Association between Bile Acids and Enterolactone & Enterodiol

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**Introduction**

Lignans are phenolic compounds that are frequently found in plant-based foods, particularly seeds, whole grains and vegetables (Primeau, 2018). In the mammalian gut, they are metabolized into two major enterolignans: estrogenic enterolactone (ENL) and enterodiol (END) by gut bacteria (Carreau et al., 2008). Plasma END and ENL are shown to be associated with reduced risk of colorectal, prostate and breast cancer, but the biological mechanism is still not fully understood (Carreau et al., 2008). Bile acids are steroid acids found predominantly in the bile of mammals and other vertebrates. They are synthesized in the liver from cholesterol and are secreted via the bile in the small intestine (Suharoschi et al, 2019). They have long been known to regulate the absorption of fat-soluble vitamins, cholesterol and lipids, but also play important roles in the modulation of epithelial cell proliferation, gene expression and metabolism. These homeostatic pathways, when disrupted, are able to promote local inflammation, systemic metabolic disorders and, ultimately, cancer (Ciaula et al., 2017).

Previous studies have shown that ENL may affect synthesis and metabolism of bile acids, thereby regulating downstream metabolic effects of bile acids (Narravo et al., 2020). Given that ENL and END are both converted from lignan via intestinal microflora, we aimed to: 1) evaluate the association between ENL and concentration of different bile acids, and 2) evaluate the association between END and concentration of different bile acids. The results of this project might bring us valuable insights into possible physiological pathways through which the two major diet-derived polyphenol metabolites can affect metabolic signaling and ultimately influence cancer risk.

**Data**

The data came from the Carbohydrate and Related Biomarkers (CARB) Study, a randomized controlled crossover feeding trial (conducted from June 2016 to July 2009) investigating the effect of diet high in whole grain vs. refined grain on circulating bile acids. The trial involved 80 healthy adults (40 men/40 women, 18-45 years) from the great Seattle Area, with equal numbers of normal weight and overweight/obese individuals. Participants consumed a diet either high in whole grain (WG diet), or high in refined grain (RG diet) for four weeks, then switched to the other diet after a four-week washout period, with randomly assigned dietary order. Plasma concentrations of 25 distinct bile acids were measured from the blood specimens collected after a 12-hour fast at the end (day 28) of each feeding period. END and ENL were measured from 24-h urine samples that were also collected at the end of each intervention period. The data also contains demographic information such as race, age, sex, and physical measurements such as height, weight, and BMI of each participant. There are nine missing observations for ENL and END, respectively, but all the missing values concentrate on five participants. No data is missing for demographic information, dietary intervention, or bile acid measurements. This data can be used cross-sectionally to investigate our associations of interest, given that the data contains measurements for ENL, END, bile acids, as well as potential confounders to adjust for. Since there are two measurements for each individual, the statistical model we select needs to account for the non-independent observations.

**Methods**

We summarized participants' baseline characteristics, based on experimental diet sequences. ENL and END were separated into low and high levels using their sample medians. We provided summary statistics on the bile acids by low/high levels of ENL and END for each dietary intervention. We explored the distributions of the bile acids, ENL and END, and log-transformed these variables because the majority were highly right-skewed.

Linear mixed models were used to assess the association between each bile acid and ENL level, as well as the association between each bile acid and END level. We used the concentration of each bile acid as the outcome and ENL level and END level as the predictor of interest, respectively. Meanwhile, we adjusted age, sex, race, and dietary intervention as fixed effects and participant ID as the random effect. We used the Benjamin-Hochberg procedure to control for multiple comparisons at a false discovery rate of 10%. Given that only five participants have missing data, complete case analyses were applied.

#### **Results**

Baseline characteristics of 80 CARB study participants completing both WG and RG diets, stratified by diet RG to diet WG and diet WG to diet RG were shown in Table 1. In general, the majority of participants were White, followed by Hispanic. There were no substantial differences in sex, baseline age, and race in the two participant groups defined by randomized dietary sequence. Table 2 showed the median and interquartile range of each bile acid by urinary excretion of ENL and END (above vs. below median) regarding each dietary intervention (RG and WG). No consistent pattern was found across different bile acid species.

We examined the association of the concentration of bile acid metabolites with enterolactone (ENL) and enterodiol (END), respectively (Table 3), adjusting for age, sex, race, and dietary intervention. ENL excretion was found to be associated linearly with three bile acids at p < 0.05. Specifically, ENL excretion was positively associated with Murocholic acid (p = 0.048), and negatively associated with Deoxycholic acid (p = 0.01) and Ursodeoxycholic acid (p = 0.011). However, none of the associations satisfied the FDR of q < 0.1 after BH adjustment. Besides, END excretion was associated linearly with one bile acid at p < 0.05. Specifically, END excretion was inversely associated with Taurocholic acid (p = 0.014). However, this association also did not satisfy the FDR threshold of q < 0.1 after BH adjustment.

**Discussion**

Using the randomized controlled crossover feeding trial data, we found that at alpha level of 0.05, ENL was positively associated with Murocholic acid, and negatively associated with Deoxylic and Ursodeoxycholic acid; END was negatively associated with Taurocholic acid. However, all of these associations were no longer significant after BH-adjustment at the false discovery rate of 0.1.

There was a prior study investigating the association between ENL and bile acids. The title of the paper is *Effect of a Flaxseed Lignan Intervention on Circulating Bile Acids in a Placebo-Controlled Randomized, Crossover Trial.* This was a randomized controlled crossover feeding trial, where the diet interventions were placebo vs. flaxseed lignan supplement. We compared our analysis results against the results reported in this article, and found no overlaps between them. There are a couple factors that can contribute to the discrepancy. The major factor is that the dietary interventions are very different between the two studies. In the CARB study, RG vs. WG diet has a high impact on fiber intake, which directly affects the diversity of gut microbial. Given that ENL is a microbially derived metabolite, RG vs. WG diet can substantially influence ENL concentration. In contrast, in the other study, the additional flaxseed lignan supplement is not expected to make an appreciable impact on ENL. Moreover, dietary factors play a key role in determining bile acid concentrations (Navarro, 2020). Given the obvious distinction in dietary interventions provided in the two trials, their results are not directly comparable. Besides, in the prior study, researchers adjusted for age, sex, body mass index, intervention sequence, assay batch, and baseline bile acid concentrations in their analysis. Whereas, we adjusted for age, sex, race and dietary intervention in our analysis. The different set of covariates adjusted in the linear mixed model may also contribute to the differences in our analysis results.

A notable advantage of this project is that the data comes from a controlled feeding trial, where the food species and nutritional intake are carefully controlled for each participant throughout the study period. The closely regulated diet substantially reduces the confounding effect of diet on our association of interest, given that dietary pattern impacts the generation of enterolignans as well as the concentration of bile acids (Trefflich, 2019). Besides, this project examines multiple associations at one time, which is an effective way of utilizing the trial data. A limitation is the moderate sample size, which might lead to insufficient power to detect true associations, especially given that adjusting for multiple comparisons requires a more stringent threshold of p-value in order to reject the null hypothesis. Another limitation is that although participants were instructed to only consume food that researchers provided for them during both intervention periods, there was no way to fully supervise participants’ eating behavior outside the laboratory setting. Hence, we cannot rule out the possibility of residual confounding effect by inter-individual variation in diet. Furthermore, given the cross-sectional nature of the analysis, causal inference cannot be drawn because other unmeasured factors and/or individual behaviors may also affect both enterolignan excretion and bile acid abundance.

There is still a need for a larger body of high-quality studies on the association between enterolignans, bile acids and cancer risk. Research in this area will help advance scientific understanding of health effects of dietary patterns, which not only sheds light on primary intervention of certain types of cancer, but also provides valuable insight into potential strategies of curbing disease progression in cancer patients.

**Tables**

**Table 1: Baseline participants’ characteristics by diet sequence**

|  |  |  |
| --- | --- | --- |
|  | **RG -> WG**  **N = 381** | **WG -> RG**  **N = 421** |
| Female | 18 (47%) | 22 (52%) |
| Age | 28 (25, 37) | 28 (21, 36) |
| Race: Asian | 3 (7.9%) | 3 (7.1%) |
| Race: Black | 8 (21%) | 9 (21%) |
| Race: Caucasian | 17 (45%) | 18 (43%) |
| Race Hispanic / Latino | 9 (24%) | 11 (26%) |
| Race Other | 1 (2.6%) | 1 (2.4%) |

n (%); Median (IQR)

**Table 2: Descriptive statistics for bile acids by diet type and a) END, b) ENL levels**

|  | **RG Diet** | | **WG Diet** | |
| --- | --- | --- | --- | --- |
|  | **Low END, N = 39** | **High END, N = 36** | **Low END, N = 38** | **High END, N = 38** |
| Chenodeoxycholic Acid | 5,782 (2,602, 12,650) | 9,065 (2,576, 17,999) | 5,747 (2,339, 14,288) | 5,515 (2,086, 13,921) |
| Cholic Acid | 2,322 (1,500, 3,963) | 3,178 (1,668, 7,491) | 2,067 (1,224, 4,152) | 2,133 (1,701, 4,238) |
| Deoxycholic Acid | 21,329 (11,942, 36,378) | 28,154 (14,075, 47,102) | 21,365 (15,204, 34,873) | 20,498 (8,753, 37,385) |
| Glycochenodeoxycholic Acid | 19,646 (11,453, 38,983) | 22,137 (15,049, 34,411) | 31,476 (18,721, 51,968) | 23,847 (15,104, 33,117) |
| Glycocholic Acid | 6,972 (4,213, 15,420) | 7,759 (5,500, 14,598) | 15,151 (7,567, 26,627) | 9,583 (5,767, 14,482) |
| Glycodeoxycholic Acid | 13,292 (6,005, 20,823) | 13,848 (9,685, 24,986) | 18,779 (10,058, 36,086) | 12,673 (4,231, 25,727) |
| Glycohyodeoxycholic Acid | 5,066 (2,070, 9,568) | 4,998 (2,994, 9,462) | 5,309 (2,143, 9,993) | 2,657 (1,677, 5,424) |
| Glycolithocholic Acid | 1,789 (1,565, 2,037) | 1,376 (1,088, 2,348) | 1,549 (1,293, 2,263) | 1,573 (1,340, 2,080) |
| Glycoursodeoxycholic Acid | 5,702 (2,449, 11,076) | 6,049 (3,147, 11,586) | 5,515 (2,919, 10,418) | 4,308 (2,372, 6,452) |
| Hyocholic Acid | 624 (375, 906) | 632 (443, 1,375) | 527 (289, 717) | 542 (250, 942) |
| Hyodeoxycholic Acid | 3,708 (2,557, 6,831) | 3,886 (2,406, 5,413) | 2,641 (2,069, 5,054) | 3,284 (2,458, 4,648) |
| Isolithocholic Acid | 3,523 (2,359, 4,955) | 2,480 (1,802, 3,939) | 3,880 (2,320, 5,252) | 3,026 (1,563, 4,506) |
| Lithocholic Acid | 2,250 (1,683, 2,670) | 1,980 (1,187, 2,411) | 2,489 (1,656, 3,190) | 1,782 (1,143, 3,113) |
| Murocholic Acid | 8,153 (6,590, 19,881) | 8,186 (5,064, 12,906) | 11,480 (6,227, 16,627) | 7,204 (5,063, 10,828) |
| Tauro alpha Muricholic Acid | 491 (243, 816) | 929 (454, 1,713) | 780 (347, 1,100) | 577 (295, 1,011) |
| Tauro omega Muricholic Acid | 228 (105, 335) | 311 (128, 700) | 305 (154, 599) | 306 (112, 588) |
| Tauro ursodeoxycholic Acid | 621 (408, 1,330) | 767 (455, 1,368) | 1,089 (486, 1,806) | 700 (508, 1,019) |
| Taurochenodeoxycholic Acid | 7,771 (4,103, 14,216) | 6,149 (4,565, 11,275) | 12,534 (5,749, 19,158) | 8,646 (3,814, 13,391) |
| Taurocholic Acid | 4,379 (3,553, 8,675) | 4,960 (3,756, 9,198) | 11,029 (5,271, 17,611) | 8,833 (4,392, 12,960) |
| Taurodeoxycholic Acid | 4,015 (1,829, 7,624) | 4,178 (2,629, 6,828) | 6,055 (3,630, 12,053) | 3,727 (1,909, 8,185) |
| Taurohyocholic Acid | 230 (110, 332) | 294 (129, 766) | 286 (143, 595) | 309 (125, 546) |
| Taurohyodeoxycholic Acid | 876 (562, 1,613) | 908 (585, 1,748) | 970 (497, 1,745) | 825 (556, 1,108) |
| Taurolithocholic Acid | 676 (343, 1,303) | 503 (251, 699) | 1,186 (616, 2,360) | 806 (387, 1,435) |
| Ursodeoxycholic Acid | 21,840 (11,929, 37,753) | 30,053 (14,439, 49,034) | 22,821 (15,342, 37,769) | 22,192 (8,122, 38,439) |
| Glycohyocholic Acid | 1,883 (1,026, 3,119) | 2,052 (1,099, 4,223) | 1,976 (1,073, 3,415) | 2,011 (972, 3,412) |

\* 5 observations missing in END have been removed

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **RG Diet** | | **WG Diet** | |
|  | **Low ENL, N = 38** | **High ENL, N = 37** | **Low ENL, N = 38** | **High ENL, N = 38** |
| Chenodeoxycholic Acid | 7,126 (2,966, 16,524) | 7,085 (2,459, 15,163) | 7,988 (2,962, 17,097) | 4,104 (1,967, 11,170) |
| Cholic Acid | 2,755 (1,538, 5,100) | 3,173 (1,677, 5,668) | 2,708 (1,379, 4,835) | 1,974 (1,642, 2,831) |
| Deoxycholic Acid | 28,154 (12,281, 40,442) | 24,610 (12,815, 38,209) | 23,025 (15,318, 43,792) | 18,648 (8,753, 33,183) |
| Glycochenodeoxycholic Acid | 21,394 (12,472, 52,217) | 21,992 (14,415, 33,972) | 31,046 (18,186, 55,496) | 23,304 (15,146, 34,261) |
| Glycocholic Acid | 6,622 (4,828, 15,807) | 7,924 (4,956, 14,381) | 13,982 (7,539, 26,632) | 9,733 (6,111, 17,146) |
| Glycodeoxycholic Acid | 12,881 (7,510, 22,257) | 14,261 (6,831, 20,172) | 19,712 (8,855, 35,799) | 11,964 (5,694, 25,870) |
| Glycohyodeoxycholic Acid | 5,682 (2,693, 9,795) | 4,845 (2,800, 6,656) | 5,309 (2,714, 10,113) | 2,304 (1,515, 5,165) |
| Glycolithocholic Acid | 1,752 (1,178, 2,285) | 1,764 (1,207, 2,004) | 1,841 (1,401, 2,434) | 1,495 (1,328, 1,953) |
| Glycoursodeoxycholic Acid | 6,989 (2,955, 13,254) | 5,465 (3,009, 7,502) | 6,055 (3,379, 10,560) | 3,696 (2,266, 5,939) |
| Hyocholic Acid | 644 (505, 1,151) | 573 (345, 911) | 489 (189, 923) | 544 (266, 762) |
| Hyodeoxycholic Acid | 4,434 (2,833, 7,326) | 3,483 (2,353, 5,433) | 3,811 (2,275, 6,710) | 2,745 (2,039, 3,450) |
| Isolithocholic Acid | 2,783 (1,796, 4,445) | 2,844 (2,186, 4,323) | 3,603 (1,807, 4,479) | 3,328 (1,823, 5,068) |
| Lithocholic Acid | 2,158 (1,579, 2,534) | 2,040 (1,322, 2,519) | 2,540 (1,522, 3,454) | 1,979 (1,220, 2,994) |
| Murocholic Acid | 8,351 (5,434, 19,814) | 8,153 (5,577, 13,156) | 11,379 (5,569, 16,611) | 7,254 (4,959, 10,828) |
| Tauro alpha Muricholic Acid | 600 (334, 1,006) | 763 (413, 1,289) | 544 (336, 990) | 730 (299, 1,374) |
| Tauro omega Muricholic Acid | 295 (124, 508) | 231 (66, 408) | 273 (125, 613) | 330 (153, 588) |
| Tauro ursodeoxycholic Acid | 667 (483, 1,544) | 688 (385, 1,042) | 1,070 (537, 2,120) | 675 (446, 1,064) |
| Taurochenodeoxycholic Acid | 6,846 (4,863, 14,023) | 8,086 (4,012, 12,807) | 9,529 (5,135, 20,383) | 8,780 (3,814, 13,837) |
| Taurocholic Acid | 4,731 (3,632, 9,951) | 4,775 (3,298, 8,568) | 9,761 (4,563, 17,271) | 10,366 (5,129, 13,857) |
| Taurodeoxycholic Acid | 4,105 (2,623, 6,568) | 4,242 (1,708, 7,500) | 6,055 (2,718, 10,159) | 4,956 (1,923, 9,417) |
| Taurohyocholic Acid | 285 (149, 527) | 252 (87, 489) | 259 (135, 551) | 311 (158, 579) |
| Taurohyodeoxycholic Acid | 891 (672, 2,123) | 822 (523, 1,433) | 970 (542, 2,083) | 821 (556, 1,275) |
| Taurolithocholic Acid | 507 (320, 899) | 676 (253, 920) | 875 (538, 1,498) | 1,173 (476, 1,845) |
| Ursodeoxycholic Acid | 30,053 (12,077, 41,668) | 26,156 (15,093, 39,633) | 24,148 (15,259, 44,977) | 20,522 (8,706, 34,526) |
| Glycohyocholic Acid | 1,963 (1,122, 3,540) | 2,061 (1,070, 3,691) | 1,848 (1,041, 3,591) | 2,050 (1,005, 3,332) |

\* 4 observations missing in ENL have been removed

**Table 3: Estimated coefficients, 95% CIs and p-values, and BH adjusted p-values**

**from linear mixed model**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Coefficient on ENL | 95% CI | p-value | Adjusted p-value |
| Deoxycholic Acid | -0.094 | (-0.165, -0.024) | 0.01 | 0.137 |
| Ursodeoxycholic Acid | -0.102 | (-0.178, -0.025) | 0.011 | 0.137 |
| Murocholic Acid | 0.05 | (0.001, 0.099) | 0.048 | 0.4 |
| Glycodeoxycholic Acid | -0.062 | (-0.144, 0.02) | 0.139 | 0.694 |
| Hyocholic Acid | -0.057 | (-0.139, 0.024) | 0.168 | 0.694 |
| Taurodeoxycholic Acid | -0.052 | (-0.133, 0.028) | 0.203 | 0.694 |
| Glycohyodeoxycholic Acid | 0.046 | (-0.024, 0.116) | 0.204 | 0.694 |
| Hyodeoxycholic Acid | -0.037 | (-0.098, 0.024) | 0.238 | 0.694 |
| Lithocholic Acid | -0.033 | (-0.089, 0.023) | 0.25 | 0.694 |
| Glycohyocholic Acid | 0.027 | (-0.025, 0.08) | 0.308 | 0.716 |
| Chenodeoxycholic Acid | -0.049 | (-0.145, 0.046) | 0.315 | 0.716 |
| Taurohyodeoxycholic Acid | -0.03 | (-0.098, 0.039) | 0.399 | 0.811 |
| Taurolithocholic Acid | -0.023 | (-0.082, 0.036) | 0.45 | 0.811 |
| Cholic Acid | -0.03 | (-0.11, 0.049) | 0.454 | 0.811 |
| Taurochenodeoxycholic Acid | 0.027 | (-0.053, 0.108) | 0.504 | 0.84 |
| Glycochenodeoxycholic Acid | 0.018 | (-0.046, 0.081) | 0.587 | 0.917 |
| Taurohyocholic Acid | 0.022 | (-0.067, 0.111) | 0.631 | 0.928 |
| Glycoursodeoxycholic Acid | 0.009 | (-0.057, 0.075) | 0.785 | 0.992 |
| Glycolithocholic Acid | -0.004 | (-0.039, 0.03) | 0.815 | 0.992 |
| Glycocholic Acid | -0.008 | (-0.077, 0.062) | 0.83 | 0.992 |
| Isolithocholic Acid | -0.007 | (-0.073, 0.06) | 0.845 | 0.992 |
| Taurocholic Acid | -0.005 | (-0.068, 0.058) | 0.877 | 0.992 |
| Tauro omega Muricholic Acid | 0.004 | (-0.075, 0.082) | 0.929 | 0.992 |
| Tauro ursodeoxycholic Acid | -0.002 | (-0.069, 0.066) | 0.961 | 0.992 |
| Tauro alpha.Muricholic Acid | 0 | (-0.067, 0.066) | 0.992 | 0.992 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Bile Acid | Coefficient on END | 95% CI | p-value | Adjusted p-value |
| Taurocholic Acid | -0.05 | (-0.089, -0.011) | 0.014 | 0.35 |
| Glycocholic Acid | -0.04 | (-0.085, 0.004) | 0.079 | 0.625 |
| Taurodeoxycholic Acid | -0.047 | (-0.099 , 0.005) | 0.08 | 0.625 |
| Lithocholic Acid | -0.031 | (-0.068, 0.006) | 0.1 | 0.625 |
| Taurochenodeoxycholic Acid | -0.033 | (-0.086, 0.019) | 0.211 | 0.853 |
| Hyodeoxycholic Acid | 0.024 | (-0.015, 0.064) | 0.227 | 0.853 |
| Glycodeoxycholic Acid | -0.032 | (-0.085, 0.022) | 0.249 | 0.853 |
| Tauro.omega.Muricholic Acid | -0.028 | (-0.079, 0.023) | 0.284 | 0.853 |
| Taurohyocholic Acid | -0.03 | (-0.088, 0.028) | 0.314 | 0.853 |
| Isolithocholic Acid | -0.021 | (-0.065, 0.022) | 0.341 | 0.853 |
| Glycohyodeoxycholic Acid | -0.018 | (-0.064, 0.028) | 0.441 | 0.873 |
| Taurolithocholic Acid | -0.014 | (-0.053, 0.025) | 0.486 | 0.873 |
| Chenodeoxycholic Acid | 0.021 | (-0.042, 0.083) | 0.517 | 0.873 |
| Glycoursodeoxycholic Acid | -0.013 | (-0.056, 0.03) | 0.548 | 0.873 |
| Glycohyocholic Acid | -0.009 | (-0.044, 0.025) | 0.592 | 0.873 |
| Tauro.ursodeoxycholic Acid | -0.011 | (-0.055, 0.034) | 0.639 | 0.873 |
| Tauro.alpha.Muricholic Acid | -0.009 | (-0.052, 0.034) | 0.688 | 0.873 |
| Glycolithocholic Acid | -0.004 | (-0.027, 0.018) | 0.714 | 0.873 |
| Cholic Acid | 0.01 | (-0.042, 0.061) | 0.716 | 0.873 |
| Glycochenodeoxycholic Acid | -0.006 | (-0.048, 0.035) | 0.771 | 0.873 |
| Deoxycholic Acid | -0.006 | (-0.054, 0.041) | 0.798 | 0.873 |
| Taurohyodeoxycholic Acid | -0.005 | (-0.05, 0.039) | 0.818 | 0.873 |
| Hyocholic Acid | -0.006 | (-0.06, 0.047) | 0.824 | 0.873 |
| Murocholic Acid | -0.003 | (-0.036, 0.029) | 0.838 | 0.873 |
| Ursodeoxycholic Acid | -0.004 | (-0.055, 0.048) | 0.884 | 0.884 |

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**Code Appendix**

bile\_acid <- read.csv("~/Downloads/bile acid.csv")

bile\_acid$period\_0first\_1second

# bile\_acid$age

# bile\_acid$race

# bile\_acid$female\_0no\_1yes

# bile\_acid$diet\_seq\_0AB\_1BA

# which(is.na(bile\_acid$endmol))

# bile\_acid$enlmol

which(colnames(bile\_acid)=="period\_0first\_1second")

library(dplyr)

data <- bile\_acid[c(1,5,4,6,7,121,19,20,43:67)]

#period 1

dietA = data %>% filter(diet\_0A\_1B == 0)

dietA = dietA[-2]

#period 2

dietB = data %>% filter(diet\_0A\_1B ==1)

dietB = dietB[-2]

#update dietA data

tertile\_limits\_end <- quantile(dietA$endmol, seq(0, 1, 1/2), na.rm = TRUE)

tertile\_limits\_enl <- quantile(dietA$enlmol, seq(0, 1, 1/2), na.rm = TRUE)

dietA$end.Tertiles <- cut(dietA$endmol, tertile\_limits\_end, c('Low', 'High'), include.lowest = TRUE)

dietA$enl.Tertiles <- cut(dietA$enlmol, tertile\_limits\_enl, c('Low', 'High'), include.lowest = TRUE)

#update dietB data

tertile\_limits\_end <- quantile(dietB$endmol, seq(0, 1, 1/2), na.rm = TRUE)

tertile\_limits\_enl <- quantile(dietB$enlmol, seq(0, 1, 1/2), na.rm = TRUE)

dietB$end.Tertiles <- cut(dietB$endmol, tertile\_limits\_end, c('Low', 'High'), include.lowest = TRUE)

dietB$enl.Tertiles <- cut(dietB$enlmol, tertile\_limits\_enl, c('Low', 'High'), include.lowest = TRUE)

#mean\_outcome = data %>% group\_by(PptID) %>% summarise(mean\_enl = mean(enlmol),mean\_end = mean(endmol))

#data %>% inner\_join(mean\_outcome, by = "PptID") %>% distinct(.)

library(gtsummary)

#dietA 5 missing

which(colnames(dietA)=="enl.Tertiles")

table1 <- tbl\_summary(dietA[-c(1:7,34)],by=end.Tertiles) #end table

table1

table2 <- tbl\_summary(dietA[-c(1:7,33)],by=enl.Tertiles) #enl table

table2

#dietB 4 missing

table4 <- tbl\_summary(dietB[-c(1:7,34)],by=end.Tertiles) #end table

table4

table5 <- tbl\_summary(dietB[-c(1:7,33)],by=enl.Tertiles) #enl table

table5

table3 <- tbl\_summary(dietA[c(2:5)],by=diet\_seq\_0AB\_1BA)#demographics end table

table3

#Code for running LMM

library(lme4)

library(lmerTest)

#fix the variable of race

bile\_acid$race.new[bile\_acid$race==5]<-'Caucasian'

bile\_acid$race.new[bile\_acid$race==4]<-"Black"

bile\_acid$race.new[bile\_acid$hispanic\_0No\_1Yes==1]<-"Hispanic/Latino"

bile\_acid$race.new[bile\_acid$race==3]<-"Asian"

bile\_acid$race.new[is.na(bile\_acid$race.new)]<-"Other"

bile\_acid<- bile\_acid[,-c(7, 8)]

acid<- bile\_acid%>%rename(race=race.new)

acid$enlmol<-log(acid$enlmol)

acid$endmol<-log(acid$endmol)

acid[,43:67]<-log(acid[,43:67])

acid<-acid%>%rename(female=female\_0no\_1yes)

acid<-acid%>%rename(dietB=diet\_0A\_1B)

#Bile acids and ENL

pvalue <- NA

coef <- NA

CI.left<-NA

CI.right<-NA

BHp <- NA

output <- matrix(NA, nrow=25, ncol=6,)

for (i in 43:67){

outcome = colnames(acid)[i]

y <- acid[, i]

lmm <- lmer(y ~ enlmol+age+race+female+dietB+(1|PptID), data=acid)

my\_summary <- summary(lmm)$coefficients

coef[i - 42] <- my\_summary["enlmol",1]%>%round(3)

CI.left[i - 42] <- round(my\_summary["enlmol",1]-1.96\*my\_summary["enlmol",2],3)

CI.right[i - 42] <- round(my\_summary["enlmol",1]+1.96\*my\_summary["enlmol",2],3)

pvalue[i - 42] <- my\_summary["enlmol",5]%>%round(3)

output[i - 42, 1] <- outcome

output[i - 42, 2] <- coef[i - 42]

output[i - 42, 3] <- CI.left[i - 42]

output[i - 42, 4] <- CI.right[i - 42]

output[i - 42, 5] <- pvalue[i - 42]

}

output<-as.data.frame(output)

colnames(output)<-c("Bile Acid", "Coefficient on ENL", "95% CI left", "95% CI right","p-value", "BHp")

output$BHp <- round(p.adjust(output$`p-value`, "BH"), 3)

output<-arrange(output, `p-value`)

write\_csv(output, file="Bile Acid and ENL.csv")

#Bile acids and END

pvalue <- NA

coef <- NA

CI.left<-NA

CI.right<-NA

BHp <- NA

output <- matrix(NA, nrow=25, ncol=6,)

for (i in 43:67){

outcome = colnames(acid)[i]

y <- acid[, i]

lmm <- lmer(y ~ endmol+age+race+female+dietB+(1|PptID), data=acid)

my\_summary <- summary(lmm)$coefficients

coef[i - 42] <- my\_summary["endmol",1]%>%round(3)

CI.left[i - 42] <- round(my\_summary["endmol",1]-1.96\*my\_summary["endmol",2],3)

CI.right[i - 42] <- round(my\_summary["endmol",1]+1.96\*my\_summary["endmol",2],3)

pvalue[i - 42] <- my\_summary["endmol",5]%>%round(3)

output[i - 42, 1] <- outcome

output[i - 42, 2] <- coef[i - 42]

output[i - 42, 3] <- CI.left[i - 42]

output[i - 42, 4] <- CI.right[i - 42]

output[i - 42, 5] <- pvalue[i - 42]

}

output<-as.data.frame(output)

colnames(output)<-c("Bile Acid", "Coefficient on END", "95% CI left", "95% CI right", "p-value", "BHp")

output$BHp <- round(p.adjust(output$`p-value`, "BH"), 3)

output<-arrange(output, `p-value`)

write\_csv(output, file="Bile Acid and END.csv")