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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References

1. Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr 2012;3(1):21–38.
2. Anderson OS, Sant KE, Dolinoy DC. Nutrition and epigenetics: an interplay of dietary methyl donors, one-carbon metabolism and DNA methylation. J Nutr Biochem 2012;23(8):853–9.
3. Mandaviya PR, Joehanes R, Brody J, Castillo-Fernandez JE, Dekkers KF, Do AN, Graff M, Hanninen IK, Tanaka T, de Jonge EAL, et al. Association of dietary folate and vitamin B-12 intake with genome-wide DNA methylation in blood: a large-scale epigenome-wide association analysis in 5841 individuals. Am J Clin Nutr 2019;110(2):437–50.
4. Chamberlain JA, Dugué P-A, Bassett JK, Hodge AM, Brinkman MT, Joo JE, Jung C-H, Makalic E, Schmidt DF, Hopper JL, et al. Dietary intake of one-carbon metabolism nutrients and DNA methylation in peripheral blood. Am J Clin Nutr 2018;108(3):611–21.
5. Milne RL, Fletcher AS, MacInnis RJ, Hodge AM, Hopkins AH, Bassett JK, Bruinsma FJ, Lynch BM, Dugué PA, Jayasekara H, et al. Cohort profile: The Melbourne Collaborative Cohort Study (Health 2020). Int J Epidemiol 2017;46(6):1757–1757i.
6. Joubert BR, den Dekker HT, Felix JF, Bohlin J, Ligthart S, Beckett E, Tiemeier H, van Meurs JB, Uitterlinden AG, Hofman A, et al. Maternal plasma folate impacts differential DNA methylation in an epigenome-wide meta-analysis of newborns. Nat Commun 2016;7:10577.
7. Geurts YM, Dugué P-A, Joo JE, Makalic E, Jung C-H, Guan W, Nguyen S, Grove ML, Wong EM, Hodge AM, et al. Novel associations between blood DNA methylation and body mass index in middle-aged and older adults. Int J Obes (Lond) 2018;42(4):887–96.
8. Dugué P-A, Wilson R, Lehne B, Jayasekara H, Wang X, Chol-Hee J, Joo JE, Makalic E, Schmidt DF, Baglietto L, et al. Alcohol consumption is associated with widespread changes in blood DNA methylation: analysis of cross-sectional and longitudinal data, Addict Biol. 2019. doi: 10.1111/adb.12855.
9. Dugué P-A, Jung C-H, Joo JE, Wang X, Wong EM, Makalic E, Schmidt DF, Baglietto L, Severi G, Southey MC, et al. Smoking and blood DNA methylation: an epigenome-wide association study and assessment of reversibility. Epigenetics 2019;25:1–11.

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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References

1. Mandaviya PR, Joehanes R, Brody J, Castillo-Fernandez JE, Dekkers KF, Do AN, Graff M, Hanninen IK, Tanaka T, de Jonge EAL, et al. Association of dietary folate and vitamin B-12 intake with genome-wide DNA methylation in blood: a large-scale epigenome-wide association analysis in 5841 individuals. Am J Clin Nutr 2019;110(2):437–50.
2. Chamberlain JA, Dugué P-A, Bassett JK, Hodge AM, Brinkman MT, Joo JE, Jung C-H, Makalic E, Schmidt DF, Hopper JL, et al. Dietary intake of one-carbon metabolism nutrients and DNA methylation in peripheral blood. Am J Clin Nutr 2018;108(3):611–21.
3. Reinius LE, Acevedo N, Joerink M, Pershagen G, Dahlen SE, Greco D, Soderhall C, Scheynius A, Kere J. Differential DNA methylation in purified human blood cells: implications for cell lineage and studies on disease susceptibility. PLoS One 2012;7(7):e41361.
4. Hebuterne X, Lemarie E, Michallet M, de Montreuil CB, Schneider SM, Goldwasser F. Prevalence of malnutrition and current use of nutrition support in patients with cancer. JPEN J Parenter Enteral Nutr 2014;38(2):196–204.
5. Dong L, Ren H. Blood-based DNA methylation biomarkers for early detection of colorectal cancer. J Proteomics Bioinform 2018;11(6):120–6.
6. Xu Z, Sandler DP, Taylor JA. Blood DNA methylation and breast cancer: a prospective case-cohort analysis in the Sister Study. J Natl Cancer Inst 2019.
7. Sandanger TM, Nost TH, Guida F, Rylander C, Campanella G, Muller DC, van Dongen J, Boomsma DI, Johansson M, Vineis P, et al. DNA methylation and associated gene expression in blood prior to lung cancer diagnosis in the Norwegian Women and Cancer cohort. Sci Rep 2018;8(1):16714.
8. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65(4 Suppl):1220S–8S; discussion 1229S–31S.