Update of use of dietary indices to control for diet in human gut microbiota studies

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Short report

Keywords: Microbiome, Microbiota, Dietary Index, Dietary covariate, Human microbiota, Food frequency questionnaire, FFQ, Healthy Eating Index, HEI, Dietary inflammation index, DII, E-DII

Posted Date: March 16th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-17325/v1

Abstract

Background In our recent publication (DOI: https://doi.org/10.1186/s40168-018-0455-y) we concluded that of the three dietary indices we studied the Healthy Eating Index (HEI) was the index of choice where researchers wish to account for the role of diet in microbiome association studies. Following correspondence from its creators, we replicated our initial study with an additional index, the Dietary inflammation index (DII ®) using an updated data analysis pipeline for microbiota comparisons that incorporate the use of Amplicon Sequence Variants (ASVs) rather than the previously utilised Operational Taxonomic Units (OTUs). The energy-adjusted DII (E-DII) reflects the inflammatory potential of an individual's diet, after controlling for total energy intake, which is of interest given the known association between the microbiota and inflammatory disease.

Results Using data from 5047 participants of the TwinsUK cohort, we observed the E-DII to be a valid dietary measure within this cohort. The E-DII had a stronger association with frailty, a measure of health deficit, than the HEI (E-DII: $\beta = 0.12$, $p < 0.001$; HEI: $\beta = -0.09$, $p < 0.001$) But not with BMI (E-DII: $\beta = 0.04$, $p < 0.01$, HEI: $\beta = -0.07$, $p < 0.001$). In a subset of individuals (n = 1853) with existing microbiota data, the E-DII had a stronger association with measures of microbiota species (alpha) diversity; the HEI had a higher number of associations with differences of taxa abundance. Of interest are the differences where the two indexes do not overlap). For example, the genus with the strongest association with the E-DII but not significantly associated with the HEI was assigned as Escherichia/Shigella (logFC=0.692, q<0.001). Both indexes were associated with PCoAs of weighted UniFrac distances.

Conclusion Both the E-DII and HEI are strong candidates for use as covariates in microbiota association studies where measures of the microbiota are captured using ASVs. The E-DII is an interesting alternative to the HEI particularly where study designs are looking to assess causality of the microbiota in driving inflammation and inflammatory disease.

Background

Last year we published an article entitled "Use of dietary indices to control for diet in human gut microbiota studies" [1]. In the work, we concluded that the Healthy Eating Index-2010 (HEI) was the index of choice to describe overall diet in gut microbiota association studies of the three we compared using dietary data derived from EPIC-NORFOLK Food Frequency Questionnaire (FFQ) data [1, 2]. The manuscript received great interest from scientists conducting similar studies seeking a means to control for diet in gut microbiota studies, along with suggestions of alternative indexes. We welcomed correspondence in particular directing us to consider Shivappa et al's 2014 [3] population-based dietary inflammatory index (DII®); the results of the subsequent collaboration are presented here.

As acknowledged in our previous submission, there are, of course, other dietary indexes that could act as summary variables for the microbiota. The three we selected in our original analysis (we compared the HEI to the Mediterranean Dietary Score and the Health Food Diversity Index) captured diet in different

ways; via consumption measured against dietary guidelines, recommended foods and dietary variety [1]. Other indexes seek to classify diet in terms of how it relates to disease (e.g., the DASH -score [4]) but we felt the use of such an index would complicate interpretation of microbiota association because it had the potential to associate with non-dietary health-related variance in the microbiota. The DII reflects the inflammatory potential of an individual's diet, and thus it falls into this category; however, given it was designed to capture the inflammatory potential of diet (i.e., rather than a specific morbidity), and given the widely reported association of the microbiota and inflammatory disease [5], we decided to consider the DII in relation to microbiota composition.

In addition to consideration of a new index, we also wanted to present the association of the HEI with gut microbiota variance as derived from study of amplicon sequence variants (ASVs), rather than the formerly used operational taxonomic units (OTUs). We agree with the assertion of McMurdie and colleagues that ASVs should replace OTUs in 16 s marker gene analysis due to improved taxonomic resolution and assignment, and because they can be more readily compared across study populations [6].

Results

Index construction and validation

Cohort statistics are presented in Table 1. The E-DII is negatively associated with health; positive values reflect a pro-inflammatory diet, negative values reflect an anti-inflammatory one [3]. In our cohort the DII mean was − 1.20 (± 1.21) with a range of -4.08 to + 4.00.

Table 1
Cohort statistics.

Characteristic (measure)	Validation cohort	Microbiota subset*
n	5047	1853
Sex (%female)	91.2	90.4
Zygosity (% MZ)	56.8	56.2
Ethnicity (% white)*	98.2	98.6
Age (at FFQ) (μ,σ^2)	58.4 (13.2)	60.56 (11.4)
BMI (μ,σ^2)*	26.2 (5)	25.8 (4.6)
FI (μ,σ^2)* ^	0.18 (0.1)	0.17 (0.1)
HEI (μ,σ^2)	60 (10.3)	60.3 (10.1)
DII	-1.20 (1.21)	-1.29 (1.17)

^ Coverage of frailty index increased since publication of last paper with further data processing, and has been updated accordingly. *Reduced n of microbiota subset compared with previous study due to changes in methodology of amplicon analysis (see methods). Ethnicity is self-reported. **Data incomplete (check how worded in original). All values rounded to 1dp.

The E-DII and HEI were significantly negatively correlated with each other (Spearman's rank correlation, rho: -0.72, $p < 0.001$, supplementary figure S1). The E-DII successfully differentiated between smokers and non-smokers, men and women, and over 60 s and under 60 s (Table 2). As a reminder, the HEI distinguished smokers and non-smokers, and men and women, but not over 50 s and under 50. The mean of the E-DII in under 60 s suggests a more inflammation associated diet than the over 60 s.

Table 2

Concurrent criterion validation of dietary indices (Wilcoxon rank sum) p values: *p < 0.05, **p < 0.01, ***p < 0.001, ⊥ = non-significant

	n	HEI	E-DII
Men vs women	443:4604	56.4:60.4***	-0.57:-1.26***
Over 60 s vs under 60 s	2543:2504	59.8:60.3⊥	-1.26:-1.15**
Smokers vs non-smokers	317:2909	55.9:61***	-0.85:-1.39***

Both indexes were significantly associated with BMI and frailty (Table 3). The E-DII was positively associated with these measures, suggesting a pro-inflammatory diet is associated with both health measures (Table 3). The HEI remained significantly associated with frailty (standardised coefficient changed from − 0.12 in previous paper due to increased coverage [1]). Interestingly, the standardised coefficient was greater with the E-DII and frailty the HEI and frailty; the opposite was true with the dietary indexes and BMI.

Table 3

E-DII and HEI scores in relation to body mass index and frailty..

	n	HEI	E-DII
BMI	4428	β = −0.07 **	β = 0.04*
FI^	4553	β = −0.09**	β = 0.12**

Two dietary indices, the Healthy Eating Index 2010 (HEI) and the Energy adjusted Dietary Inflammation Index (E-DII) were assessed for their correlation with two health measures; body mass index (BMI kg/m^2) and Rockwood's frailty index (FI) via nested linear regression models (adjusting for age, sex and zygosity). p values: p < 0.01*, p < 0.001**. ^ Coverage of frailty index increased since publication of last paper with further data processing, and has been updated accordingly.

Microbiota assessment

Similarly to the first study, the HEI was significantly associated with the three measures of species diversity considered here; the standardised coefficients are greater here than in the previous paper reflecting differences in ASVs vs OTUs and methodological differences in calculating diversity measures.

Table 4
Standardised coefficients from linear mixed effects models.

Diversity measure	Healthy Eating Index (HEI)	Energy-adjusted Dietary Inflammation Index (E-DII)
Richness (observed ASVs)	0.095***	-0.122***
Shannon	0.11***	-0.135***
Simpson	0.071**	-0.095***
p-values =***p < 0.001 **p < 0.01 Species diversity measures were calculated on untrimmed, unadjusted ASVs and adjusted for sequencing depth, age, sex, BMI and technical variables (see Methods)		

Both the HEI and E-DII were associated significantly with abundances of ASVs, genus and phylum following FDR-correction (Fig. 1, full results table and ASV sequences in supplementary material S2.). As a reminder, increase in the E-DII indicates a more pro-inflammatory diet and is generally negatively associated with health, whereas the HEI is positively associated. The HEI was associated with more ASVs than the E-DII (Table 5) and the E-DII was associated with slightly more genus. Many of the association were the same and reflected typical associations with diet (e.g. associations with Ruminococcaceae and Lachnospiraceae). Of interest is the difference between the HEI and E-DII in terms of the associations observed (Fig. 1). For example, the genus with the strongest association with the E-DII but not significantly associated with the HEI was assigned as Escherichia/Shigella (logFC = 0.692, q < 0.001). Erysipelatoclostridium and Erysipelotrichaceae_UCG-003 at both genus and ASV level were reduced in association with the HEI but not the E-DII, whereas Oscillospira and Oscillobacter were associated with the E-DII and not the HEI (Fig. 1, supplementary material S2).

Table 5
Number of taxonomic associations observed with dietary indices

Taxonomic level	HEI	E-DII
ASVs	43	36
Genus	33	34
Phylum	2	2

Both the HEI and E-DII were significantly associated with the first 3 PCoAs of weighted UniFrac distance, suggesting they explain significant inter-individual differences in microbiota composition (supplementary material S3). Interestingly, only the HEI was associated with the 4th PCoA (beta = -0.09, $p < 0.001$) whereas the E-DII was associated with the 5th (beta = 0.08, $p < 0.001$) suggesting the indexes are capturing different aspects of microbiota variance.

A total of 213 twin pairs were discordant on their HEI score, 210 were discordant on their E-DII. Of the 43 ASVs (those significant in the earlier analysis above) considered for difference in HEI-discordant twin pairs, only one was significant and was higher in abundance in twins eating a more health associated diet; an ASV belonging to the Lachnospiraceae family ($q = 0.043$). Of the 36 ASVs considered for difference in E-DII-discordant twin pairs, again an ASV assigned to Lachnospiraceae family (although not the same ASV; sequences and results tables are in supplementary material S4).

Discussion

The purpose of the initial study was to identify a dietary index that can be best utilised as a dietary summary measure to describe differences in microbiota. Here, we aimed to extend this using the DII. Results of validation tests suggest it is an appropriate measure within our cohort. The E-DII performed better than the HEI in concurrent criterion validation tests, and had a stronger association with frailty, although not BMI. Both indexes captured significant amounts of microbiota variance in all the ways considered here, suggesting they both could be considered as a dietary control; the HEI captured slightly more microbiota variance than the E-DII, although many of the relationships were stronger with the E-DII.

The differences in microbiota results in association with HEI here and in the previous paper are due to the differences in processing of amplicon sequences to ASVs rather than OTUs, and, in the case of diversity and taxa analysis, differences in analytical pipeline [1, 6]. ASVs offer better resolution and reproducibility than OTUs; this along with divergences in methodology from the previous paper to better account for the structure of microbiota datasets accounts for the difference in association between this and the first paper (for more detail on these methodological differences, refer to other published reports [7–9]). When considered in terms of the reproduction of associations with microbiota variance, the HEI and other dietary indexes are demonstrably robust to difference in analytical choice.

Given research to date on the composition of the microbiota, it is perhaps unsurprising that dietary indexes capture large amounts of microbiota variance in a manner associated with health. Of greater interest may be the difference in the associations between the two indexes, suggesting they each might offer different utility depending on the research question of interest. For example, the E-DII exhibited significant associations with known pathobiontic species, such as the Shigella and Escheria genus (genetic analysis of Shigella and Escheria coli suggests they are closely related and should likely be considered as the same genera, and in some cases the same species depending on the strain [10]). The E-DII might be more useful in study designs looking to assess causality of the microbiota in driving

inflammation and inflammatory disease, although how this might confound, or be utilised in study of, microbiota-by-diet effects on the host should be considered.

There are multiple measures of dietary quality that could be considered, including an updated HEI [11]. An important advantage of the DII/E-DII over the HEI is that it was defined using an approach that accounted for greater dietary diversity and is therefore more applicable to non-Western populations. Both indexes were derived from self-reported dietary data which are known to be problematic in accurately reflecting an individual's true diet [12]; this should be a consideration of future studies.

Conclusion

We have found that both the DII and HEI dietary patterns relate differently to microbiota, as measured using ASV method which, has better taxonomic resolution and assignment than previously used OTUs. This highlights the importance of dietary variability on microbiota composition and that dietary indices are relevant for controlling for diet with over-saturation models

Methods

Data

We used the same cohort of 5047 individuals from the TwinsUK cohort as in the original paper to create and validate the dietary indexes. TwinsUK is the UK's largest cohort of mono- and di-zygotic twins. All participants with a completed Food Frequency Questionnaire (FFQ) as of 2016 were included in analysis.

Dietary index creation

The original Healthy Eating Index (HEI) methodology created by Guenther and colleagues [13] was modified to consider dietary data derived from an FFQ designed for UK populations; we use the same variable created for the previous paper.

Dietary Inflammatory Index (DII) creation

The DII is based on literature published through 2010 linking diet to inflammation. Details regarding DII are described in details elsewhere [3]. Briefly, 1,943 eligible peer-reviewed primary research articles published through 2010 on the effect of dietary factors on six inflammatory markers (IL-1β, IL-4, IL-6, IL-10, tumour necrosis factor-a (TNF-α), and CRP) were identified and scored to derive the component-

specific inflammatory effect scores for 45 dietary factors (i.e., components of DII), which comprised macronutrients, micronutrients and some foods or bioactive components such as spices and tea. To avoid the arbitrariness resulting from simply using raw intake amounts, all individual self-reports for each DII component in the study were standardized to a world database consisting of dietary intake from 11 populations living in different countries across the world. The standardized dietary intake was then multiplied by the literature-derived inflammatory effect score for each DII component, and summed across all components to obtain the overall DII score. Higher DII scores represent more pro-inflammatory diets, while lower (i.e., more negative) DII scores indicate more anti-inflammatory diets [3]. Here we present results for the DII adjusted for energy (E-DII) using the density approach wherein all nutrients are converted to per 1000 kcal intake and as a result energy is not part of the E-DII calculation. This was a similar approach to that in the HEI and we felt it the better comparator.

Validation of the DII in our cohort

As before, we validated the DII in our study using Wilcoxon rank sum tests to assess the extent to which the DII distinguished smokers from non-smokers (n=3226, due to longitudinal differences in sample questionnaires), those over and under 50 years of age, and men and women. Indices were assessed as the primary explanatory variable against health measures, body mass index (BMI-weight(kg)/height(m)^2) (n=4428) and a frailty index calculated using the Rockwood method [14] (n=4553); coverage of the frailty index has increased since the last submission due to increase in received questionnaire data; all data are thought to be missing at random and due to differences in longitudinal sampling. Linear models including BMI and frailty were adjusted for age, twin relatedness and sex.

Microbiota analysis

In contrast to the original paper, here we present results from microbiota data where 16S sequences have been re-analysed to produce amplicon sequence variants (ASVs). ASVs were created following the following the DADA2 pipeline [7]. Briefly, DNA sequences were demultiplexed, and separate forward- and reverse-read files were generated for each sample. The DADA2 pipeline was applied to each sequencing run separately, until the final merge step. Quality of sequences was assessed, with ends trimmed to remove poor quality reads, error estimated within-sample for forward and reverse reads, and then the ASV algorithm applied. Forward and reverse ASVs were joined, and the total dataset merged. Chimeras were removed. Taxonomic assignment was via SILVA 1.3.2.

Due to the differences in analytical pipelines of ASVs in this analysis rather than OTUs in the previous, more samples failed to achieve quality control thresholds. We wanted only to use samples considered in the previous study (i.e., rather than rematching individuals to different samples); as such, this resulted in 1853 samples used for analysis rather than 2070 used in the original study. In the previous study we considered four indexes of species diversity, here, Chao1 was not considered as it is an inappropriate index to use with ASVs (due to the lack of singletons); a richness measure remains as 'Observed ASVs', as does use of Shannon diversity and Simpson's Index. All indexes were calculated using the 'phyloseq' package in RStudio v1.1.423 [15,16] on untrimmed, untransformed ASV tables following suggestion of McMurdie and colleagues [8]. Mixed effects models were constructed in RStudio using the 'lme4' package [17] with each alpha diversity measure as the response variable and the primary explanatory variable as the HEI or the E-DII were adjusted for age at microbiome sample, BMI at microbiome sample and sequencing depth. Only the technician who extracted the sample was considered as a random factor; the other random factors considered in the initial analysis explained so little of the variance associated with the indexes that they prevented appropriate model fit, and were thus not included. All variables were scaled prior to model inclusion and standardised coefficients are reported.

Differences in abundance of ASVs, genus and phylum (i.e., ASVs collapsed to genus and phylum level) were assessed using DeSeq2 [18], adjusted for library size. Similar to the previous analysis, the first 10 PCoAs of weighted-UniFrac distance (calculated on variance transformed ASVs with negative abundances set to 0 for the ordination) were extracted and used as response variables in mixed effects models, adjusted as in ASV diversity analysis, with the only random effect considered (again, because inclusion of those in the first analysis prevented adequate model fit) to be the sequencing run. Finally, twin pairs with greater than one standard deviation and within different quintiles for their dietary score (HEI and E-DII) were identified, and differences in ASV abundances were compared using paired Wilcoxon rank-sum tests, and false discovery rate adjusted using the Benjamin-Hochberg method.

Abbreviations

ASV – Amplicon sequence variant

DII – Dietary inflammatory index

HEI – Healthy Eating Index (2010)

FFQ – Food Frequency Questionnaire

OTU – Operational Taxonomic Unit

Declarations

Ethics approval and consent to participate

Favourable ethical opinion was granted by the formerly known St. Thomas' Hospital Research Ethics Committee (REC). Following restructure and merging of REC, subsequent amendments were approved by the NRES Committee London—Westminster (TwinsUK, REC ref: EC04/015, 1 November 2011); use of microbiota samples was granted NRES Committee London—Westminster (The Flora Twin Study, REC ref: 12/LO/0227, 1 November 2011)

Consent for publication

Not applicable

Availability of data and material

The European Bioinformatics Institute (EBI) accession numbers for the sequences reported in this paper is ERP015317.

According to Wellcome Trust's Policy on data, software and materials management and sharing, all data supporting this study will be openly available following reasonable request to the TwinsUK data access committee. Information on data access and how to apply is available at http://www.twinsuk.ac.uk/data-access/submission-procedure-2/. Please contact the corresponding author for further detail.

Competing interests

JRH owns controlling interest in Connecting Health Innovations LLC (CHI), a company that has licensed the right to his invention of the dietary inflammatory index (DII®) from the University of South Carolina in order to develop computer and smart phone applications for patient counseling and dietary intervention in clinical settings. NS is an employee of CHI. The subject matter of this paper will not have any direct bearing on that work, nor has that activity exerted any influence on this project.

TDS is a co-founder of Zoe Global Ltd, a precision nutrition company.

Funding

Twins UK receives funding from the Wellcome Trust (WT081878MA), the National Institute for Health Research (NIHR) Clinical Research Facility at Guy's & St Thomas' NHS Foundation Trust and NIHR Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. This work was also supported by the Chronic Disease Research Foundation.

Authors' contributions

RB prepared and performed analysis of the data used here. NS and JRH created the E-DII for use in this analysis and contributed to manuscript development. PW created the ASVs used in this analysis. All

authors contributed towards writing the manuscript and read and approved the final manuscript.

Acknowledgements

The authors of this paper wish to express our appreciation to all study participants of the TwinsUK cohort for donating their samples and time. TwinsUK is funded by the Wellcome Trust, Medical Research Council, European Union, the National Institute for Health Research (NIHR)-funded BioResource, Clinical Research Facility and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London.

We thank Dr. Julia K Goodrich, Dr. Ruth E Ley and the Cornell technical team for generating the microbial data. Thanks also to Dr Jane Skinner of the University of East Anglia and Dr Tess Pallister for their assistance with processing the FFQ data and Dr Matthew Jackson for his guidance in the original analysis which informed this one.

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Figures

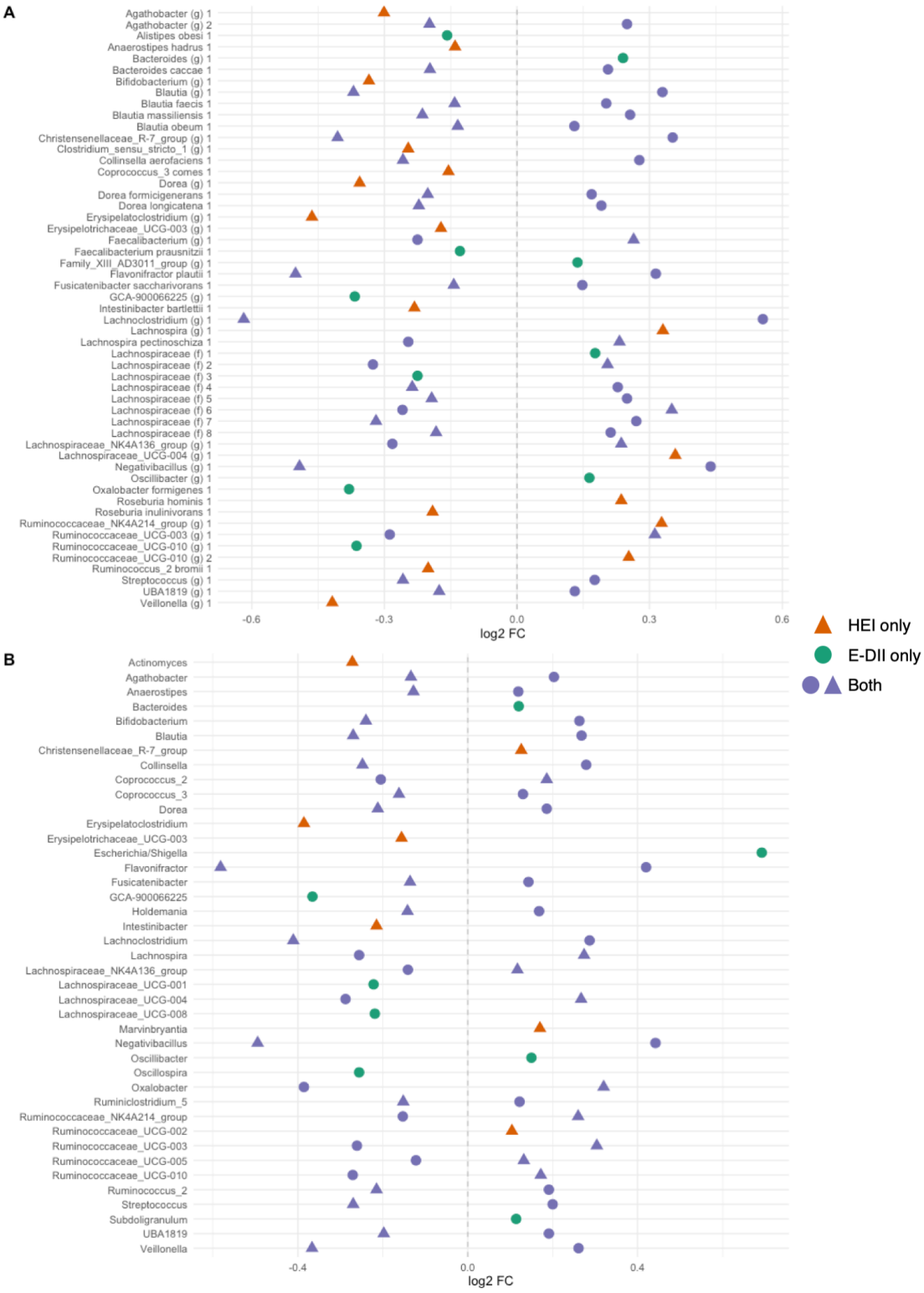

Figure 1

. Differences in abundances (q < 0.05) of A. ASVs and B. Genus in association with two dietary indexes the HEI and E-DII calculated using DeSeq2. Shapes indicate the index of association. Green indicates that

the ASV/genus was only associated significantly with the E-DII; orange indicates that the ASV/genus only associated with the HEI; purple indicates that the ASV/genus associates with both.

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