Gut and Serum Metabolites and Alzheimer's: Exploring the Link Between Metabolite Levels and the Risk of Developing Alzheimer's

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***Abstract*—Alzheimer's disease is a fatal neurodegenerative disorder marked by inflammation and gradual cognitive decline. Recent research has revealed that gut microbiome affect the functioning of the brain, while at the same time, disease, and inflammation can also alter the gut microbiota. The composition, and function patterns of the gut microbiome was also found to be varied between the sexes during late adulthood, with it being mainly determined by biological sex. Analyzing the gut and serum metabolite data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database, this exploratory analysis indicated that several gut bacteria-modulated metabolites are sex-dependent even at late adulthood stage. Moreover, several gut metabolites highlight possible correlation with the risk of developing Alzheimer's disease, prior to symptom development. As the demographic distribution of subjects with normal baseline who later developed cognitive impairment was low, the results should be interpreted cautiously. This work has implications in the predictive modeling of Alzheimer's risk.**

Keywords—Alzheimer's disease, gut metabolites, serum metabolites, ADNI

I Introduction

Alzheimer's disease is a neurodegenerative disease threatening elderly health and life quality [1]–[4]. The defining features of Alzheimer disease (AD) include neuroinflammation and gradual cognitive decline [2], [3]. The behavioral symptoms of AD correlate with the accumulation of plaques and tangles, and they are a direct consequence of the damage and destruction of synapses that mediate memory and cognition [2].

Recent research showed evidence that the gut microbiota is involved in the pathogenesis of Alzheimer's disease [3], [5], [6] via a constant bidirectional communication through the gut-brain axis [3], [7], [8]. The gut microbiota consists of a commensal grouping of microbes that inhabit the gastro-intestinal tract [3]. Various studies show that intestinal microbes affect the functioning of the brain, such as learning, and memory functions [1], [3], [8], [9]. Changes in the gut microbiota, collectively known as dysbiosis, was found to increase the permeability of the intestine, alter the permeability of the blood-brain barrier, and elevate proinflammatory mediators causing neurodegeneration, also associated with Alzheimer's disease [3], [7], [10]–[14]. In addition, gut flora-regulated metabolites were identified in cerebrospinal fluid, showing correlation with AD [15]. Furthermore, metabolic syndrome and associated serum metabolites such as lipoproteins, lipid molecules, fatty acids, and glycoproteins also feature an association with the risk of Alzheimer's [16]–[20].

In addition to gut and serum metabolites, current literature highlights some differences in the microbiota between sexes during adulthood [21]. These differences are said to be minimized during late adulthood (after menopause), where the gut microbiota taxonomy, and functionality present in women is more similar to men [21]. On the other hand, research show that there are equally notable alterations in aging populations that likely impact signalling between the gut microbiome and brain [22]. Age-related changes to gut microbes has been reported in relation to measures of frailty, nutritional status, metabolic profiles, and markers of inflammation [22]–[24]. Moreover, the composition and function patterns of the gut microbiome was also found to be varied between the sexes during late adulthood [25], [26] , with male gut microbiome exhibiting greater resistance to oxidative stress compared to females, while the species associated with healthy aging dominated among healthy females [26]. Research also consider that factors driving female-male differences in the microbiota seem to be mainly determined by biological sex [21], [27] Hence, association with sex should be considered for studies concerning gut metabolites and disease [23], [25].

Early risk detection of Alzheimer's from when subjects are still cognitively normal could improve prognosis and intervention strategies. Successful therapeutic intervention would benefit from early likelihood detection before cognitive impairment become evident [2].

This study aimed to explore the link between the gut and serum metabolites, and the risk of developing Alzheimer's disease. Specifically, it aimed to determine if there are specific microbe-mediated metabolite that can be used as predictors in the mathematical modelling of the likelihood of getting Alzheimer's disease. In addition, possible sex-based interaction with the metabolite levels was also evaluated.

II Data and Methods

II.A Data Sources

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD) [28].

- *Human participant data*

  The demographic data was obtained from the adnimerge dataset. This table merges together several of the key variables from various case report forms and biomarker lab summaries across the ADNI protocols (ADNI1 , ADNIGO, and ADNI2) [29].

- *Gut microbial metabolite data*

  The data was obtained from the ADMC U Hawaii UPLC-MS/MS Gut Metabolites Serum Longitudinal [ADNI1,GO,2] dataset. A panel of 104 representative gut microbial metabolites including bile acids in human serum samples was measured using both ultra-performance liquid chromatography coupled with mass spectrometry (UPLC-MS/MS) [3].

- *Serum metabolite data*

  The data was from the ADNINIGHTINGALELONG, and the ADNINIGHTINGALE2 datasets of the ADNI database (adni.loni.usc.edu). Blood biomarkers were analyzed using Nuclear Magnetic Resonance (NMR) assay based on the methods developed by the Nightingale Health. The assay quantified 250 metabolic biomarkers, covering biomarkers multiple biological pathways including lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and various low-molecular metabolites including amino acids, ketone bodies and gluconeogenesis-related metabolites from the ADNI 1/GO/2 cohorts' human serum samples [31], [32].

II.B Data Processing

The metabolites dataset were merged with the corresponding patient baseline diagnoses, gender, and age. The duplicate patient entries were imputed with the mean of the corresponding metabolite values. Moreover, the metabolite data was screened to include only the patient entries with a normal baseline diagnosis. This was done with the aim of finding metabolites that can be used as a predictor of Alzheimer's when the patients are still of normal cognitive performance. All analyses were done using Pandas [33], Matplotlib [34] , Statsmodels [35] in Python 3.11.4.

II.C Data Exploration

The patients are classified based on their corresponding diagnoses at the end of the longitudinal study. In order to determine if there are possible Alzheimer's predictors out of the quantified gut and serum metabolites, the histogram for each metabolite, were visually analyzed, to see if there are distinct data clusters based on the end diagoses of the patients. These distinct data clusters are important because distinct separation lead to a more defined decision boundary, which then minimizes the classification errors in the predictive model [36]. All analyses were done using Pandas [33], Matplotlib [34] , Statsmodels [35], Seaborn [37], and Plotly [38] in Python 3.11.4.

II.D Statistical Analysis

The sex-dependency of the metabolite values were analyzed with one-way analysis of variance (ANOVA) at $\alpha = 0.05$ to see if there exists a statistically significant difference in the mean metabolite values between male and female patients. This was performed using the metabolite data from the patients that were normal at baseline and did not develop any cognitive impairment at the end of the longitudinal study. This was designed in order to exclude bidirectional interactions of health (diagnosis) on the gut metabolite levels, and look solely on the sex-dependency of the metabolite levels on normal healthy subjects. The difference between groups was also quantified using the Cohen's d.

To supplement the data exploration results, each metabolite was tested with one-way analysis of variance (ANOVA) at $\alpha = 0.05$ to see if there exists a statistically significant difference in the mean metabolite values between the (normal baseline) patients that stayed normal, developed MCI, and those that were finally diagnosed with AD at the end of the longitudinal study. If the metabolite showed statistically significant differences between sex groups, the ANOVA was done for corresponding sex. The Bonferroni correction ($\alpha = 0.0167$) was also applied as a post hoc adjustment in order to control Type I error when comparing multiple groups. The difference between groups was also quantified using the Cohen's d.

All analyses were done using Pandas [33], Matplotlib [34], Statsmodels [35], Numpy [39], and Seaborn [37] in Python 3.11.4.

III Results

III.A Lack of Longitudinal Data

Fig. 1 provides the patient demographics for the gut metabolites from the ADNI database. There were 352 patients that started with a healthy baseline, of which, 171 (48.6 %) are female, and 181 (51.4 %) are male. Out of the female patients, 23 (13 %) developed MCI and 8 (5 %) developed AD, whereas in male patients, 35 (19 %) developed MCI, and 9 (5 %) developed AD at the end of the longitudinal study.

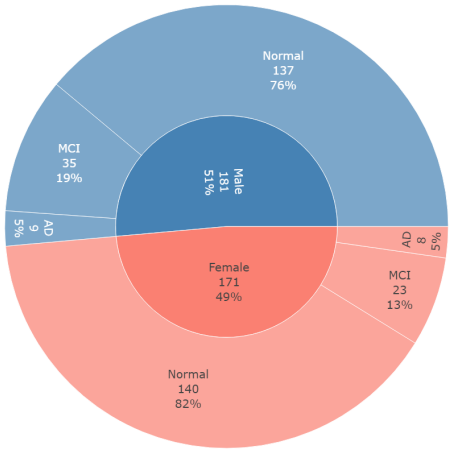

Fig. 1 Distribution of subjects who started normal at baseline in the gut metabolites data.

The patient demographics for the serum metabolites from the ADNI database is provided in Fig. 2. There were 458 patients that started with a normal baseline, of which, 233 (50.9 %) are female, and 225 (49.1 %) are male. Out of the female patients, 26 (11 %) developed MCI and 8 (3 %) developed AD, whereas 43 (19 %) male patients developed MCI, and 10 male patients (4 %) developed AD at the end of the longitudinal study.

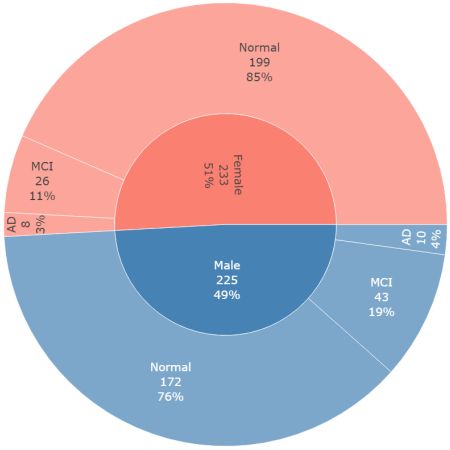

Fig. 2 Distribution of subjects who started normal at baseline in the serum metabolites data.

III.B Sex-dependent Gut Metabolites

The gut metabolites that showed statistically significant difference are shown in Table I. The test statistic for other gut metabolites are tabulated in Appendix A.

TABLE I GUT METABOLITES THAT SHOWED STATISTICALLY SIGNIFICANT DIFFERENCE IN THE MEAN AT BASELINE BETWEEN MALE (N = 137) AND FEMALE (N = 140) PATIENTS THAT DID NOT DEVELOP ANY TYPE OF COGNITIVE DISORDER.

Metabolite	p-value α= 0.05	Cohen's d	Group with higher mean
3-Hydroxybutyric Acid (C4H8O3)	0.0488	-0.2108	Female
Pelargonic Acid (C8H1802)	0.0300	-0.2323	Female
Decanoic Acid (C10H20O2)	0.0006	-0.3684	Female
Dodecanoic Acid (C12H24O2)	0.0074	-0.2873	Female
Tridecanoic Acid (C13H26O2)	0.0038	-0.3112	Female
Myristic Acid (C14H28O2)	0.0000	-0.8233	Female
Pentadecanoic Acid (C15H30O2)	0.0000	-0.4531	Female
Palmitic Acid (C16H32O2)	0.0001	-0.4120	Female
Palmitoleic Acid (C16H30O2)	0.0000	-1.0869	Female
Heptadecanoic Acid (C17H34O2)	0.0036	-0.3127	Female
10Z-Heptadecenoic Acid (C17H31O2)	0.0000	-0.8600	Female
Stearic Acid (C18H36O2)	0.0030	-0.3190	Female
Oleic Acid (C18H34O2)	0.0000	-0.7098	Female
Linoleic Acid (C18H32O2)	0.0000	-0.6577	Female
Gamma-Linolenic Acid (C18H30O2)	0.0000	-0.8388	Female
Alpha-Linolenic Acid (C18H30O2)	0.0000	-0.7452	Female
10,13-Nonadecadienoic Acid (C18H34O)	0.0001	-0.4347	Female
Eicosadienoic Acid (C20H36O2)	0.0000	-0.5231	Female
8,11,14-Eicosatrienoic Acid (C20H34O2)	0.0002	-0.4069	Female
Arachidonic Acid (C20H32O2)	0.0000	-0.4436	Female
Eicosapentaenoic Acid (C20H30O2)	0.0023	-0.3275	Female
Docosahexaenoic Acid (C22H32O2)	0.0000	-0.4386	Female
Docosatetraenoic Acid (C22H36O2)	0.0000	-0.5949	Female
Docosapentaenoic Acid (C22H34O2)	0.0000	-0.5309	Female
Docosapentaenoic Acid (C22H34O2)	0.0000	-0.6171	Female
Beta Alanine	0.0000	0.7216	Male
L-Aspartic Acid	0.0361	-0.2243	Female
L-Proline	0.0000	0.5622	Male
L-Valine	0.0000	0.4453	Male
L-Methionine	0.0000	0.5354	Male
L-Leucine	0.0000	0.4937	Male
L-Tryptophan	0.0011	0.3514	Male
3-Hydroxyisovaleric Acid	0.0004	0.3806	Male
Indoleacetic Acid	0.0033	0.3151	Male
3-Methyl-2-oxovaleric Acid	0.0000	0.4845	Male
Cholic Acid	0.0030	0.3192	Male
Chenodeoxycholic Acid	0.0016	0.3400	Male
Muricholic Acid/Hyocholic Acid	0.0020	0.3320	Male
Isolithocholic Acid	0.0090	0.2800	Male
Murocholic Acid	0.0129	0.2665	Male
Norcholic Acid	0.0234	0.2428	Male
Cis-Aconitic Acid	0.0407	-0.2471	Female
Glycochodeoxycholic Acid	0.0327	0.2579	Male
Apocholic Acid	0.0234	-0.2428	Female
Lithocholic Acid 3-Sulfate	0.0040	0.3089	Male

III.C Sex-dependent Serum Metabolites

TABLE II SERUM METABOLITES THAT SHOWED STATISTICALLY SIGNIFICANT DIFFERENCE IN THE MEAN AT BASELINE BETWEEN MALE (N = 172) AND FEMALE (N = 199) PATIENTS THAT DID NOT DEVELOP ANY COGNITIVE IMPAIRMENT.

Metabolite	p-value α= 0.05	Cohen's d	Group with higher mean
Total Cholesterol	0.0000	-0.6733	Female
Non-HDL Cholesterol	0.0000	-0.4335	Female
Remnant cholesterol; non-HDL, non-LDL - cholesterol	0.0000	-0.4352	Female
Clinical LDL Cholesterol	0.0001	-0.4060	Female
Cholesterol in LDL	0.0001	-0.4104	Female
Cholesterol in HDL	0.0000	-0.7732	Female
Triglycerides in LDL particles	0.0003	-0.3783	Female
Triglycerides in HDL particles	0.0011	-0.3419	Female
Total Phospholipids	0.0000	-0.8719	Female
Phospholipids in LDL particles	0.0003	-0.3845	Female
Phospholipids in HDL particles	0.0000	-0.8901	Female
Total Cholesteryl Esters	0.0000	-0.6945	Female
Cholesteryl Esters in HDL particles	0.0264	-0.2321	Female
Cholesteryl Esters in HDL particles	0.0001	-0.3990	Female
Cholesteryl Esters in HDL particles	0.0000	-0.7568	Female
Total Free Cholesterol	0.0000	-0.5994	Female
Free Cholesterol in LDL particles	0.0001	-0.4250	Female
Free Cholesterol in HDL particles	0.0000	-0.8104	Female
Total Lipids	0.0000	-0.6690	Female
Lipids in LDL particles	0.0001	-0.4153	Female
Lipids in HDL particles	0.0000	-0.8641	Female
Total Lipoprotein Particle Concentrations	0.0000	-0.8939	Female
Very Low Density Lipoprotein Particles	0.0459	-0.2085	Female
Low Density Lipoprotein Particles	0.0072	-0.2815	Female
High Density Lipoprotein Particles	0.0000	-0.8827	Female
Size of VLDL	0.0382	0.2166	Male
Size of LDL	0.0013	-0.3363	Female
Size of HDL	0.0000	-0.5332	Female
Apolipoprotein B	0.0026	-0.3157	Female
Apolipoprotein A-1	0.0000	-0.8984	Female
Ratio of Apolipoprotein B to A-1	0.0067	0.2841	Male
Phosphoglycerides	0.0000	-0.9667	Female
Ratio of Triglycerides to Phosphoglycerides	0.0088	0.2742	Male
Cholines	0.0000	-0.9616	Female
Phosphatidylcholines	0.0000	-0.9443	Female
Sphingomyelins	0.0000	-0.8381	Female
Total Fatty Acids	0.0000	-0.6459	Female
Estimated Degree of Unsaturation	0.0000	-0.4546	Female
Omega-3 Fatty Acids	0.0000	-0.5970	Female
Omega-6 Fatty Acids	0.0000	-0.7159	Female
Polyunsaturated Fatty Acids (mmol/l)	0.0000	-0.7664	Female
Monounsaturated Fatty Acids; 16:1, 18:1	0.0000	-0.4471	Female
Saturated Fatty Acids (mmol/l)	0.0000	-0.5967	Female
18:2, Linoleic Acid (mmol/l)	0.0000	-0.5547	Female
22:6, Docosahexaenoic Acid (mmol/l)	0.0000	-0.6803	Female
Glutamine (mmol/l)	0.0227	0.2382	Male
Glycine (mmol/l)	0.0000	-0.5507	Female
Histidine (mmol/l)	0.0049	0.2950	Male
Total Branched-Chain Amino Acids	0.0000	0.6596	Male
Isoleucine	0.0000	0.8205	Male
Leucine	0.0000	0.5849	Male
Valine	0.0000	0.5455	Male
Tyrosine	0.0114	0.2646	Male
Glucose	0.0150	0.2545	Male
GlycA Inflammatory Biomarker	0.0002	-0.3951	Female
Total cholesterol in medium VLDL	0.0073	-0.2811	Female
Free cholesterol in medium VLDL	0.0276	-0.2303	Female
Free cholesterol in small VLDL	0.0232	-0.2374	Female
Concentration of very small VLDL particles	0.0000	-0.4601	Female
Total lipids in very small VLDL	0.0000	-0.4533	Female
Phospholipids in very small VLDL	0.0002	-0.3983	Female
Total cholesterol in very small VLDL	0.0000	-0.4935	Female
Cholesterol esters in very small VLDL	0.0000	-0.5035	Female
Free cholesterol in very small VLDL	0.0000	-0.4324	Female
Triglycerides in very small VLDL	0.0170	-0.2496	Female

Metabolite	p-value α= 0.05	Cohen's d	Group with higher mean
Concentration of IDL particles	0.0000	-0.4544	Female
Total lipids in IDL	0.0000	-0.6014	Female
Phospholipids in IDL	0.0000	-0.5615	Female
Total cholesterol in IDL	0.0000	-0.5854	Female
Cholesterol esters in IDL	0.0000	-0.5833	Female
Free cholesterol in IDL	0.0000	-0.5781	Female
Triglycerides in IDL	0.0001	-0.4191	Female
Concentration of large LDL particles	0.0014	-0.3355	Female
Total lipids in large LDL	0.0000	-0.4866	Female
Phospholipids in large LDL	0.0000	-0.4446	Female
Total cholesterol in large LDL	0.0000	-0.4802	Female
Cholesterol esters in large LDL	0.0000	-0.4702	Female
Free cholesterol in large LDL	0.0000	-0.4945	Female
Triglycerides in large LDL	0.0000	-0.4514	Female
Total lipids in medium LDL particles	0.0070	-0.2825	Female
Phospholipids in medium LDL particles	0.0038	-0.3030	Female
Total cholesterol in medium LDL particles	0.0114	-0.2647	Female
Cholesterol esters in medium LDL particles	0.0236	-0.2367	Female
Free cholesterol in medium LDL particles	0.0021	-0.3225	Female
Triglycerides in medium LDL particles	0.0042	-0.2997	Female
Concentration of small LDL particles	0.0205	-0.2422	Female
Total lipids in small LDL particles	0.0109	-0.2664	Female
Phospholipids in small LDL particles	0.0178	-0.2478	Female
Total cholesterol in small LDL particles	0.0103	-0.2686	Female
Cholesterol esters in small LDL particles	0.0073	-0.2807	Female
Free cholesterol in small LDL particles	0.0407	-0.2138	Female
Concentration of very large HDL particles	0.0000	-0.4532	Female
Total lipids in very large HDL particles	0.0001	-0.4139	Female
Phospholipids in very large HDL particles	0.0001	-0.4083	Female
Total cholesterol in very large HDL particles	0.0002	-0.3972	Female
Cholesterol esters in very large HDL particles	0.0000	-0.4348	Female
Free cholesterol in very large HDL particles	0.0188	-0.2457	Female
Triglycerides in very large HDL particles	0.0001	-0.4011	Female
Concentration of large HDL particles	0.0000	-0.6181	Female
Total lipids in large HDL particles	0.0000	-0.6254	Female
(Phospholipids in large HDL particles	0.0000	-0.6572	Female
Total cholesterol in large HDL particles	0.0000	-0.5723	Female
Cholesterol esters in large HDL particles	0.0000	-0.5663	Female
Free cholesterol in large HDL particles	0.0000	-0.5890	Female
Triglycerides in large HDL particles (	0.0000	-0.5731	Female
Concentration of medium HDL particles	0.0000	-0.8921	Female
Total lipids in medium HDL particles	0.0000	-0.9104	Female
Phospholipids in medium HDL particles	0.0000	-0.9257	Female
Total cholesterol in medium HDL particles	0.0000	-0.8426	Female
Cholesterol esters in medium HDL particles	0.0000	-0.8248	Female
Free cholesterol in medium HDL particles	0.0000	-0.8963	Female
Triglycerides in medium HDL particles	0.0005	-0.3683	Female
Concentration of small HDL particles	0.0000	-0.5694	Female
Total lipids in small HDL particles	0.0000	-0.6660	Female
Phospholipids in small HDL particles	0.0000	-0.7318	Female
Total cholesterol in small HDL particles	0.0000	-0.5919	Female
Cholesterol esters in small HDL particles	0.0000	-0.5066	Female
Free cholesterol in small HDL particles	0.0000	-0.7834	Female

The test statistic for other serum Metabolites are shown in Appendix B.

III.D Gut and Serum Metabolites showing possible relation with Alzheimer's disease

TABLE III GUT AND SERUM METABOLITES THAT SHOWED SIGNIFICANT DIFFERENCES AT BASELINE FOR FEMALE PATIENTS

Metabolite	End Diagnosis Comparison	Corrected p-value	η^2	Cohen's d
Glycine[a]	MCI vs AD	0.0033	0.0421	-1.4881
Indoleacetic Acid[a]	Normal vs AD	0.0085	0.0676	-1.1036
Allolithocholic Acid[a]	Normal vs AD	0.0005	0.0574	-1.3978
Monounsaturated Fatty Acids (%)[b]	Normal vs. MCI	0.0128	0.0382	0.6019

[a] Gut metabolite [b] Serum metabolite

TABLE IV GUT METABOLITES THAT SHOWED SIGNIFICANT DIFFERENCES AT BASELINE FOR MALE PATIENTS

Metabolite	End Diagnosis Comparison	Corrected p-value	η^2	Cohen's d
Caprylic Acid	Normal vs AD	0.0056	0.0667	-1.0914
Hippuric Acid	Normal vs AD	0.0021	0.0346	-1.1928
Indoleacetic Acid	Normal vs MCI	0.0026	0.0653	-0.6432

Indoleacetic acid (p-value = 0.0085), allolithocholic acid (p-value = 0.0005) showed statistically significant difference in the baseline mean of female patients (n = 171) that stayed normal (n = 140) and those that developed AD (n = 8). Moreover, baseline glycine levels of those that developed MCI (n = 23) and those that developed AD also showed a significant difference (p-value = 0.0033). Furthermore, statistical analysis revealed a meaningful difference in the mean (p-value = 0.0128) of the baseline serum concentration of monounsaturated fatty acids in the female patients (n = 233) that developed MCI (n = 26) and those that did not develop the cognitive impairment (n = 199). There results are summarized in Table III.

In male patients (n = 181) there was a significant difference between mean values at the baseline levels of caprylic acid (p-value = 0.0056), and hippuric acid (p-value = 0.0021) between patients that developed AD, and those who stayed cognitively normal (Table IV). Additionally, indole acetic acid (p-value = 0.0026) at baseline showed statistically validated difference between normal patients and those that developed MCI.

When the whole cohort of normal baseline patients were analyzed without separating for sex, the baseline glycine metabolite level showed statistically significant difference (p-value = 0.0090) between patients that stayed normal and those who developed MCI.

IV DISCUSSION

The potential for early quantification of the risk of developing Alzheimer's disease is crucial for the elderly population. Currently diagosis involves the collection of cerebrospinal fluid (CSF) via a spinal tap where the levels of proteins associated with Alzheimer's and related dementias are measured [40]. Brain scans, such as computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography (PET), are also done to support an Alzheimer's diagnosis or rule out other possible causes for symptoms [40]. These tests are expensive, and are usually done when symptoms are apparent to the patient. Hence, it is of an advantage to find a good predictor of Alzheimer's risk, before signs of neurodegeneration appear.

In this study, the lack of longitudinal data on patients that had a normal baseline and later progressed to MCI or AD made it difficult to explore the relationship of gut and serum metabolites to the risk of developing neurodegenerative disease. In this regard, it is difficult to use the measurement values as possible predictor for the quantification of the likelihood of getting the disease in the future. Another hurdle in designing the predictive model is the lack of distinctive end diagnosis clusters at baseline. This could be attributed to the underrepresentation of patients who started without any cognitive impairment, and later on progressed to MCI or AD.

Several studies point out that the observed sex-differences in gut microbiota in the population of old adults to be more related to biological sex than sex hormones alone [25]–[27]. Moreover, the gut microbiome communicates

bidirectionally with the brain, termed as the microbiota-gut-brain axis, shown to likely contrubute to sex differeces in the susceptibility to neuropsychiatric conditions [27]. In this study, multiple gut and serum metabolites showed statistically significant difference in normal male and female patients. This shows that sex differences should be accounted for when considering metabolite levels in the prediction of the risk of developing AD.

Recent research also show increasing evidence on the role of bile acids in neuroinflammation, and stress response through bidirectional interactions within the gut-brain axis[3], [7], [8], [41]. Bile acids are the end products of human cholesterol metabolism, which is produced by human and gut microbiota [1], [41]–[43]. The presence of primary and secondary bile acid metabolites at the serum level were found to be related with amyloid proteins and tau in the cerebrospinal fluid of AD patients, as well as with brain atrophy and cerebral glucose metabolism dysfunction [1]. Bile acids can enter the systematic circulation, and cross the blood–brain barrier [7], [13], [41], [44]. The bile acid pool and composition is manipulated by the gut microbiota, being dependent on the metabolic capacity of the microbial flora, antibiotics, and diet [43], [45], [46]. Primary bile acids are metabolized by bacteria in the gut to secondary bile acids, such as deoxycholic acid (DCA), and lithocholic acid (LCA) [10], [43]. While specific studies on allolithocholic acid are limited, research has shown that alterations in bile acid profiles, including secondary bile acids like lithocholic acid, are associated with cognitive impairment in Alzheimer's disease [44], [47].

Other bacteria-modulated metabolites including hippurate and indoleacetate, the conjugate bases of hippuric acid, and indoleacetic acid, were also found to be detectable within the cerebrospinal fluid [15]. Indoleacetic acid is a gut microbial metabolite derived from tryptophan. Tryptophan metabolites were also shown to correlate with neurological disorders suggesting a connection between gut microbial metabolites, and cognitive impairment [4], [10], [13], [48]. Hippuric acid was also found to be statistically different in male patients that later developed MCI, and those that stayed normal. Hippuric acid is a metabolite resulting from the glycine conjugation of benzoic acid or from the gut bacterial metabolism of phenylalanine [49]. Hippuric acid levels has been proposed as an aging biomarker as serum hippuric acid level can be influenced by the presence of age-related conditions, including frailty, and cognitive impairment [49]. Another study within the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort also found that higher plasma levels of caprylic acid were inversely associated with the risk of developing MCI among cognitively normal individuals [50].

Glycine is the smallest nonessential amino acid, and has been associated with neurotherapeutic effects by inhibiting oxidative stress [51]. It has also been shown to influence synaptic plasticity, and cognitive functions [52]. In oxidative stress and neurotoxicity associated with AD, glycine residues within the amyloid betapeptide were found to be implicated [53]. Although the aforementioned studies suggest that glycine, and its interactions within the brain may play a role in Alzheimer's disease, there are not many studies describing a direct association of glycine with the risk of AD, warranting further research into its potential therapeutic applications in this field.

For serum metabolites, serum lipid levels has been associated with current AD biomarkers and AD clinical symtpoms [54]–[57]. Polyunsaturated fatty acids and docosahexaenoic acid (DHA) were found to be lower on both MCI and AD patients which suggest a role in Alzheimer's pathology [56]. Dietary intake of monounsaturated fatty acids was also shown to be associated with reduced risk of AD [56], [58] On the other hand, some found no significant association between serum monounsaturated fatty acids, and the risk of AD [55], [56]. Overall, while some studies suggest a protective role of monounsaturated fatty acids against cognitive decline and AD, others do not find a significant association. Additionally, the gut microbiome also show a biderectional interaction

In summary, it is difficult to attribute specific gut and serum metabolites to the risk of developing AD as the metabolic pathways are often overlapping, and a full understanding of how these metabolites interact with the host is still lacking [10]. The results also did not lead to specific metabolites that can consistently distinguish between all diagnostic groups, prior to symptom development. However, this data exploration showed interesting findings that could be looked at more closely in the future.

V Conclusion and Recommendation

This exploratory analysis indicated that several gut bacteria-modulated metabolites are sex-dependent even at late adulthood stage. Hence, it would be useful to factor in sex-differences when using metabolite levels in predicting the risk of AD. Moreover, several gut metabolites including allolithocholic acid, indoleacetic acid, hippuric acid, and glycine showed significant difference between end diagnostic groups, highlighting possible correlation with the risk of developing Alzheimer's diesease, prior to symptom development. As the demographic distribution of subjects with normal baseline and later developed cognitive impairment was low, the results should be interpreted cautiously. Additionally, the metabolite values were measured as the serum abundace, which may not be directly comparable to other research results which studied gut metabolites using the stool sample. Most published research also performed the measurements between diagnostic groups when the patients already exhibited cognitive decline, which does not elliminate the effects of altered gut microbiome as caused by inflammation, neurodegenerative disease, and frailty. Further research is needed in order to verify the suitability of specific serum metabolites in the predictive modelling of the likelihood of future Alzheimer's diagnosis.

Future investigation should focus on longitudinal studies which also cover the baseline values before symptoms of neurodegeneration occur, for patients who later developed cognitive impairment. This will increase the quality of the comparison between future diagnostic groups at baseline level, as well as the possibility of finding suitable predictors that can consistently be used for the predictive modelling of AD risk.

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REFERENCES

[1] Y. He, B. Li, D. Sun, and S. Chen, "Gut Microbiota: Implications in Alzheimer's Disease," *J. Clin. Med. 2020, Vol. 9, Page 2042*, vol. 9, no. 7, p. 2042, Jun. 2020, doi: 10.3390/JCM9072042.

[2] G. S. Bloom, "Amyloid-β and Tau: The Trigger and Bullet in Alzheimer Disease Pathogenesis," *JAMA Neurol.*, vol. 71, no. 4, pp. 505–508, Apr. 2014, doi: 10.1001/JAMANEUROL.2013.5847.

[3] V. Peddinti *et al.*, "Gut instincts: Unveiling the connection between gut microbiota and Alzheimer's disease," 2024, doi: 10.1016/j.clnesp.2024.02.019.

[4] L. Wu *et al.*, "Altered Gut Microbial Metabolites in Amnestic Mild Cognitive Impairment and Alzheimer's Disease: Signals in Host–Microbe Interplay," *Nutr. 2021, Vol. 13, Page 228*, vol. 13, no. 1, p. 228, Jan. 2021, doi: 10.3390/NU13010228.

[5] C. H. Chang, C. H. Lin, and H. Y. Lane, "d-glutamate and Gut Microbiota in Alzheimer's Disease," *Int. J. Mol. Sci.*, vol. 21, no. 8, Apr. 2020, doi: 10.3390/IJMS21082676.

[6] Y. Wang, "The Role of the Gut Microbiota and Microbial Metabolites in the Pathogenesis of Alzheimer's Disease," *CNS Neurol. Disord. Drug Targets*, vol. 22, no. 4, pp. 577–598, Apr. 2023, doi: 10.2174/1871527321666220417005115.

[7] V. V Giridharan, C. E. Barichello De Quevedo, and F. Petronilho, "Microbiota-gut-brain axis in the Alzheimer's disease pathology-an overview," *Neurosci. Res.*, vol. 181, pp. 17–21, 2022, doi: 10.1016/j.neures.2022.05.003.

[8] [R. Pluta, M. Ulamek-Koziol, S. Januszewski, and S. J. Czuczwar, "Gut microbiota and pro/prebiotics in Alzheimer's disease," *Aging (Albany. NY).*, vol. 12, no. 6, pp. 5539–5550, Mar. 2020, doi: 10.18632/AGING.102930.

[9] J. Dai *et al.*, "Gut Microbiota Changes are associated with abnormal metabolism activity in Children and Adolescents with Obsessive-compulsive Disorder," *J. Psychiatr. Res.*, 2024, doi: 10.1016/j.jpsychires.2024.12.041.

[10] M. Akbar, P. Toppo, and A. Nazir, "Ageing, proteostasis, and the gut: Insights into neurological health and disease," 2024, doi: 10.1016/j.arr.2024.102504.

[11] J. Zhang *et al.*, "including those for text and data mining, AI training, and similar technologies. NMN synbiotics intervention modulates gut microbiota and metabolism in APP/PS1 Alzheimer's disease mouse models," *Biochem. Biophys. Res. Commun.*, vol. 726, p. 150274, 2024, doi: 10.1016/j.bbrc.2024.150274.

[12] Y. Qiu *et al.*, "Systematic characterization of multi-omics landscape between gut microbial metabolites and GPCRome in Alzheimer's disease", doi: 10.1016/j.celrep.2024.114128.

[13] J. W. Kang, V. Vemuganti, J. F. Kuehn, T. K. Ulland, F. E. Rey, and B. B. Bendlin, "Gut microbial metabolism in Alzheimer's disease and related dementias," 2024, doi: 10.1016/j.neurot.2024.e00470.

[14] E. L. Paley, "Relation of human gut Alzheimer disease associated sequence (ADAS) with shikimate pathway metabolites," 2021, doi: 10.1016/B978-0-323-88445-7.00011-7.

[15] M. B. Heston *et al.*, "GUT MICROBIAL METABOLITES IN CEREBROSPINAL FLUID ARE ASSOCIATED WITH BIOMARKERS OF ALZHEIMER'S DISEASE PATHOLOGY," *Alzheimer's Dement.*, vol. 15, no. 7, pp. P673–P675, Jul. 2019, doi: 10.1016/J.JALZ.2019.06.2648.

[16] T. Tukiainen *et al.*, "A multi-metabolite analysis of serum by 1H NMR spectroscopy: Early systemic signs of Alzheimer's disease," *Biochem. Biophys. Res. Commun.*, vol. 375, no. 3, pp. 356–361, Oct. 2008, doi: 10.1016/J.BBRC.2008.08.007.

[17] N. Purandare *et al.*, "Serum Metabolite Biomarkers In Alzheimer's Disease," *Alzheimer's Dement.*, vol. 5, no. 4, p. e21, Jul. 2009, doi: 10.1016/J.JALZ.2009.07.089.

[18] Y. Gao, H. Zhang, and Y. Hu, "Baseline serum glutamate: Implications for diagnosis and prediction in mild cognitive impairment and Alzheimer's disease of the Alzheimer's Disease Neuroimaging Initiative," *J. Clin. Neurosci.*, vol. 129, p. 110828, Nov. 2024, doi: 10.1016/J.JOCN.2024.110828.

[19] B. A. Şanlı, K. J. Whittaker, G. K. Motsi, E. Shen, T. H. Julian, and J. Cooper-Knock, "Unbiased metabolome screen links serum urate to risk of Alzheimer's disease," *Neurobiol. Aging*, vol. 120, pp. 167–176, Dec. 2022, doi: 10.1016/J.NEUROBIOLAGING.2022.09.004.

[20] H. Wang *et al.*, "Association between serum total bilirubin and Alzheimer's disease: A bidirectional Mendelian randomization study," *Arch. Gerontol. Geriatr.*, vol. 103, p. 104786, Nov. 2022, doi: 10.1016/J.ARCHGER.2022.104786.

[21] Á. del Castillo-Izquierdo, J. Mayneris-Perxachs, and J. M. Fernández-Real, "Bidirectional relationships between the gut microbiome and sexual traits," *Am. J. Physiol. - Cell Physiol.*, vol. 322, no. 6, pp. C1223–C1229, Jun. 2022, doi: 10.1152/AJPCELL.00116.2022/ASSET/IMAGES/LARGE/AJPCELL.00116.2022_F001.JPEG.

[22] E. Jašarević, K. E. Morrison, and T. L. Bale, "Sex differences in the gut microbiome - Brain axis across the lifespan," *Philos. Trans. R. Soc. B Biol. Sci.*, vol. 371, no. 1688, Feb. 2016, doi: 10.1098/RSTB.2015.0122.

[23] K. Hatayama, K. Kono, K. Okuma, K. Hasuko, H. Masuyama, and Y. Benno, "Sex Differences in Intestinal Microbiota and Their Association with Some Diseases in a Japanese Population Observed by Analysis Using a Large Dataset," *Biomedicines*, vol. 11, no. 2, p. 376, Feb. 2023, doi: 10.3390/BIOMEDICINES11020376/S1.

[24] C. Peng *et al.*, "Sex-specific association between the gut microbiome and high-fat diet-induced metabolic disorders in mice," *Biol. Sex Differ.*, vol. 11, no. 1, pp. 1–12, Jan. 2020, doi: 10.1186/S13293-020-0281-3/FIGURES/6.

[25] M. Costanzo, M. Caterino, G. Sotgiu, M. Ruoppolo, F. Franconi, and I. Campesi, "Sex differences in the human metabolome," *Biol. Sex Differ.*, vol. 13, no. 1, pp. 1–18, Dec. 2022, doi: 10.1186/S13293-022-00440-4/TABLES/7.

[26] Z. Luan *et al.*, "A metagenomics study reveals the gut microbiome as a sex-specific modulator of healthy aging in Hainan centenarians," *Exp. Gerontol.*, vol. 186, Feb. 2024, doi: 10.1016/J.EXGER.2023.112356.

[27] R. Vemuri *et al.*, "The microgenderome revealed: sex differences in bidirectional interactions between the microbiota, hormones, immunity and disease susceptibility," *Semin. Immunopathol.*, vol. 41, no. 2, pp. 265–275, Mar. 2019, doi: 10.1007/S00281-018-0716-7.

[28] "ADNI Manuscript Citations," 2016, [Online]. Available: https://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Manuscript_Citations.pdf

[29] M. C. Donohue and C. Sun, "ADNIMERGE : Key ADNI variables." pp. 20–22, 2013.

[30] H. Zhang, "Quantitative measurement of gut microbial metabolites in human serum samples ADNI1, 2 and GO cohorts".

[31] P. Artimo, "NMR Analysis of Human Serum Samples from the Alzheimer's Disease Neuroimaging Initiative Using the Nightingale Health Platform," pp. 4–6, 2021.

[32] P. Soininen, "NMR Analysis of Human Serum Samples from the Alzheimer's Disease Neuroimaging Initiative Using the Nightingale Health Platform," pp. 4–6, 2020.

[33] The pandas development team, “pandas-dev/pandas: Pandas (v2.2.3).” 2024.

[34] J. D. Hunter, “Matplotlib: A 2D graphics environment,” *Comput. Sci. Eng.*, vol. 8, no. 3, pp. 90–95, 2007, doi: https://doi.org/10.5281/zenodo.13308876.

[35] S. Seabold and J. Perktold, “statsmodels: Econometric and statistical modeling with python,” in *9th Python in Science Conference*, 2010.

[36] K. P. . Murphy, “Logistic Regression,” in *Probabilistic machine learning : an introduction*, The MIT Press, 2021, pp. 333–337.

[37] M. Waskom, “seaborn: statistical data visualization,” *J. Open Source Softw.*, vol. 6, no. 60, p. 3021, 2021, doi: 10.21105/joss.03021.

[38] Plotly Technologies Inc, “Collaborative Data Science.” [Online]. Available: https://plot.ly

[39] C. R. Harris *et al.*, “Array programming with NumPy,” *Nat. 2020 5857825*, vol. 585, no. 7825, pp. 357–362, Sep. 2020, doi: 10.1038/s41586-020-2649-2.

[40] “How Is Alzheimer’s Disease Diagnosed? | National Institute on Aging.” Accessed: Jan. 05, 2025. [Online]. Available: https://www.nia.nih.gov/health/alzheimers-symptoms-and-diagnosis/how-alzheimers-disease-diagnosed

[41] M. Wu *et al.*, “Molecular mechanism and therapeutic strategy of bile acids in Alzheimer’s disease from the emerging perspective of the microbiota-gut-brain axis,” 2024, doi: 10.1016/j.biopha.2024.117228.

[42] M. McMillin and S. DeMorrow, “Effects of bile acids on neurological function and disease,” *FASEB J.*, vol. 30, no. 11, p. 3658, Nov. 2016, doi: 10.1096/FJ.201600275R.

[43] J. Tang, W. Xu, Y. Yu, S. Yin, B.-C. Ye, and Y. Zhou, “The role of the gut microbial metabolism of sterols and bile acids in human health including those for text and data mining, AI training, and similar technologies,” 2024, doi: 10.1016/j.biochi.2024.11.003.

[44] J. K. Pasupalak, P. Rajput, and G. L. Gupta, “Gut microbiota and Alzheimer’s disease: Exploring natural product intervention and the Gut-Brain axis for therapeutic strategies,” 2024, doi: 10.1016/j.ejphar.2024.177022.

[45] S. L. Collins, J. G. Stine, J. E. Bisanz, C. D. Okafor, and A. D. Patterson, “Bile acids and the gut microbiota: metabolic interactions and impacts on disease,” *Nat. Rev. Microbiol.*, vol. 21, no. 4, pp. 236–247, Apr. 2023, doi: 10.1038/S41579-022-00805-X.

[46] C. Xing *et al.*, “Neuroprotective effects of mesenchymal stromal cells in mouse models of Alzheimer’s Disease: The Mediating role of gut microbes and their metabolites via the Microbiome-Gut-Brain axis,” 2024, doi: 10.1016/j.bbi.2024.08.047.

[47] S. MahmoudianDehkordi *et al.*, “Altered bile acid profile associates with cognitive impairment in Alzheimer’s disease-An emerging role for gut microbiome,” *Alzheimers. Dement.*, vol. 15, no. 1, pp. 76–92, Jan. 2019, doi: 10.1016/J.JALZ.2018.07.217.

[48] H. Choi and I. Mook-Jung, “Functional effects of gut microbiota-derived metabolites in Alzheimer’s disease,” 2023, doi: 10.1016/j.conb.2023.102730.

[49] A. Ticinesi, A. Guerra, A. Nouvenne, T. Meschi, and S. Maggi, “Disentangling the Complexity of Nutrition, Frailty and Gut Microbial Pathways during Aging: A Focus on Hippuric Acid,” *Nutrients*, vol. 15, no. 5, p. 1138, Mar. 2023, doi: 10.3390/NU15051138.

[50] L. Fan *et al.*, “Association of Circulating Caprylic Acid with Risk of Mild Cognitive Impairment and Alzheimer’s Disease in the Alzheimer’s Disease Neuroimaging Initiative (ADNI) Cohort,” *J. Prev. Alzheimer’s Dis.*, vol. 10, no. 3, pp. 513–522, Sep. 2023, doi: 10.14283/JPAD.2023.37.

[51] R. Ullah *et al.*, “Glycine, the smallest amino acid, confers neuroprotection against d-galactose-induced neurodegeneration and memory impairment by regulating c-Jun N-terminal kinase in the mouse brain,” *J. Neuroinflammation*, vol. 17, no. 1, pp. 1–21, Dec. 2020, doi: 10.1186/S12974-020-01989-W/FIGURES/8.

[52] R. Roesler, J. Quevedo, R. Walz, F. D. Pizzol, and F. Kapczinski, “Drugs acting at the glycine site on the NMDA receptor as cognitive enhancers in patients with Alzheimer’s disease [2],” *Neurology*, vol. 50, no. 4, p. 1195, 1998, doi: 10.1212/WNL.50.4.1195.

[53] J. Kanski, S. Varadarajan, M. Aksenova, and D. A. Butterfield, “Role of glycine-33 and methionine-35 in Alzheimer’s amyloid β-peptide 1-42-associated oxidative stress and neurotoxicity,” *Biochim. Biophys. Acta - Mol. Basis Dis.*, vol. 1586, no. 2, pp. 190–198, Mar. 2002, doi: 10.1016/S0925-4439(01)00097-7.

[54] [54] D. K. Barupal *et al.*, “Sets of coregulated serum lipids are associated with Alzheimer’s disease pathophysiology,” *Alzheimer’s Dement. Diagnosis, Assess. Dis. Monit.*, vol. 11, p. 619, Dec. 2019, doi: 10.1016/J.DADM.2019.07.002.

[55] E. Rönnemaa, B. Zethelius, B. Vessby, L. Lannfelt, L. Byberg, and L. Kilander, “Serum fatty-acid composition and the risk of Alzheimer’s disease: a longitudinal population-based study,” *Eur. J. Clin. Nutr.*, vol. 66, no. 8, pp. 885–890, Aug. 2012, doi: 10.1038/EJCN.2012.63.

[56] M. Hosseini, A. Poljak, N. Braidy, J. Crawford, and P. Sachdev, “Blood fatty acids in Alzheimer’s disease and mild cognitive impairment: A meta-analysis and systematic review,” *Ageing Res. Rev.*, vol. 60, Jul. 2020, doi: 10.1016/J.ARR.2020.101043.

[57] Y. Zeng, S. Cao, J. Tang, and G. Lin, “Effects of saturated and monounsaturated fatty acids on cognitive impairment: evidence from Mendelian randomization study,” *Eur. J. Clin. Nutr. 2024 787*, vol. 78, no. 7, pp. 585–590, Apr. 2024, doi: 10.1038/s41430-024-01437-5.

[58] M. C. Morris *et al.*, “Dietary Fats and the Risk of Incident Alzheimer Disease,” *Arch. Neurol.*, vol. 60, no. 2, pp. 194–200, Feb. 2003, doi: 10.1001/ARCHNEUR.60.2.194.

VI Appendix A

Gut metabolites that did not show statistically significant difference in the mean at baseline between male (N = 137) and female (N = 140) patients that did not develop any type of cognitive disorder.

Variable	Corrected p-value
Hyodeoxycholic Acid	0.7985
Methylsuccinic Acid	0.9087
Formic Acid (HCOOH)	0.6769
Ursocholic Acid	0.2387
7-Ketolithocholic Acid	0.7527
Glycoursodeoxycholic Acid	0.4308
Adipic Acid	0.0914
3-Ursodeoxycholic Acid (Isoursodeoxycholic acid)	0.1298
Glycocholic Acid	0.799
Glycohyodeoxycholic Acid	0.1061
Deoxycholic Acid	0.0952
Tauroursodeoxycholic Acid	0.1183
Lithocholic Acid	0.7442
Heptanoic Acid (C7H1402)	0.1678
Caprylic Acid (C8H1602)	0.6018
Glycolic Acid	0.7299
L-Lysine	0.775
Butyric Acid (C4H803)	0.6019
Taurodeoxycholic Acid	0.3396
L-Alpha Amiobutyric Acid	0.284
Undecanoic Acid (C11H22O2)	0.9234
L-Malic Acid	0.5178
Glycohyocholic Acid	0.906
Valeric Acid	0.1758
Isocitric Acid	0.5574
7-Dehydrocholic Acid	0.1485
Ursodeoxycholic Acid	0.6982
Glycolithocholic Acid	0.6588
Glycine	0.0721
Taurocholic Acid	0.6223
Tauro-muricholic Acid	0.871
Suberic acid	0.7058
Propionic Acid (C3H6O2)	0.4132
Undecylenic Acid (C11H20O2)	0.3621
Oxoglutaric Acid	0.1884
Glyceric Acid	0.8934
Succinic acid	0.1355
Allolithocholic Acid (Isoallolithocholic acid)	0.0994
Glycodeoxycholic Acid	0.953
L-Histidine	0.0974
L-Tyrosine	0.2048
Pyroglutamic Acid	0.1994
Hexanoic Acid (C6H1202)	0.0795
3-Dehydrocholic Acid	0.1539
Dehydrolithocholic Acid	0.4099
Isobutyric Acid (C4H803)	0.823
L-Aspargine	0.7384
Indolepropionic Acid	0.8299
p-Cresol Sulfate	0.5647
12-Ketolithocholic Acid	0.5887
L-Alanine	0.1179
Citric Acid	0.0848
23-Nordeoxycholic Acid	0.2862
Hippuric Acid	0.948
Acetic Acid (C2H4O2)	0.3881
L-Serine	0.7126
Taurochenodeoxycholic Acid	0.6758

VII Appendix B

Serum metabolites that did not show statistically significant difference in the mean at baseline between male (n = 172) and female (n = 199) patients that did not develop any cognitive impairment.

Variable	p-value
Triglycerides in very large HDL particles (%)	0.985
Free Cholesterol in VLDL particles (mmol/l)	0.1781
Cholesterol esters in IDL particles (%)	0.2986
Free cholesterol in IDL particles (%)	0.0748
Total cholesterol in large VLDL particles (%)	0.8043
Citrate (mmol/l)	0.1506
Concentration of medium LDL particles (mmol/l)	0.127
Free cholesterol in medium LDL particles (%)	0.3161
Triglycerides in large VLDL (mmol/l)	0.7939
Triglycerides in VLDL particles (mmol/l)	0.9933
Triglycerides in very large VLDL (mmol/l)	0.5655
Phospholipids in medium LDL particles (%)	0.5927
Phospholipids in small LDL particles (%)	0.3028
Cholesterol esters in small VLDL particle (%)	0.8579
Total Triglycerides (mmol/l)	0.505
Total lipids in chylomicrons and extremely large VLDL (mmol/l)	0.4162
β-hydroxybutyrate (mmol/l)	0.6409
Phospholipids in VLDL particles (mmol/l)	0.2515
Monounsaturated Fatty Acids (%)	0.6227
Cholesterol esters in small VLDL (mmol/l)	0.1942
Triglycerides in medium HDL particles (%)	0.5219
Total lipids in large VLDL (mmol/l)	0.8329
Total lipids in small VLDL (mmol/l)	0.1676
Cholesterol esters in extremely large VLDL particles (%)	0.2223
Cholesterol esters in very large VLDL particles (%)	0.3109
Triglycerides in IDL particles (%)	0.2673
Saturated Fatty Acids (%)	0.206
Triglycerides in very large VLDL particles (%)	0.3317
Triglycerides in very small VLDL particles (%)	0.0636
Cholesterol esters in medium LDL particles (%)	0.1203
Phospholipids in very large VLDL particles (%)	0.4212
Free cholesterol in small LDL particles (%)	0.3113
Triglycerides in small VLDL (mmol/l)	0.4658
Acetoacetate (mmol/l)	0.1355
Free cholesterol in large LDL particles (%)	0.0876
Phospholipids in IDL particles (%)	0.1254
Concentration of very large VLDL particles (mmol/l)	0.6847
Cholesterol esters in medium HDL particles (%)	0.5322
Alanine (mmol/l)	0.1539
Concentration of large VLDL particles(mmol/l)	0.9062
Phospholipids in large HDL particles (%)	0.6373
Cholesterol esters in chylomicrons and extremely large VLDL (mmol/l)	0.7244
Triglycerides in small LDL particles (%)	0.9688
Acetate (mmol/l)	0.1474
18:2, Linoleic Acid (%)	0.1156
Cholesterol esters in small LDL particles (%)	0.0606
Total cholesterol in medium HDL particles (%)	0.2779
Chylomicrons and extremely large VLDL Particles (mmol/l)	0.5
Triglycerides in chylomicrons and extremely large VLDL (mmol/l)	0.3518
Phospholipids in medium HDL particles (%)	0.0903
Ratio of Polyunsaturated to Monounsaturated Fatty Acids	0.9712
Cholesterol esters in large HDL particles (%)	0.5537
Total lipids in very large VLDL (mmol/l)	0.6407
Triglycerides in small LDL particles (mmol/l)	0.1474
Pyruvate (mmol/l)	0.2275
Phospholipids in large VLDL (mmol/l)	0.719
Free cholesterol in large VLDL (mmol/l)	0.8166
Concentration of medium VLDL particles (mmol/l)	0.0774
Cholesterol esters in very large HDL particles (%)	0.8768
Total cholesterol in extremely large VLDL particles (%)	0.2281
Cholesterol esters in very small VLDL particles (%)	0.0773
Phospholipids in large VLDL particles (%)	0.0549
Total cholesterol in very large VLDL (mmol/l)	0.9302
Cholesterol in VLDL particles (mmol/l)	0.0623
Total cholesterol in medium LDL particles (%)	0.3525
Total lipids in medium VLDL (mmol/l)	0.1293
Lipids in VLDL particles (mmol/l)	0.4558
Cholesterol esters in very large VLDL (mmol/l)	0.8718
Total cholesterol in large VLDL (mmol/l)	0.9804
Triglycerides in small HDL particles (%)	0.0529
Total cholesterol in small LDL particles (%)	0.3848
Lactate (mmol/l)	0.8677
Free cholesterol in extremely large VLDL particles (%)	0.6839

APPENDIX B

SERUM METABOLITES THAT DID NOT SHOW STATISTICALLY SIGNIFICANT DIFFERENCE IN THE MEAN AT BASELINE BETWEEN MALE (N = 172) AND FEMALE (N = 199) PATIENTS THAT DID NOT DEVELOP ANY COGNITIVE IMPAIRMENT.

Variable	p-value
Phospholipids in small VLDL particle (%)	0.0509
Total cholesterol in very small VLDL particles (%)	0.0872
Phospholipids in small VLDL (mmol/l)	0.057
Phospholipids in very large HDL particles (%)	0.0509
Free cholesterol in very large VLDL particles (%)	0.617
Triglycerides in large VLDL particles (%)	0.371
Phospholipids in very small VLDL particles (%)	0.8777
Phospholipids in chylomicrons and extremely large VLDL (mmol/l)	0.4402
Triglycerides in extremely large VLDL particles (%)	0.5556
Polyunsaturated Fatty Acids (%)	0.6553
Omega-6 Fatty Acids (%)	0.0577
Free cholesterol in very large VLDL (mmol/l)	0.7321
Total cholesterol in IDL particles (%)	0.1164
Total cholesterol in very large VLDL particles (%)	0.2611
Concentration of small VLDL particles (mmol/l)	0.2848
Phenylalanine (mmol/l)	0.8053
Total cholesterol in chylomicrons and extremely large VLDL (mmol/l)	0.6449
Free cholesterol in large VLDL particles (%)	0.1918
Total cholesterol in large HDL particles (%)	0.749
Total cholesterol in small VLDL (mmol/l)	0.0957
Ratio of Omega-6 to Omega-3 Fatty Acids	0.0556
Cholesterol esters in large VLDL particles (%)	0.2454
Cholesterol esters in large LDL particles (%)	0.3607
Phospholipids in very large VLDL (mmol/l)	0.6119
Total cholesterol in small VLDL particle (%)	0.3527
Triglycerides in small VLDL particles (%)	0.1996
Free cholesterol in very small VLDL particles (%)	0.6173
Albumin (g/l)	0.1556
Free cholesterol in chylomicrons and extremely large VLDL (mmol/l)	0.5533
Triglycerides in large LDL particles (%)	0.8905
Triglycerides in large HDL particles (%)	0.2874
Triglycerides in medium VLDL (mmol/l)	0.567
Triglycerides in small HDL particles (mmol/l)	0.4946
Triglycerides in medium LDL particles (%)	0.4561