entire feeding histories since birth) are important, not just the cross-sectional proportion of children breastfed at a particular age during infancy.

In designing PROBIT, we were fully cognizant that any effect of breastfeeding would be attenuated because of the overlap in breastfeeding behavior in our experimental and control groups. This is precisely why we required a large sample size. That an intention-to-treat analysis with an appropriately randomized intervention can indeed detect causal effects of the experimental intervention is clearly shown by the substantial and statistically significant effects we have reported on gastrointestinal infection, atopic eczema, and growth in infancy (1, 2) and on cognitive development at the age of 6.5 y (3). The absence of any observed effect of the intervention on mean body mass index (BMI; in kg/m²) or risk of obesity cannot, therefore, be attributed to insufficient statistical power. Although we agree that “absence of evidence does not imply evidence of absence,” we observed no trend toward reduced mean BMI in the experimental group (15.6 in both groups), and the narrow CI around the cluster-adjusted difference [+0.1 (95% CI: −0.2 to +0.3)] gives no hint of such an effect.

We must therefore conclude that the overall difference in breastfeeding we achieved in our experimental group compared with the control group was sufficient to cause detectable effects on a number of important health outcomes, yet insufficient to cause differences in mean BMI or risk of obesity. In other words, the effect of increased exclusivity and duration of breastfeeding must be smaller for BMI and obesity outcomes than for those for which statistically significant effects were observed.

Rückinger and von Kries mention the systematic review and meta-analysis by Owen et al (4). They cite a reported difference in BMI between subjects who had been breastfed and formula-fed of −0.19; in fact, however, Owen et al’s individual-subject data meta-analysis of the 11 observational studies, in which they were able to control for confounding by socioeconomic status, maternal BMI, and maternal smoking during pregnancy, yielded a nonsignificant difference of −0.01 (95% CI: −0.05 to +0.03) (4). Thus, when confounding was adequately controlled for those 11 studies, no effect of breastfeeding was observed.

In summary, despite our detection of statistically significant and clinically important effects on several other outcomes, both during infancy and at 6.5 y of age, we observed no such effect on mean BMI or obesity risk among PROBIT children. This result is consistent with an individual-subject data meta-analysis of observational studies. The problem does not seem to be a lack of statistical power but rather a lack of causal effect.

No conflicts of interest were reported.

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