and white blood cell proportions; and similar study design. Of note, previous analyses of blood samples from MCCS participants showed substantial replication of previously identified signals and discovered highly replicable novel associations for other health risk factors such as BMI (7), alcohol consumption (8), and tobacco smoking (9).

Taken together, our data and those presented in Mandaviya et al. would mean that there is at best weak evidence for an association between FFQ-derived intakes of folate and vitamin B-12 and DNA methylation in peripheral blood. This suggests that blood DNA methylation might not mediate, nor be a useful marker of, the association between intake of these nutrients and disease risk and shows the complexity of the one-carbon metabolism pathway in terms of, e.g., the number of nutrients involved, their interactions, and existing interindividual differences in nutrient absorption and metabolism.

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Reply to P-A Dugué et al.

Dear Editor:

Mandaviya et al. have attempted to replicate our findings of the association of folate and vitamin B-12 intake with differentially methylated positions and differentially methylated regions (DMRs) (1). They used a previously published data set, in which they performed a similar study to investigate associations between one-carbon nutrients (including folate and vitamin B-12) and DNA methylation in peripheral blood, and could not replicate our findings (2). They therefore concluded that there is weak evidence for an association between intake of folate and vitamin B-12 and differences in genome-wide DNA methylation in peripheral blood. We welcome the effort of Dugué et al. and, in general, we agree with the overall conclusion that there is at most weak association between folate and vitamin B-12 intake and circulating DNA methylation levels.

Nevertheless, we believe that there are large differences between the study of Dugué et al. and ours, apart from the ones already mentioned by Dugué et al. First, we used whole blood leukocytes to measure DNA methylation, whereas Dugué et al. used either peripheral blood mononuclear cells (PBMCs), buffy coats, or dried blood spots. PBMCs are lymphocytes and monocytes and lack granulocytes. Because DNA methylation patterns greatly vary between different cell types and with varying cell-type proportions (3), the results from Dugué et al.'s study and ours are difficult to directly compare. Second, we conducted our study in population-based cohorts and specifically excluded prevalent cancer of any type. In contrast, the study from Dugué et al. consisted of multiple nested case–control sets including cases with different forms of cancer. We excluded subjects with cancer cases because they potentially change dietary patterns in response to their disease (4) and have different

methylation patterns in blood (5–7). Third, to investigate differential methylation, we used the lowest compared with the highest tertiles of dietary intakes, whereas Dugué et al. divided dietary intakes into quintiles and used the lowest compared with the middle three quintiles (to evaluate deficiency) and the highest compared with the middle three quintiles (to evaluate excess).

Fourth, a further difference in analysis between Dugué et al.'s study and ours is the fact that we include vitamin supplement use (B-vitamins, multivitamins, or folic acid supplements) as a covariate in both of our continuous and categorical models because this could confound the association between dietary folate or vitamin B-12 and DNA methylation. Dugué et al. did not use this covariate owing to absence of these data. Although only 16% of the Melbourne Collaborative Cohort Study reported using multivitamins, there might still be some impact to their results. Fifth, concerning the replication of the DMRs, Dugué et al. attempted to replicate our identified DMRs by a look-up of the single CpGs of the DMRs. However, to replicate DMRs, they need to be analyzed as a whole region together and, therefore, one can use tools that are specific to finding DMRs in order to replicate a complete region.

Lastly, we used the residual method to adjust for total energy intake, whereas Dugué et al. used total energy intake as a covariate in the model (standard multivariate method). In the residual method, total energy intake is adjusted using linear regression with continuous variables before dividing it into categories, whereas in the standard multivariate model, total energy intake is adjusted insufficiently owing to categorical variables and loss of individuals by excluding quantiles, leading to a decrease in power (8). Therefore, the residual method is considered more optimal when using categorical models.

We believe that it is worthwhile to replicate our results by using similar analytic approaches, sample types, cohort types (controls without cancer prevalence of any type), and covariates in both studies. However, with all the efforts and evidence we have to date, we agree that there seems to be little association between dietary intake of folate or vitamin B-12 and DNA methylation.

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