Frequency of discontinuation of contraceptive use: results from a French population-based cohort

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BACKGROUND: Despite the widespread use of highly effective contraceptive methods in France, one in every three pregnancies is unintended. Among women experiencing an unintended pregnancy leading to an abortion, half had changed their contraceptive method in the 6 months preceding the abortion, in most cases switching to a less-effective method or to no method at all. This study provides estimates of method-specific contraceptive discontinuation rates for any reason and for method-related reasons among French women.

METHODS: The data were drawn from the COCON survey (2000–2004), a population-based French prospective cohort, comprising a representative sample of 2863 women aged 18–44. We estimated Kaplan–Meier life-table probabilities of contraceptive discontinuation during the 4 years of follow-up and tested for differences by intrauterine device (IUD) type and pill composition.

RESULTS: Probabilities of contraceptive discontinuation for method-related reasons varied widely by method: IUDs were associated with the lowest probabilities of discontinuation (11% within 12 months, 30% within 4 years), followed by the pill (22% and 48%, respectively). Discontinuation rates were significantly higher for all other methods (condoms, withdrawal, fertility awareness methods and spermicides). We found no differences in discontinuation rates by the type of IUD (levonorgestrel-IUD versus copper-IUD) and increasing rates of pill discontinuation with decreasing dosage in estrogen.

CONCLUSIONS: Contraceptive discontinuation rates among French women are substantially lower than those reported for US women. Comparing the determinants of contraceptive discontinuation and the role of healthcare providers in helping women make these changes would improve our understanding of the reasons for such variation.

Key words: contraception / discontinuation / cohort / France / population-based study

Introduction

In France, despite the fact that the vast majority of women (82% of contraceptive users) use highly effective medical methods of contraception, unintended pregnancies still comprise an estimated 33% of pregnancies (Bajos et al., 2003), of which 60% end in abortion (Bajos et al., 2004a, b). Under the conditions observed in 2000, it is estimated that 40% of women in France undergo an abortion in the course of their reproductive years (Bajos et al., 2004b). These figures, drawn from the COCON study, a population-based analysis of a cohort of women of reproductive age conducted in France in 2000, reveal the difficulties women experience with their daily use of contraceptives. Such difficulties translate into substantial differences between typical-use and perfect-use contraceptive failure rates, mostly due to inconsistent or incorrect use of the methods (Trussell, 2004; Moreau et al., 2007a; Kost et al., 2008). Typical-use failure rates generally decline over time, reflecting the weeding out of the most fecund women over time and perhaps an improvement in the consistency of the use of method (Ranjit et al., 2001; Moreau et al., 2007a). In addition to contraceptive failure, discontinuation of contraceptive use may be a significant contributor to unintended pregnancies in industrialized countries.

Using data from the 1995 National Survey of Family Growth in the USA, Trussell and Vaughan estimated that the typical woman who uses reversible methods of contraception continuously from her 15th to her 45th birthday will discontinue contraceptive use for...
method-related reasons nearly 10 times (Trussell and Vaughan, 1999). The authors conclude that ‘such high rates of discontinuation almost surely reflect dissatisfaction with current methods’. While the vast majority of women resume contraceptive use shortly after discontinuing their previous method (Trussell and Vaughan, 1999), the transition period between contraceptive methods leaves these women exposed to the risk of unintended pregnancy. These results give rise to concern, especially since studies focusing on oral contraceptive users suggest that a significant proportion of women switch to less-effective methods or no method at all (Rosenberg, 1995; Grady et al., 2002; Huber et al., 2006). Each year, nearly one in four of US woman at risk of unintended pregnancy experience one or more months of contraceptive non-use (Frost et al., 2008). These results are also reflected in a French analysis of patterns of contraceptive use by women who have undergone an abortion: half of these women had changed their contraceptive method in the 6 months preceding the abortion, in most cases switching to a less-effective method or to no method at all (Bajos et al., 2006).

In order to improve contraceptive efficacy, it is critical to understand the frequency, the determinants and motives underlying discontinuation. In this paper, we estimate probabilities of contraceptive method discontinuation for method-related reasons in France.

**Population and Methods**

The data for this study are drawn from the COCON survey, designed to measure contraceptive practices and rates of abortion in France in a population-based cohort. A national two-stage probability sampling design was used to identify a representative sample of 2863 French-speaking women of reproductive age (18–44 years) (Bajos et al., 2004a). An initial sample of households including at least one eligible woman between the ages of 18 and 44 years was selected at random from the telephone directory in 2000. The response rate was 74.6%. The sampling design specified unequal probabilities of inclusion in order to over-represent women who had undergone an abortion or an unintended pregnancy in the 5 years prior to the survey (sampling fraction = 100%, n = 1034), whereas only a fraction of the other women were selected at random (sampling fraction = 19%, n = 1829).

Following the first telephone interview in 2000 upon entry into the cohort, women who agreed to participate were interviewed once per year for 4 years (2001–2004) to investigate contraceptive changes that had occurred since the previous interview. Of the initial sample, 2217 women completed the first follow-up questionnaire in 2001 and 1569 completed all 4 years of follow-up. While this substantial reduction in the sample size affects the precision of the statistical analysis, the attrition of the cohort studied between 2000 and 2002 was not found to suffer from selection bias for the variables of interest (contraceptive histories and current patterns of use) (Razafindratsima et al., 2004).

This analysis is based on the data collected during the follow-up interviews (2001–2004). Each follow-up questionnaire provided a detailed description of pregnancies and contraceptive use since the last interview, in the form of a series of contraceptive episodes of use (including episodes of non-use of contraception).

- For each ‘episode’, women described the contraceptive method used, the start date and end date, and the reasons for stopping (including side effects and method failure) if the method was discontinued.
- For each pregnancy, women described the outcome, the date the pregnancy ended, whether the pregnancy was intended or not and the contraceptive used at the time the pregnancy started if the pregnancy was unintended.

Using the above information, we were able to construct the history of contraceptive use and reproduction for each woman over the course of the 4-year follow-up.

In the first part of the analysis, we estimated probabilities of discontinuation for any reason and for method-related reasons for the principal contraceptive method used (IUD, pill, condom, withdrawal, fertility-awareness-based methods, spermicides), regardless of the method composition (type of IUD or pill) or dual method use. Method-related discontinuation included all reasons that a woman states for discontinuing a contraceptive method other than intention to become pregnant or being no longer at risk due to absence of a male partner.

- A woman describing the following three sequences of contraceptive use (pill alone—pill + condom—pill alone) was considered to have a single episode of pill use in the analysis of pill discontinuation.
- A woman who switched from copper IUD to levonorgestrel-IUD with no time in between (described in the data as two continuous episodes of IUD) was considered to have a single episode of IUD use in the analysis of IUD discontinuation.

A description of these contraceptive-use episodes is provided in Table I. The reasons for method discontinuation were missing for 1.7% of pill, 2.5% of condom, 6.8% of IUD, 3.6% of withdrawal, 7.9% of fertility awareness and 12.2% of spermicide discontinuation events. Most missing information was due to a change of methods between the last reported method in year n and the first method reported in the year n + 1. These episodes were considered to have been discontinued for method-related reasons unless they were followed by a period of contraceptive non-use preceding a wanted pregnancy or if they were followed by a period of contraceptive non-use during which the women had no sexual partner.

We also provide an estimate of contraceptive discontinuation for method-related reasons followed by a period of non-use of contraception while still at risk, which focuses attention on contraceptive disruption leading to subsequent high risk of pregnancy. We first examine the probability of abandonment of any contraceptives for method-related reasons. In that case, a single contraceptive episode is defined as an uninterrupted use of any method which can include a series of methods. We then break down the analysis to estimate the probability of discontinuing highly effective methods followed by a period of non-use of contraception while still at risk of an unintended pregnancy and the probability of discontinuation of barrier or natural contraception for method-related reasons followed by a period of non-use of contraception while still at risk. In this subanalysis, an episode of highly effective contraception is defined as an uninterrupted use of one or more highly effective method (pill, IUD, other hormonal methods), and an episode of barrier or natural contraception as an uninterrupted use of one or more of the following methods: condom, spermicides or sponges, withdrawal, fertility awareness methods.
Contraceptive discontinuation rates among French women

Table I Description of contraceptive-use episodes and episodes discontinued

<table>
<thead>
<tr>
<th>Contraceptive method</th>
<th>Episodes (n)</th>
<th>Total time of exposure (months)</th>
<th>Episodes discontinued for any reason (n)</th>
<th>Episodes discontinued for method-related reasons (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pill</td>
<td>1876</td>
<td>42 177.38</td>
<td>1011</td>
<td>569</td>
</tr>
<tr>
<td>IUD</td>
<td>702</td>
<td>20 429.77</td>
<td>206</td>
<td>144</td>
</tr>
<tr>
<td>Condom</td>
<td>793</td>
<td>12 188.95</td>
<td>534</td>
<td>369</td>
</tr>
<tr>
<td>Withdrawal</td>
<td>255</td>
<td>3878.92</td>
<td>158</td>
<td>119</td>
</tr>
<tr>
<td>Spermicides/sponges</td>
<td>57</td>
<td>795.8</td>
<td>36</td>
<td>29</td>
</tr>
<tr>
<td>Fertility-awareness*</td>
<td>198</td>
<td>3015.7</td>
<td>124</td>
<td>93</td>
</tr>
</tbody>
</table>

*Discontinuation followed by no contraceptive use while still at risk.

Data analysis

We estimated Kaplan–Meier life-table probabilities of method-specific contraceptive discontinuation for the first 4 years of use. A cluster effect was introduced to account for the fact that an individual woman could contribute more than one episode to the calculation. Using the same models, we tested for differences in discontinuation rates by IUD composition and pill composition (log-rank test).

In all analyses, we used weighted observations. Weights were used in order to take the sampling design into account as well as to reflect the social and demographic composition (age, marital status, professional activity and level of education) of the French population in the 1999 census. The total numbers reported in the tables are unweighted, i.e. the number of contraceptive episodes reported. The probabilities of contraceptive discontinuation are weighted.

Statistical analyses were performed using Stata, version 10 (Stata Corporation, College Station, TX, USA). The study received the approval of the relevant French government oversight agency (the Commission Nationale de l’Informatique et des Libertés).

Results

The risk of contraceptive discontinuation varied widely by contraceptive method (Tables II and III): IUDs were associated with the lowest probabilities of discontinuation (15% within 1 year, 43% within 4 years), followed by the pill (31% and 69%, respectively). Condoms (53% and 88%, respectively) and spermicides (62% and 97%, respectively) exhibited the highest rates of discontinuation.

Most women discontinued their method for method-related reasons (ranging from 51% in the case of pill discontinuation to 84% in the case of withdrawal; results not shown). In all, 22% of women had stopped using the pill for method-related reasons within the first year of use (accounting for 70% of all first year discontinuation of the method) and 48% had discontinued the method after 4 years of use (Tables II and III). The estimates were lower for IUD users, 11% of whom had stopped the IUD for method-related reasons within the first year (accounting for 71% of first year of use discontinuations) and 30% after 4 years. Discontinuation risks for method-related reasons were significantly higher for all other methods, with more than half the women stopping use of the condom, withdrawal, spermicides or fertility-awareness-based methods within the first 2 years of use.

While the risk of discontinuing use of contraceptive methods may be high, results suggest that most of the disruption is due to method switching rather than contraceptive abandonment altogether. Thus, the probability of contraceptive discontinuation with no subsequent contraceptive use while still at risk was substantially lower than the overall probability of discontinuation (Table III). Only ~6.0% of women had stopped using contraceptives for method-related reasons within the first year of use without adopting a new method while still at potential risk of an unintended pregnancy.

Table II Percentage of women discontinuing contraceptive use for any reason

<table>
<thead>
<tr>
<th>Contraceptive method</th>
<th>Duration of use</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
<th>36 months</th>
<th>48 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pill</td>
<td></td>
<td>19.6 (17.9–21.4)</td>
<td>30.7 (28.8–32.7)</td>
<td>49.0 (47.2–50.9)</td>
<td>59.9 (58.2–61.6)</td>
<td>68.5 (67.0–70.0)</td>
</tr>
<tr>
<td>IUD</td>
<td></td>
<td>10.2 (8.2–12.6)</td>
<td>15.0 (12.7–17.6)</td>
<td>26.2 (23.5–29.0)</td>
<td>36.4 (33.7–39.3)</td>
<td>43.4 (40.7–46.1)</td>
</tr>
<tr>
<td>Condom</td>
<td></td>
<td>39.1 (36.7–41.5)</td>
<td>52.9 (50.6–55.3)</td>
<td>70.3 (68.3–72.4)</td>
<td>81.1 (79.4–82.8)</td>
<td>88.1 (86.6–89.5)</td>
</tr>
<tr>
<td>Withdrawal</td>
<td></td>
<td>32.9 (29.1–37.0)</td>
<td>44.8 (40.8–49.1)</td>
<td>67.5 (63.3–71.5)</td>
<td>76.1 (72.1–79.9)</td>
<td>81.4 (76.9–85.4)</td>
</tr>
<tr>
<td>Spermicides/sponges</td>
<td></td>
<td>51.4 (40.6–63.3)</td>
<td>62.1 (50.9–73.4)</td>
<td>67.7 (56.6–78.4)</td>
<td>75.4 (64.4–85.1)</td>
<td>96.6 (91.4–99.0)</td>
</tr>
<tr>
<td>Fertility-awareness*</td>
<td></td>
<td>31.0 (26.5–36.2)</td>
<td>47.9 (42.8–53.1)</td>
<td>70.9 (66.2–75.5)</td>
<td>80.2 (75.9–84.1)</td>
<td>85.9 (82.1–89.2)</td>
</tr>
</tbody>
</table>

*Discontinuation followed by no contraceptive use while still at risk.
The cumulative probability of contraceptive abandonment altogether rose to 12.3% after 4 years. Interestingly, women were more likely to abandon contraceptive use if they had been using highly effective methods then if they had been using barrier or natural methods: 14.0% (12.7–15.3%) had stopped using the pill, the IUD or other hormonal methods and switched to non-contraceptive use within the first 4 years of use versus 5.8% (4.4–7.7%) of women using barrier or natural methods (results not shown).

In a further analysis of contraceptive discontinuation by pill and IUD composition (Figs 1 and 2), we found no significant variation by the type of IUD (levonorgestrel versus copper IUD, \( P = 0.69 \)), but higher discontinuation rates for method-related reasons for progestin-only pills and very low-dose estrogen pills compared with higher dose estrogen pills \( (P < 0.001) \). More than half of the women \( [57.9\% (53.0–62.9\%)] \) using progestin-only pills had stopped their pill for method-related reasons within the first year of use and 86.3% \( (83.5–88.8\% \) ) had discontinued their method within 4 years of use. Combined oral contraceptive pills exhibited lower rates of discontinuation: 33.4% \( (29.5–37.6\% \) ) and 74.1% \( (71.3–76.9\% \) ) at 1 and 4 years, respectively, for very low-dose estrogen pills (\( \leq 20 \mu g \) estrogen) and 24.0% \( (21.8–26.3\% \) ) and 58.0% \( (55.8–60.1\% \) ), respectively, for higher dose estrogen pills (\( > 20 \mu g \) estrogen). The difference between the two categories of combined oral contraceptive pills was significant \( (P < 0.001) \).

### Discussion

Our results suggest high rates of discontinuation of contraceptive methods for method-related reasons among French women. However, most of the discontinuation found in this study was method switching rather than contraceptive abandonment altogether. Our estimates are higher than those generally reported in clinical trials, from which the majority of estimates of contraceptive continuation originate. Clinical trials have the advantage of collecting prospective data on the timing and reasons of contraceptive discontinuation; however, they include only a selected group of women and are therefore unlikely to reflect the typical conditions of contraceptive use in the general population. Few population-based surveys, such as the National Survey of Family Growth (NSFG) in the USA or the Australian Family Project, provide retrospective contraceptive biographies which enable the study of contraceptive discontinuation in the general population of industrialized countries. Expanding on this

### Table III Percentage of women discontinuing contraceptive use for method-related reasons

<table>
<thead>
<tr>
<th>Contraceptive method</th>
<th>Duration of use</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
<th>36 months</th>
<th>48 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pill</td>
<td>13.4 (11.9–15.0)</td>
<td>21.5 (19.8–23.3)</td>
<td>32.7 (30.9–34.6)</td>
<td>41.6 (39.7–43.5)</td>
<td>47.7 (45.8–49.6)</td>
<td></td>
</tr>
<tr>
<td>IUD</td>
<td>7.5 (5.8–9.7)</td>
<td>10.7 (8.7–13.0)</td>
<td>17.6 (15.2–20.2)</td>
<td>24.9 (22.4–27.7)</td>
<td>29.8 (27.2–32.6)</td>
<td></td>
</tr>
<tr>
<td>Condom</td>
<td>27.4 (25.2–29.8)</td>
<td>38.3 (35.9–40.8)</td>
<td>53.8 (51.3–56.3)</td>
<td>67.5 (65.0–69.9)</td>
<td>76.7 (74.3–79.0)</td>
<td></td>
</tr>
<tr>
<td>Withdrawal</td>
<td>27.4 (23.8–31.5)</td>
<td>38.9 (34.8–43.2)</td>
<td>59.5 (54.9–64.1)</td>
<td>69.5 (64.9–74.0)</td>
<td>74.7 (69.5–79.6)</td>
<td></td>
</tr>
<tr>
<td>Spermicides/sponges</td>
<td>45.8 (34.6–58.6)</td>
<td>57.7 (45.8–70.1)</td>
<td>61.3 (49.3–73.4)</td>
<td>70.5 (58.4–81.8)</td>
<td>93.9 (85.4–98.2)</td>
<td></td>
</tr>
<tr>
<td>Fertility awareness</td>
<td>25.7 (21.4–30.7)</td>
<td>34.5 (29.6–39.9)</td>
<td>56.0 (50.3–61.8)</td>
<td>68.1 (62.5–73.7)</td>
<td>75.6 (70.1–80.8)</td>
<td></td>
</tr>
<tr>
<td>Method abandonment*</td>
<td>3.8 (3.0–4.9)</td>
<td>6.0 (5.0–7.1)</td>
<td>8.1 (6.4–8.5)</td>
<td>10.5 (7.1–9.4)</td>
<td>12.3 (11.1–13.6)</td>
<td></td>
</tr>
</tbody>
</table>

*Discontinuation followed by no contraceptive use while still at risk.

### Figure 1 Probability of contraceptive discontinuation by pill composition (Kaplan–Meier estimates).

### Figure 2 Probability of contraceptive discontinuation by IUD composition (Kaplan–Meier estimates).
population approach, our study takes a step further by combining the advantages of both the longitudinal study design, which limits recall bias, and the population-based setting, allowing for measurement of the probabilities and reasons for contraceptive disruption in the general population.

One limitation to our approach is the sizeable proportion of women lost to follow-up, which may lead to potential distortion of the study population. An analysis of the attrition of the COCON cohort shows no impact on the overall distribution of contraceptive methods used, but this says little about its effect on contraceptive discontinuation (Razafindratsima et al., 2004). A further limitation of our study lies in the underreporting of abortions, estimated to be around 50% in the COCON survey (Moreau et al., 2004). This reporting rate is close to that found in the 2002 NSFG survey in the USA (Jones and Kost, 2007). Underreporting of abortions affects the estimates of contraceptive failures (Fu et al., 1999; Kost et al., 2008), but this has little effect on discontinuation rates, since contraceptive failures only account for a small fraction of contraceptive discontinuation (Vaughan et al., 2008). Finally, our estimates are based on self-reported contraception histories, which in the case of natural or barrier-methods have mediocre validity and cannot be verified. The fact that the analysis is based on data collected every year rather than, like most demographic surveys, as a 5-year retrospective contraception history allows a more precise account of the timing and reasons for contraceptive discontinuation.

Despite these methodological issues, our results are consistent with those of earlier population-based studies, showing that barrier methods and other natural methods, used by 11% of women in France in 2000 (Bajos et al., 2003), exhibit the highest rates of discontinuation for method-related reasons. These estimates, in the range of those reported for US women (Trussell and Vaughan, 1999; Vaughan, et al. 2008), suggest that barrier or natural methods are primarily used as temporary contraceptive options related to an individual’s life circumstances. This hypothesis is corroborated by a study in the US showing that barrier methods were more likely to be used in the early stage of a relationship (Beckman and Harvey, 1996). In the context of the HIV epidemic, couples are likely to abandon condom use as the relationship stabilizes and they disclose their HIV status. Reassuringly, most women in our study who had discontinued these methods for method-related reasons switched to more effective contraceptives (only 12% abandoned contraceptive use within 4 years while still at risk). Nevertheless, as barrier methods remain the single means of reducing the risk of sexually transmitted diseases, further analysis of the determinants and reasons for stopping these methods, including method failure which is relatively frequent (Moreau et al., 2007a; Kost et al., 2008), would improve our understanding of the dynamics of use of these methods; failures of these methods account for 35% of unintended pregnancies in France (Bajos et al., 2003).

The probabilities of discontinuing highly effective methods (the pill and the IUD) for method-related reasons among French women are strikingly lower than those reported for US women, for both early discontinuation (1 year) and after longer durations of use (2 years) (Trussell and Vaughan, 1999; Vaughan et al., 2008). US women were three times as likely to have stopped the IUD for method-related reasons and one and a half times as likely to have discontinued the pill within the first year of use. Ultimately, if discontinuation is a marker for prior poor adherence, these differences may translate to the lower contraceptive failure rates observed among French women compared with US women (Moreau et al., 2007a, Kost et al., 2008). Cross-national differentials in contraceptive discontinuation rates were also seen in the comparison with the Australian survey, although they did not follow a systematic pattern (Bracher and Santow, 1992). Australian women were twice as likely to have stopped the IUD for method-related reasons, but half as likely to have stopped the pill within the first year of use. As in Australia, our probabilities of contraceptive discontinuation for method-related reasons declined over time, which has been shown in the Australian study to result from both a decline in failure rates and a decline of contraceptive suspension because of side effects (Bracher and Santow, 1992).

Adding to this literature, our study examines discontinuation rates by method formulation. To our knowledge, only one population-based survey provides such estimates, although the study design was different and focused solely on the first 3 months of use (Murphy and Brixner, 2008). Consistent with the results of this study, we found increasing discontinuation rates with decreasing estrogen dosage. Our findings also mirror the conclusion of a recent Cochrane review of randomized controlled trials indicating that combined oral contraceptives containing >20 μg estrogen had lower rates of discontinuation that those containing 20 μg estrogen (Gallo et al., 2005). Possible explanations could be found in the increased risk of bleeding disturbances found in very low-dose estrogen pills compared with higher estrogen dosage pills (Moreau et al., 2007b; Gallo et al., 2005). Another reason, however, could relate to the higher likelihood of insurance reimbursement for second generation pills compared with third generation pills in France, the latter being the only category to offer very low-dose estrogen formulations. Such results should be brought to the attention of health care professionals in order for them to anticipate difficulties likely to be experienced with oral contraception (OCs) and to encourage women in more discussion about alternative effective contraceptive options. Although few studies have shown a direct improvement in contraceptive continuation due to interventions on the part of health care professionals (Halpern et al., 2006), raising providers’ awareness about the frequency and reasons for OC discontinuation is important, as women who cease to use the pill rarely consult their health care providers and usually switch to less effective methods (Rosenberg, 1998; Grady et al., 2002; Huber et al., 2006).

In contrast to the pill, we found no variation in IUD discontinuation by the type of IUD. To our knowledge, these results are the first to be obtained in a population-based setting and therefore should be confirmed in future studies. They are nevertheless consistent with the conclusions of a recent Cochrane review of randomized controlled trials showing no difference in discontinuation rates by the type of IUD (Grimes et al., 2007). Further analysis of reasons for stopping IUDs as well as resumption of use should be undertaken to complement these initial results.

In conclusion, this study shows great variation in contraceptive discontinuation rates by method, with lower probabilities of discontinuing highly effective methods. In view of the substantial differences demonstrated across countries, more cross-national comparisons on the dynamics of contraceptive use, including reasons for ceasing highly effective methods and resumption of use, would shed new light on the causes of such variation. Future research should also focus on factors related to the health care system, in terms of access to and
counseling about contraception, which could contribute to the differences observed.

Authors’ contribution

C.M. was involved in designing the study, analyzing the data and wrote the paper. J.T. and J.B. were involved in analyzing the data and writing the paper. J.B. also contributed to the study design. As principal investigator of the Cocon study, N.B. was responsible for designing the study. She revised the manuscript. G.R. contributed to the statistical analysis, and revised the manuscript. All the authors of the paper approved the current version of the manuscript.

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