

Disorders in lipid metabolism, oxidative stress, and antioxidants in patients with amnestic mild cognitive impairment without major depression

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Abstract

Introduction: Amnestic mild cognitive impairment (aMCI) is characterized by changes in lipids and oxidative stress (OS). It is crucial to exclude patients with major depression (MDD) to accurately evaluate these biomarkers in aMCI.

Aims: To examine lipid and oxidative stress biomarkers associated with aMCI versus normal controls.

Materials and methods: We performed a case-control analysis involving 61 individuals with aMCI (without MDD) and 60 healthy controls. We assessed the severity of aMCI, distress symptoms of old age, and lipid/OS biomarkers.

Results: The levels of serum sulfhydryl (-SH) groups were significantly higher in individuals with aMCI, while the levels of malondialdehyde (MDA) were significantly lower in the same group. Serum advanced oxidation protein products, glutathione, and folic acid did not show any notable variations. In individuals with aMCI, we observed an elevated apolipoprotein B (ApoB)/apolipoprotein A (ApoA) ratio, as well as decreased levels of high-density lipoprotein cholesterol (HDL), ApoA, and a reverse cholesterol transport (RCT) index. The simultaneous presence of aMCI and subclinical depressive symptoms is marked by elevated levels of triglycerides and ApoB, as well as decreased levels of ApoA and HDL. A significant portion of the variability (24.9%) in a quantitative MCI severity score can be attributed to -SH groups, age (positively), MDA, and education (inversely).

Conclusion: The alterations in MDA and -SH levels in aMCI may potentially disrupt redox signaling, which can affect cell signaling and homeostatic setpoints. The interaction between aMCI and subclinical depressive symptoms can lead to increased atherogenicity and reduced antiatherogenic protection.

Keywords

biomarkers, depression, oxidative and nitrosative stress, pathophysiology, physiological stress, neurocognition

Introduction

Mild cognitive impairment (MCI) is the intermediate, transitional phase between dementia and typical aging, affecting 10%–15% of people aged 65 and older.^[1] MCI is identified by (1) subjective cognitive complaints by the patient and/or informant; (2) poorer cognitive performance compared to normal adults of the same age and background as assessed via clinical objective evaluation; (3) reports of cognitive decline from the past year; (4) preserved general cognitive functioning with unaffected daily activities of living; and (5) absence of dementia.^[2,3] Currently, the global prevalence of MCI is around 19.7%^[4], and at least 10%–15% of MCI cases progress to Alzheimer's dementia (AD)^[5].

A meta-analysis reported the prevalence of major depressive disorder (MDD) in MCI is approximately 32%^[6], and 41% in those with AD^[7]. Similarly, Kohler et al.^[8] reported that 29.8% of people with MCI had depression, 18.3% had sleep disturbances, and 15.2% had apathy. A recent study^[9] on MCI participants uncovered two separate clinical dimensions (quantitative scores) within older adults who showed no signs of major depression (MDD). The first dimension is 'mild cognitive dysfunctions,' represented through a quantified MCI score (qMCI) indicating the extent of objective cognitive decline. The second dimension is referred to as 'distress symptoms of old age (DSOA), which encompasses manifestations of anxiety, tension, and neuroticism. This dimension is linked to negative life events and adverse childhood experiences (ACEs).

Several studies report that blood biomarkers are associated with the pathology of MCI, including mechanisms involving lipids, oxidative stress, antioxidant defenses, and lipid peroxidation. Previous studies found changes in lipid profiles in aMCI and AD reminiscent of atherogenicity and lowered anti-atherogenic defenses, including increased triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and ApoB, and lowered high-density lipoprotein cholesterol (HDL-C) and ApoA1.^[10–15] Additionally, an imbalance between lowered levels of detoxifying antioxidant defenses and increased reactive oxygen (ROS) and nitrogen (RNS) species has also been linked to MCI. For example, one recent meta-analysis reported increased oxidative stress toxicity due to increased lipid peroxidation, coupled with reduced levels of antioxidant defenses, including glutathione (GSH) in MCI compared to controls.^[16] In separate studies, malondialdehyde accumulation that occurs with aging has been associated with an increased risk of both MCI and AD^[17], and plasma levels of advanced oxidation protein products

(AOPP) were elevated in individuals with MCI and AD compared to controls^[18]. Furthermore, a simultaneous decline in plasma glutathione levels and cognitive function was found.^[19] Lower serum total thiol (-SH) levels in MCI individuals were reported compared to controls.^[20] From another meta-analysis, AD patients had lower levels of folate and vitamins A, B12, C, and E compared to controls, indicating compromised antioxidant status.^[21] However, the oxidative stress toxicity/antioxidant defenses (OSTOX/ANTIOX) and lipid correlates of the two dimensions of aMCI (qMCI and DSOA) have remained elusive.

Evidence has suggested that MDD patients have significant disorders in lipid metabolism and an increased OSTOX/ANTIOX ratio as well. A recent systematic review demonstrated increased atherogenicity biomarkers in MDD together with lowered lipid-associated antioxidant defenses and a reverse cholesterol transport (RCT) system with lowered lecithin-cholesterol acyl transferase (LCAT) activity and ApoA levels.^[22] Additionally, MDD has been associated with low lecithin-cholesterol acyl transferase, high free cholesterol, and high ApoE levels compared to controls in another study.^[23] Moreover, MDD patients were reported to have a higher Castelli risk index 1, which is the ratio between total cholesterol and high-density lipoprotein cholesterol (HDL-C).^[24] Importantly, previous reviews demonstrated that the oxidative and nitrosative stress (O&NS) processes are integral factors underlying MDD.^[25] Taken together, it can be deduced that the OSTOX/ANTIOX profiles of aMCI cannot be evaluated if patients with MDD are included in the same study sample. Subsequently, associations between the lipid and O&NS biomarkers, neurocognitive functioning, and qMCI and DSOA scores should be examined in aMCI after excluding MDD subjects to get a more accurate understanding of the relationship between these factors.

Aim

Hence, the objective of this study is twofold. Firstly, it seeks to investigate the specific lipid and OSTOX/ANTIOX biomarkers of aMCI without MDD, and normal controls. Secondly, it will also examine the effects of these biomarkers on the severity of both aMCI (including qMCI scores) and DSOA scores. The study hypothesizes that individuals with aMCI (without MDD) will show: (1) elevated oxidative stress markers (e.g., MDA, AOPP), (2) reduced antioxidant defenses (-SH, GSH, folic acid), and (3) increased atherogenic lipid profiles (e.g., ApoB, Castelli index) with decreased protective lipids (HDL, ApoA1, LCAT).

Materials and methods

Participants

An analysis was conducted to determine the required sample size, considering several factors such as the effect size, significance level, power, number of groups, and covariates. Based on this analysis, a sample size of 90 participants would be sufficient to conduct an ANCOVA with a power of 80%. To enhance the robustness of our analysis and account for potential dropouts, we decided to include an additional 30 subjects. As a result, the final study sample size consisted of 120 participants, all of whom were between the ages of 60 and 75.

The study included 61 aMCI cases referred from Thailand's King Chulalongkorn Memorial Hospital, as well as 60 healthy controls. The two groups were selected from the same geographical region, specifically Bangkok, Thailand. Subjects with aMCI were recruited from various departments at King Chulalongkorn Memorial Hospital, including the Geriatric Clinic, the Cognitive Fitness Center unit, the Geriatric Psychiatry clinic, and the Neuroscience Center. The control group included a variety of individuals, such as senior Red Cross volunteers, attendees of health check-up clinics, members of neighborhood senior organizations, and healthy elderly caregivers of aMCI patients who visited the Dementia Clinic.

An efficient screening process was conducted to identify eligible individuals for enrollment. Patients diagnosed with aMCI underwent assessment and met the criteria established by Petersen.^[26] Additionally, they obtained a Clinical Dementia Rating (CDR) score of 0.5. These criteria encompass the identification of both subjective and objective memory impairments, the exclusion of dementia, and alterations in activities of daily living (ADL).

Individuals with neurological conditions such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, epilepsy, or stroke were deemed ineligible for participation in the study for both groups. In addition, the study excluded patients who had been diagnosed with various neuropsychiatric disorders such as substance use disorders, chronic fatigue syndrome, bipolar disorder, MDD, post-traumatic stress disorder, schizophrenia, obsessive-compulsive disorder, autism spectrum disorders, and generalized anxiety disorder. Individuals with chronic kidney disease, cancer, metabolic syndrome, chronic inflammatory bowel disease, HIV infection, hepatitis, or chronic obstructive pulmonary disease were not eligible to participate. People with communication difficulties, visual impairments, hearing loss, physical conditions affecting their ability to sit or stand, or mobility issues were also not included.

The study received approval from the Institutional Review Board committee of the Faculty of Medicine, Chulalongkorn University, and the ethical committee of King Chulalongkorn Memorial Hospital (886/64). Prior to any data collection, all participants provided written informed consent.

Clinical measurements

This study utilized a case-control design, consisting of 60 control participants and 61 individuals with aMCI.^[9,27] We conducted a comprehensive interview to collect demographic and clinical information, utilizing a range of questionnaires. The Mini International Neuropsychiatric Interview (M.I.N.I.)^[28] was utilized for the purpose of establishing axis-1 diagnoses, specifically MDD. To assess the extent of neurocognitive impairments, we employed the Montreal Cognitive Assessment (MoCA) scale^[29] and the Thai version of the Mini-Mental State Examination (MMSE)^[30]. Additionally, we utilized the modified CDR score.^[9,31] The qMCI score, which serves as the primary principal component, was derived from the scores on the MoCA, MMSE, and the modified CDR score.^[9]

For the assessment of distress, depression, and anxiety symptoms, a variety of standardized scales were utilized. These included the Perceived Stress Scale (PSS) developed by Wongpakaran and Wongpakaran^[32]; the State-Trait Anxiety Inventory (STAI) developed by Spielberger et al.^[33]; the Thai Geriatric Depression Scale (TGDS) developed by Yesavage et al.^[34] and translated into the Thai version by Pongwarin^[35]; the depression (HADS-D) and anxiety (HADS-A) subscales of the Hospital Anxiety and Depression Scale developed by Nilchaikovit^[36]; and the neuroticism trait score from the Five Factor Model standardized psychometric pool of items (IPIP-NEO) developed by Yomaboot and Cooper.^[37] We organized the study group into smaller groups based on a HADS-D score of ≥ 8 , resulting in two subgroups: one with higher depressive symptoms ($n=26$) and one with lower depressive symptoms ($n=95$). In a study conducted by Tran-Chi et al.^[9], a distress symptom of old age (DSOA) dimension was constructed through principal component analysis (PCA) on stress-affective symptoms. This dimension was derived as the first principal component extracted from various subscales, including PSS, STAI, TGDS, HADS-D, HADS-A, and the neuroticism score. In previous research, it was found that there was no significant association between the DSOA and qMCI dimensional scores.

In addition, a specific group of individuals with cognitive impairments was identified through cluster analysis. This advanced machine learning technique effectively removed subjects who displayed symptoms associated with the DSOA dimension in aMCI subjects, as demonstrated in the study by Tran-Chi et al.^[9] This two-step cluster analysis indicated that the study sample based on the aMCI diagnostic criteria is overinclusive because part of the individuals with DSOA symptoms were incorrectly classified as aMCI. As such, the newly formed MCI subgroup (denoted as mCoDy, or mild cognitive dysfunction) removed subjects with higher DSOA scores, thereby generating a more homogenous group comprising people with only episodic and semantic memory disorders.^[9] Therefore, a more specific subset of aMCI, referred to as mCoDy, was established. In this study, we will discuss the findings from a group of

individuals with aMCI (n=61) as well as a more specific subgroup called mCoDy (n=52), which is more restricted and homogeneous. Furthermore, we employed the established Thai translation of the Adverse Childhood Experiences (ACEs) Questionnaire^[38] to evaluate five dimensions of ACE, including emotional, physical, and sexual abuse, as well as emotional and physical neglect. The Negative Event (Hassle) Scale, developed by Maybery and Neale^[39], was utilized to assess negative life events (NLEs). The Alcohol, Smoking, and Substance Involvement Screening Test (AS-SIST)^[40] was utilized to identify and eliminate individuals who engaged in substance use, including tobacco.

Assays

Biological samples

Blood samples were obtained from the antecubital vein following an overnight fast between 8.00 and 9 a.m. Qualified nurses collected blood samples for the assay of lipids and O&NS biomarkers. All serum/plasma samples were kept at -80°C until thawed for analyses. We employed the Alinity C (Abbott Laboratories, USA; Otawara-Shi, Tochigi-Ken, Japan) to assay total TC, HDL-C, TG, and direct LDL-C.^[41] The inter-assay CV values were 2.3%, 2.6%, 2.3%, and 4.5% for TC, HDL-C, TG, and LDL-C, respectively. ApoA1 and ApoB were measured using an immunoturbidimetric assay using the Roche Cobas 6000 and c501 module (Roche, Rotkreuz, Switzerland). The intra-assay CV values were 1.75% and 2.64% for ApoA1 and ApoB, respectively. Free cholesterol (FC) was assayed using the Free Cholesterol Colorimetric Assay Kit (Elabscience, catalogue No: E-BC-K004-M), as explained previously.^[41] Based on these measurements, we determined the cholesterol esterification rate (CER) using the following formula: $1 - (\text{free cholesterol}/\text{total cholesterol}) \times 100$.^[41] Accordingly, the reverse cholesterol ratio was computed as: the z transformation of ApoA (zApoA) + z HDL-C + z CER.^[41]

The Thiol Quantification Assay Kit, Fluorometric (Abcam, catalogue number: ab112158), was employed to assay free thiol. Briefly, 50 μL of the reaction mixture (1 \times thiol green indicator stock solution in the assay buffer) and 50 μL of the protein standards or samples were added to a solid black 96-well microplate. Then, the plate was incubated at room temperature for 60 minutes in the dark. The fluorescence increase was measured at Ex/Em = 490/520 nm with a microplate reader (Varioskan Flash Multimode, Thermo). A blank well was used as the control and was subtracted with the values of the protein standards and samples. The amount of free thiol in test samples was calculated from the values of the standard curve.

The MDA assay kit, Colorimetric (Abcam, catalogue number: ab118970), was used for measuring the lipid peroxidation in plasma. For sample preparation, 20 μL of plasma and 500 μL of 42 mM H_2SO_4 were added to a microcentrifuge tube. Then, 125 μL of phosphotungstic acid solution was added to their reaction, mixed using a vortex

mixer, and incubated at room temperature for 5 minutes. The pellet was collected and suspended on ice with 200 μL of sterile water (with 2 μL BHT Stock/BHT (100 \times)). For the assay procedure, 200 μL of the developer VII/TBA reagent was added to each microcentrifuge tube containing 200 μL standard or 200 μL sample. The reaction mixes were heated at 95°C for 60 minutes and then cooled to room temperature before the detection. The heated samples were taken into a clear 96-well plate with a flat bottom. The absorbance was measured at 532 nm using a microplate reader (Varioskan Flash Multimode, Thermo). The MDA concentration was calculated according to the manufacturer's instructions and following the formula: (mmol/mL).

The presence of GSH in serum was detected using the GSH assay kit, Colorimetric (Abcam, catalogue number: ab239727). Briefly, 20 μL of diluted serums or standard proteins were added to a clear 96-well plate with a flat bottom. Then, 80 μL of the reaction mix was added to each well. The plate immediately measured the absorbance at 450 nm in kinetic mode at room temperature for 60 minutes using a microplate reader (Varioskan Flash Multimode, Thermo). For data analysis, the two time points (t1 and t2) in the linear range of the plot were selected and the corresponding absorbance values (OD1 and OD2) were obtained. The amount of GSH in test samples was calculated from the values of their standard curve following the calculation of the manufacturer's instructions.^[42,43]

The advanced oxidation protein products (AOPPs) in plasma were detected using the AOPP Assay Kit (Abcam, catalogue number: ab242295). 200 μL of test samples or standard proteins were prepared on a clear 96-well plate with a flat bottom. Then, 10 μL of chloramine Reaction Initiator was added to each well and incubated on the orbital shaker at room temperature for 5 minutes. After incubation, the stop solution was added to each well. The absorbance was immediately read at 340 nm using a microplate reader (Varioskan Flash Multimode, Thermo). Data analysis was calculated from the standard curve. The standard curve was created for each assay performed.

Statistical analysis

An analysis of variance was employed to investigate the variations in continuous variables among various diagnostic groups. Associations between the diagnosis of aMCI and the biomarkers were evaluated using multivariate and univariate GLM analysis. We implemented false discovery rate (FDR) *p*-correction on the biomarkers, which included multiple comparisons. In addition, we examined the effects of the interaction pattern between the diagnosis of aMCI x HADS-D groups. The chi-square test or Fisher's exact probability test was used to compare nominal variables across distinct categories. Stepwise automatic binary logistic regression analysis was performed with inclusion and exclusion *p*-values set at 0.05 and 0.06, respectively, to delineate the most relevant biomarkers (with demographic, ACEs, and NLEs data) of the diagnosis of aMCI (dependent

variable with controls as reference group). We employed manual and automatic multiple regression analyses to determine the most accurate predictors of quantitative scores (e.g., qMCI, MMSE, DSOA) using biomarkers and demographic data. We also utilized a forward stepwise automatic regression method with inclusion and exclusion *p*-values set at 0.05 and 0.06, respectively. The final regression models included standardized coefficients, *t*-statistics, and exact *p*-values for each explanatory variable. Additionally, the total variance was represented by effect size measures such as R^2 or partial eta squared and *F* statistics (along with their corresponding *p*-values). We employed both the White and modified Breusch-Pagan tests to evaluate the heteroskedasticity of the errors. An analysis was conducted to examine collinearity and multicollinearity. Various measures, such as tolerance (with a cut-off value of less than 0.25), the variance inflation factor (with a cut-off value of greater than 4), the condition index, and variance proportions from the collinearity diagnostics table, were utilized to assess the presence of heteroskedasticity. Furthermore, the data underwent z-score transformation to enhance their interpretability, and we generated z-unit-weighted composite scores that accurately depict distinct immune profiles. The data distributions were subjected, where necessary, to various

normalization processes, such as logarithmic or rank-based transformations. In each investigation mentioned, a two-tailed design was used to determine statistical significance at an α value of 0.05. The software used for analysis was IBM Windows SPSS version 29.

The a priori sample size was calculated using G*Power 3.1.9.4. The primary outcome analysis was a multiple regression analysis with the qMCI score as the dependent variable and biomarker data as the input variables. The minimum sample size required was 78, based on an *f* value of 0.179 (corresponding to approximately 15% of the explained variance), 5 explanatory variables, an alpha value of 0.05, and a power of 0.8.

Results

Socio-demographic and clinical features of the MCI participants

Table 1 displays the socio-demographic and clinical features of aMCI and control individuals in the current study. The MoCA and MMSE scores were significantly lower in

Table 1. Clinical data of subjects with amnesic mild cognitive impairment (aMCI) and healthy controls (HC)

Variables	HC (n=60)	aMCI (n=61)	F/ χ^2 /FET	df	P
Age (years)	66.80 (4.07)	68.72 (3.83)	7.13	1/119	0.009
Sex (male/female)	13/47	17/44	0.62	1	0.430 ^a
Marital status (S/M/D) (n)	15/36/9	14/40/7	0.48	2	0.784 ^a
Education (years)	15.88 (2.94)	13.70 (4.46)	10.01	1/119	0.002
Occupations (N/O/E/B/Ot)	30/13/1/5/11	25/8/3/14/11	6.90	4	0.141 ^a
BMI (kg/m ²)	22.66 (2.92)	23.26 (3.50)	1.07	1/119	0.303
Total MoCA	27.43 (1.51)	22.33 (1.65)	314.65	1/119	<0.001
Total MMSE	28.70 (1.23)	26.39 (2.27)	47.73	1/119	<0.001
IPIP-NEO (neuroticism)	14.33 (4.60)	15.90 (5.13)	3.12	1/119	0.080
Perceived Stress scale	12.25 (6.39)	15.03 (5.59)	6.49	1/119	0.012
HADS-A	4.53 (2.49)	5.43 (3.12)	3.00	1/119	0.085
HADS-D	3.97 (3.63)	4.52 (3.77)	0.68	1/118	0.411
STAI	36.10 (7.39)	37.18 (8.81)	0.53	1/119	0.467
NLEs (health+money)	0.84(0.13)	0.88 (0.16)	2.02	1/118	0.157
NLEs (total)	15.69 (14.72)	14.91(12.94)	0.09	1/118	0.759
ACEs (neglect)	18.00 (5.63)	19.67(6.23)	2.39	1/119	0.125
5ACEs (z score)	-0.19(0.89)	0.19(1.06)	4.86	1/119	0.029
TGDS	4.08 (3.16)	6.18 (4.57)	8.55	1/119	0.004
ApoE (E2/E3, E3/E3, E3/E4)	13/31/15	10/38/11	1.71	2/118	0.424 ^a

All results are shown as means (SD). All results of analyses of variance (F), except ^a: χ^2 – test, analysis of contingency analyses. Marital status – S: single, M: married, D: separate/divorced/widowed; Occupations (N/O/E/B/Ot): None, Government official, Employee, Private business, Other; MoCA: Montreal Cognitive Assessment; MMSE: Mini Mental State Examination; IPIP-NEO neuroticism: The International Personality Item Pool-NEO, neuroticism domain; HADS-A: Hospital Anxiety and Depression Scale-Anxiety scores; HADD-D: Hospital Anxiety and Depression Scale, Depression score; STAI: the State-Trait Anxiety Inventory; NLEs: negative life events; ACEs (neglect), adverse childhood experiences (neglect); TGDS: Thai Geriatric Depression Scale; ApoE: apolipoprotein E (gene).

aMCI subjects than in controls. The PSS score was slightly increased in aMCI, although there were no significant differences in the HADS depression and anxiety, STAI, and neuroticism scores among aMCI and control individuals. Emotional neglect and negative life events scores did not significantly differ between aMCI subjects and controls.

Differences between aMCI and controls

Table 2 shows the results of the O&NS measurements. The -SH groups were significantly higher in aMCI participants than in controls, while MDA was significantly lower in aMCI than in controls. There were no significant differences in folate, AOPP, and GSH (all results of GLM analysis with age, sex, BMI, and the drug status as covariates) and ApoE genotypes among the study groups. No significant interactions between the aMCI and HADS-D groups were found. The differences shown in **Table 2** remained significant after FDR p correction (at $p < 0.0125$).

We conducted a thorough investigation of the potential impact of the medicines and supplements that the participants were consuming on their oxidative stress profile. There were no significant differences in the use of antihypertensive drugs between the control group and the group with aMCI (43/17 versus 35/26, $p = 0.129$). Similarly, there

were no significant differences in the use of antidiabetic agents (54/6 versus 51/10, $p = 0.422$), cholesterol lowering drugs (24/36 versus 25/36), vitamins (26/34 versus 31/30, $p = 0.409$), fish oil (55/5 versus 51/10, $p = 0.270$), and calcium supplements (40/20 versus 46/15, $p = 0.321$). We have analyzed the potential impact of these medicines and supplements on the biomarkers of oxidative stress using multivariate and univariate GLM analysis, as presented in **Supplementary Table 1** in the **Supplement** (see after References). The multivariate GLM analysis revealed a significant impact of cholesterol-lowering drugs; the univariate GLM analysis indicated that this impact was specifically observed in the -SH groups, which were significantly reduced. This observed effect remained statistically significant after applying the FDR p-correction at $p = 0.04$.

Table 2 also shows the measurements of TC, FC, triglycerides, LDL-C, ApoB, LCAT, and the Castelli index in aMCI patients and control subjects. The results of GLM analyses, which were adjusted for age, sex, BMI, and drug status (as covariates), showed significant interaction patterns between the aMCI and HADS-D groups. We found significant interaction patterns for triglycerides, HDL, ApoA, ApoB, ApoB/ApoA ratio, RCT, and CRI-I. All differences in the lipid markers shown in **Table 2** remained significant following FDR p-correction (at $p < 0.0282$). **Supplementa-**

Table 2. Lipids, oxidative stress, and antioxidant levels of subjects with amnesic mild cognitive impairment (aMCI) and healthy controls (HC)

Variables	HC= 60	aMCI=61	F (F), χ^2	df	P
Folate (ng/mL)	13.54 (0.70)	13.87 (0.58)	2.37 *	1/108	0.127
Glutathione (reduced) (nmol/L)	5.95 (0.65)	4.87 (0.55)	1.05 *	1/108	0.309
-SH (thiol) groups ($\mu\text{mol/L}$)	100.19 (6.26)	133.20 (5.22)	13.36 *	1/108	<0.001
Malondialdehyde ($\mu\text{g/L}$)	142.75 (8.62)	123.38 (7.19)	8.37 *	1/108	0.005
Advanced oxidation protein products ($\mu\text{mol/L}$)	99.34 (5.68)	94.17 (4.73)	0.07 *	1/108	0.799
ApoE (E2-E3 / E3-E3 / E3-E4)	13/31/15	10/38/11	1.71	2	0.424 ^a
Total cholesterol (mg/dL)	209.98 (7.05)	206.01 (5.88)	0.39 (0.68) #	1/104	0.535 (0.412)
Free cholesterol (mg/dL)	40.37 (1.61)	38.97 (1.34)	0.23 (0.89) #	1/104	0.636 (0.347)
Triglycerides (mg/dL)	93.47 (7.90)	99.41 (6.59)	7.72 (0.04) #	1/104	0.006 (0.844)
High density lipoprotein (mg/dL)	64.07 (2.36)	57.44 (1.97)	7.63 (4.20) #	1/104	0.007 (0.043)
Low density lipoprotein (mg/dL)	124.14 (6.11)	127.35 (5.09)	2.81 (0.00) #	1/104	0.097 (0.950)
Apolipoprotein A ($\mu\text{g/mL}$)	166.80 (4.16)	147.03 (3.47)	6.26 (12.22) #	1/104	0.014 (<0.001)
Apolipoprotein B ($\mu\text{g/mL}$)	86.30 (3.88)	91.08 (3.24)	5.75 (0.54) #	1/104	0.018 (0.466)
ApoB/ApoA ratio	0.532 (0.033)	0.636 (0.028)	9.66 (6.90) #	1/104	0.002 (0.010)
Lecithin-cholesterol acyltransferase (%)	80.79 (0.51)	80.99 (0.42)	0.01 (0.11) #	1/104	0.936 (0.742)
Reverse cholesterol transport index (z score)	0.390 (0.171)	-0.144 (0.142)	5.74 (5.24) #	1/104	0.018 (0.024)
Castelli risk index 1	3.40 (3.71)	3.71 (0.15)	6.66 (1.38) #	1/104	0.011 (0.243)

All results are shown as means (SD). *All results of GLM analyses after covarying for age, sex, body mass index, and the drug status of the subjects. # All results of GLM analyses considering the interaction patterns between aMCI and HADS-D groups and after covarying for age, sex, body mass index and the drug status of the subjects. The first F value indicates the interaction pattern and the F value between brackets denotes the comparison between aMCI and controls. There were no significant differences in any of the biomarkers between the HADS-D groups; ^a ApoE genotypes: results of χ^2 -test, analysis of contingency analyses; HADS-D: Hospital Anxiety and Depression Scale-Depression score.

ry **Figures 1 through 7** illustrate the interaction patterns. While for people without depressive symptoms there were no significant differences in HDL, ApoA, and RCT between aMCI and control subjects, these three markers were lower in aMCI subjects with depressive symptoms compared to those without. While in people without depressive symptoms there were no significant differences in ApoB, ApoB/ApoA ratio, and Castelli risk index 1 between aMCI and control subjects, these markers were higher in subjects with higher HADS-D scores compared with their counterparts. HDL, ApoA, and the RCT index were significantly lower in aMCI than in controls, whilst the ApoB/ApoA ratio was significantly higher in aMCI than in controls.

We investigated the effect of the treatment on the participants' lipid profiles, and the findings are presented in **Supplementary Table 2**. The multivariate GLM analysis did not reveal any statistically significant effects on the lipid biomarkers. However, antidiabetic medications showed notable inhibitory effects on TC, LDL-C, ApoB, the ApoB/ApoA ratio, and free cholesterol based on univariate GLM. The FDR *p*-correction revealed that the impacts on TC, LDL-C, and ApoB remained statistically significant with a *p*-value of 0.0266. Regardless, we have calculated the residualized values for all biomarkers and assessed if utilizing these adjusted data will alter any outcomes. Nevertheless, there were no notable disparities observed when comparing the utilization of the unprocessed data with the residualized values.

Table 3 shows the results of a binary logistic regression analysis when the aMCI diagnosis is the dependent variable with the healthy control group as the reference group. **Table 3**, model #1, shows that aMCI was predicted

by 4 variables, namely -SH groups (positively), education, MDA, and ApoB (all inversely), with a Nagelkerke value of 0.519 and an accuracy of 78.5%. We also examined whether the same variables would predict the restricted study group of mCoDy versus controls. **Table 3**, model #2, shows that mCoDy was predicted by the same variables with a Nagelkerke value of 0.456 and an accuracy of 79.2%. Model #3 shows that two variables predicted the subgroup with increased HADS-D scores, specifically AOPP and NLEs health+money, with an accuracy of 79.5%.

Results of multiple regression analysis

Table 4 shows the results of multiple regression analyses with qMCI, MoCA, MMSE, DSAO, and HADS-D scores as dependent variables. Model #1 shows that 26.9% of the variance in the qMCI score was explained by the regression on education and MDA (both inversely) and the -SH groups and age (both positively). Model #2 shows that 24.9% of the variance in the MoCA score was explained by the same four variables. We found that 13.9% of the variance in the MMSE score was explained by two variables, namely education and MDA (both positively). Finally, we also regressed the DSOA and HADS-D scores on all biomarkers, psychological data (ACEs and NLEs), and demographic data. Not one of the biomarker data was significantly associated with the DSOA or HADS-D scores. For example, model #4 shows that NLEs health+money, education, and ACE neglect were associated with the score DSAO and explained 31.6% of its variance. Model #5 shows that NLEs health+money, and ACEs neglect were related to the HADS-D score and explained 16.4% of the variance.

Table 3. Results of binary logistic regression analyses with amnesic mild cognitive impairment (aMCI) or mild cognitive dysfunction (mCoDy) as the dependent variables and controls as reference group

Dependent variable	Explanatory variables	B	SE	Wald	P	OR	95% CI	χ^2 (df)	p	Nagelkerke
Model #1										
aMCI	Education (years)	-0.185	0.071	6.83	0.009	0.83	0.72-0.96	59.72 (4)	<0.001	0.519
	-SH groups	1.279	0.298	18.40	<0.001	3.59	2.00-6.43			
	MDA	-2.728	0.740	13.60	<0.001	0.07	0.02-0.28			
	ApoA	-0.827	0.278	8.86	0.003	0.44	0.25-0.75			
Model #2										
mCoDy	Education (years)	-0.287	0.078	13.45	<0.001	0.75	0.64-0.88	44.36 (4)	<0.001	0.456
	-SH groups	1.102	0.314	12.31	<0.001	3.01	1.63-5.57			
	MDA	-0.906	0.340	7.09	0.008	0.40	0.21-0.79			
	ApoA	-0.858	0.296	8.40	0.004	0.42	0.24-0.76			
Model #3										
High HADS-D	AOPP	0.529	0.238	4.93	0.026	1.70	1.06-2.71	14.46 (2)	0.001	0.182
	NLEs_ health+money	0.780	0.258	9.13	0.003	2.18	1.32-3.62			

-SH: thiol groups; MDA: malondialdehyde; ApoA: apolipoprotein A; AOPP: advanced oxidation protein products; NLEs_ health+money: sum negative life events (health and money); HADS-D: Hospital Anxiety and Depression Scale-Depression score.

Table 4. Results of multiple regression analysis with quantitative rating scores as dependent variables

Dependent variables	Explanatory variables	Coefficients of input variables			Model statistics			
		β	t	p	R ²	F	df	p
qMCI	Model #1				0.269	10.28	4/112	<0.001
	Education (years)	-0.317	-3.73	<0.001				
	MDA	-0.270	-3.19	0.002				
	-SH groups	0.224	2.67	0.009				
	Age	0.185	2.16	0.033				
MoCA	Model #2				0.249	66.46	4/112	<0.001
	Education (years)	0.290	3.37	0.001				
	MDA	0.246	2.87	0.005				
	-SH groups	-0.265	-3.31	0.002				
	Age	-0.177	-2.05	0.043				
MMSE	Model #3				0.139	9.22	2/114	<0.001
	Education (years)	0.322	3.71	<0.001				
	MDA	0.193	2.23	0.028				
DSOA	Model #4				0.316	16.78	3/109	<0.001
	NLEs (health+money)	0.433	5.30	<0.001				
	ACEs (neglect)	0.249	2.97	0.004				
	Education (years)	0.216	2.65	0.009				
HADS-D	Model #5				0.164	11.060	2/113	<0.001
	NLEs (health+money)	0.324	3.66	<0.001				
	ACEs (neglect)	0.180	2.04	0.044				

qMCI: quantitative mild cognitive impairment score; MoCA: the Montreal Cognitive Assessment; MMSE: the Mini Mental State Examination; DSOA: distress symptoms of old age; MDA: malondialdehyde; -SH: thiol groups; NLEs: negative life events (health and money); ACEs (neglect): adverse childhood experiences (neglect); HADS-D: Hospital Anxiety and Depression Scale-Depression scores

Discussion

MDA in aMCI

Our study discovered that individuals with aMCI had lower MDA concentrations compared to the control group. Additionally, there were no notable variations in AOPP and antioxidants (GSH and folic acid) between the two groups. These findings challenge the initial hypothesis we had. According to a previous meta-analysis conducted by Nantachai, Vasupanrajit^[16], it was found that individuals with MCI had elevated levels of MDA and lipid hydroperoxides (LOOH) compared to the control group. The latter study examined elevated levels of other oxidative stress biomarkers, such as homocysteine and carbonyls, alongside reduced levels of antioxidant biomarkers, including vitamins, GSH, and GSH peroxidase.^[16]

One explanation for the differences is that subjects with MDD were not always excluded from the previous studies. MDD is known to be associated with higher levels of oxidative stress, such as increased MDA and AOPP levels, as well as lower levels of antioxidants like GSH and GSH

peroxidase.^[25] Therefore, research on aMCI that includes patients with MDD may not provide a clear understanding of the true levels of oxidative stress and antioxidant biomarkers in aMCI.

MDA is formed through the degradation of arachidonic acid and other polyunsaturated fatty acids.^[44] It exhibits enhanced chemical stability and membrane permeability in comparison to reactive oxygen species (ROS) and demonstrates lower toxicity than other aldehyde compounds such as 4-hydroxy-2-nonenal (4-HNE) and methylglyoxal. In addition, several papers have proposed that MDA might offer protective effects, indicating that reduced MDA levels could potentially contribute to the pathophysiology of certain conditions. In fact, MDA can have a range of effects on the body, from potentially causing harm to offering protection.

MDA and 4-HNE function as signaling molecules that can stimulate gene expression and promote cell survival. However, they can also inhibit gene expression and contribute to cell death, as observed in studies conducted by Ayala et al.^[45] A recent study found that MDA acts as a signaling messenger, specifically influencing islet glucose-stimulated insulin secretion (GSIS) through the Wnt pathway. According to a study by Wang et al.^[46], it was discovered

that MDA levels of 5 and 10 μM have a moderate effect on enhancing islet GSIS. These levels were also found to increase the ATP/ADP ratio and cytosolic calcium levels, as well as influence gene expression and the production of proteins/activity associated with key regulators of GSIS.

The response of aldehyde dehydrogenase, which is activated by oxidative stress in mammals, is of utmost importance in determining the consequences of both immediate and prolonged elevations in aldehydes, such as MDA.^[47] The outcome is contingent upon several factors, including the extent and duration of MDA elevations, as well as the reaction of ALDH to oxidative stress. In a study conducted by Ayala et al.^[45], it was shown that lower levels of MDA could potentially interfere with important redox mechanisms in the body. Moreover, in the case of human beings, MDA represents a distinct epitope linked to oxidation, which is present on lipoproteins and cells that are undergoing apoptosis.^[48] This elicits an intrinsic immune response facilitated by IgM antibodies, which plays a crucial role in upholding homeostasis.^[48,49]

It is interesting to observe that within the plant kingdom, there is a captivating occurrence where short-lasting small increases in MDA levels can initiate a signaling process. This signaling process is of utmost importance in the regulation of cellular redox balance, the activation of the antioxidant system, and the plant's ability to survive in challenging environments.^[50] Continuous increases in MDA, on the other hand, lead to genotoxicity, a decrease in membrane fluidity, and various detrimental effects.^[50]

Based on this observation, it is possible that the decreased MDA concentrations in individuals with aMCI may be linked to a reduced ability of redox homeostatic processes.

-SH groups and aMCI

Our study also unexpectedly revealed an elevation, rather than a reduction, in -SH groups among individuals diagnosed with aMCI. This association is consistent with a study that found higher thiol concentrations in MCI participants than in controls.^[51] Interestingly, elevated levels of thiol protein groups in individuals with depression are linked to reduced efficiency in declarative memory, working memory, and verbal fluency as well.^[52]

None of our subjects took particular supplements known to elevate thiol groups (known as thiol supplements), such as lipoic acid, bromelain, glucosamine, selenium, cysteine, glutathione, and N-acetyl cysteine.^[53] Therefore, the increased levels of -SH groups in aMCI are not induced by intake of such supplements. The question is whether increased thiol accumulation may have a pathophysiological role, and if this were the case, why could this predispose individuals to develop aMCI? Enhanced -SH aggregation might coincide with heightened levels of S-prenylation^[54] and increased glutathionylated protein levels^[55]. Furthermore, an elevation in S-nitrosylation due to increased nitric oxide levels could also occur.^[54] The

latter mechanisms may exert deleterious effects, potentially contributing to age-related processes and neurodegeneration, including AD.^[55] It is important to emphasize that the prolonged administration of GSH or NAC to both young and aged animals may disrupt overall gene expression, suppress SKN-1-mediated transcription, and hasten the aging process.^[56] On the contrary, restricting the intake of dietary thiols may prolong lifespan.^[56] Homocysteine, a thiol compound, constitutes a part of the overall plasma thiol pool, which comprises low molecular weight thiols, cysteine, glutathione, and cysteinylglycine.^[57] Elevated levels of homocysteine are linked to neurocognitive deficits in older individuals and AD.^[58] Finally, high thiol antioxidant amounts could lead to increased oxidative damage, misfolded proteins, endoplasmic reticulum (ER) stress, and accelerated aging.^[56,59]

Lipid metabolism in aMCI

Our lipid profile results were more in line with the a priori hypothesis; there were lower HDL-C and ApoA concentrations and a higher ApoB/ApoA ratio in aMCI participants compared to controls. Collectively, these findings indicate lowered antiatherogenic but increased atherogenesis processes. A prior meta-analysis examining correlations between lipid profiles and aMCI revealed that elevated TC and LDL-C levels were associated with increased cognitive impairment.^[14] There was a trend towards increased TC levels in aMCI, whilst there were no significant changes in LDL-C, TG, and HDL-C. Plasma TG levels were found to be higher in MCI than control subjects in another case-control investigations.^[10,11,60] Metabolic syndrome biomarkers (TG/HDL-C ratio and lowered ApoA1) correlated with a swifter deterioration of cognitive functions in subjects with aMCI.^[13] Meta-analyses studies performed in AD showed that this illness is accompanied by increased TC and LDL-C^[14], and lowered serum/plasma ApoA1.^[11,12]

In addition to the observed associations between the lipid profiles and aMCI diagnosis, this study also found that the co-occurrence of aMCI and subclinical depressive symptoms was characterized by lowered HDL, ApoA, a reverse cholesterol transport index, and increased ApoB, ApoB/ApoA ratio, and a Castelli risk index 1. Increased ApoB/ApoA and Castelli risk index ratios are important predictors of increased risk of cardiovascular disease.^[61] Thus, subclinical depression already is sufficient in increasing atherogenicity when coupled with aMCI. Previous literature has mentioned that individuals diagnosed with MDD were found to exhibit significantly higher atherogenicity indices and a lowered reverse cholesterol transport (including HDL-C and ApoA1 levels) compared to control subjects.^[24,62-64] Moreover, Morelli et al.^[65] found that, in MDD, elevated atherosclerosis and insulin resistance were largely influenced by reactive oxygen and nitrogen species.

According to past research, atherogenicity is consid-

ered a risk factor for cognitive deficits, and elevated atherogenic lipoproteins have been associated with impaired cognition.^[66] Additionally, low global cognitive performance, memory, and executive function have also been reported in those with atherogenic dyslipidemia (characterized by elevated triglyceride levels, increased LDL concentrations, and reduced HDL concentrations).^[67] The relationship between dyslipidemia and cognitive deficits may be due to the role that dyslipidemia plays in the formation of atherosclerotic plaques, leading to ischemic injuries in the brain.^[21]

Limitations

This study was performed on Thai individuals with aMCI and, therefore, may not be generalizable to other ethnicities or countries. Therefore, the results deserved to be rep-

the GSH/GSSG and NADPH/NADP⁺ ratios and monocyte and neutrophil oxidative bursts.

Conclusions

Fig. 1 summarizes the findings of this study. Collectively looking at our results, the study found decreased levels of MDA instead of increased MDA levels. In addition, there were no significant changes in antioxidant defenses, such as GSH, whilst the -SH groups were even increased. This indicates a more reduced extracellular redox state and does not indicate increased reductive or oxidative stress in aMCI. Although these results are contrary to our a priori hypothesis, it could potentially be explained by the underlying complex roles of MDA and -SH groups and different mechanisms of these biomarkers leading to alterations in homeostatic setpoints. Regarding the atherogenic lipid

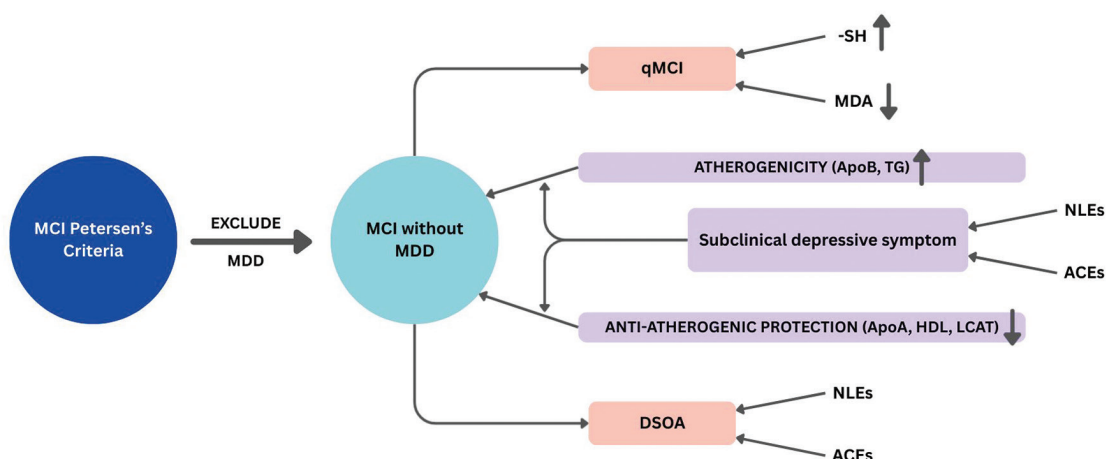


Figure 1. Summary of the results. This study is performed on subjects with amnesic mild cognitive impairment (aMCI) and controls after excluding those with major depressive disorder (MDD). Outcome data were the diagnosis of aMCI versus controls, and two quantitative scores, namely qMCI (a quantitative cognitive impairment score) and DSOA (distress symptoms of old age). The former dimension is associated with increased thiol (-SH) groups and lowered malondialdehyde (MDA). The second dimension is associated with psychological stressors, including NLEs (negative life events) and ACEs (adverse childhood experiences). The interaction of aMCI with subclinical depressive symptoms is associated with increased atherogenicity and lower antiatherogenic protection. Apo: apolipoprotein; TG: triglycerides; HDL: high-density lipoprotein; LCAT: lecithin-cholesterol acyltransferase.

licated in other countries and ethnicities. Future research should construct new models of MCI integrating the reported plasma biomarkers with brain neuroimaging data within the concept of the nomothetic networks.^[68] Recent research indicates that aMCI and the transition to dementia may be conceptualized as arising from alterations in nighttime dampening and resetting.^[69] Aging is associated with a dramatic 10-fold decrease in pineal gland melatonin at night between the 2nd and 9th decades^[70], leading to a loss of melatonin's antioxidant, anti-inflammatory, and mitochondria-optimizing effects. The effect of pineal melatonin and local melatonergic pathways requires clarification in future studies. Future research should exclude the presence of reductive stress, for example, via measuring

profile of aMCI, our study results agreed with the previous literature. Apart from this, we also report that the co-occurrence of aMCI with subclinical depressive symptoms could be characterized by increasing atherogenicity and lowered antiatherogenic protection. Studies that fail to exclude patients with MDD and neglect to control for subclinical depressive symptoms are unable to accurately determine the oxidative stress, antioxidant, and lipid profiles of aMCI. The results show that increased atherogenicity (increased TG, ApoB) and lowered antiatherogenic protection (lower HDL and ApoA1 and LCAT activity index) are new drug targets to treat aMCI, especially when subclinical depressive symptoms are present. Furthermore, drugs that normalize the aberrations in redox signaling

could be of potential importance.

Ethical approval

The research project (IRB No. 886/64) received approval from the Institutional Review Board (IRB) at Chulalongkorn University's Ethics Board in Bangkok, Thailand. This approval is in accordance with the International Guidelines for the Protection of Human Research Participants, as mandated by the Declaration of Helsinki, the Belmont Report, CIOMS Guidelines, and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP). All participants signed the appropriate institutional informed consent forms before data collection.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that all participants signed the appropriate institutional informed consent forms before data collection.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalized human and animal cell lines were used in the present study.

Conflict of interest

The authors declare no competing financial or non-financial interests.

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Use of AI

No use of AI was reported.

Data availability

The corresponding authors MM and CT will provide the data file used in the present study upon receiving an appropriate request once the authors have fully utilized the data.

Author contributions

GN: data curation, investigation, project administration, first draft writing; MM: design, methodology, conceptualization, statistical analysis, visualization, writing, review, supervision; V-LT-C: data curation, investigation, project administration; AV: pre-data processing; AA, and SH: editing; CT: design, methodology, review, and editing. All authors approved the submitted draft of this paper.

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Supplement

Supplementary Tables

Multivariate Tests 3

Supplementary Table 1. Effects of medications and supplements on oxidative stress variables

Partial Eta

Effect		Value	F	Hypothesis df	Error df	Sig.	Squared
Intercept	Pillai's Trace	0.165	4.028 ^b	5.000	102.000	0.002	0.165
	Wilks' Lambda	0.835	4.028 ^b	5.000	102.000	0.002	0.165
	Hotelling's Trace	0.197	4.028 ^b	5.000	102.000	0.002	0.165
	Roy's Largest Root	0.197	4.028 ^b	5.000	102.000	0.002	0.165
Sex	Pillai's Trace	0.126	2.939 ^b	5.000	102.000	0.016	0.126
	Wilks' Lambda	0.874	2.939 ^b	5.000	102.000	0.016	0.126
	Hotelling's Trace	0.144	2.939 ^b	5.000	102.000	0.016	0.126
	Roy's Largest Root	0.144	2.939 ^b	5.000	102.000	0.016	0.126
Age	Pillai's Trace	0.053	1.144 ^b	5.000	102.000	0.342	0.053
	Wilks' Lambda	0.947	1.144 ^b	5.000	102.000	0.342	0.053
	Hotelling's Trace	0.056	1.144 ^b	5.000	102.000	0.342	0.053
	Roy's Largest Root	0.056	1.144 ^b	5.000	102.000	0.342	0.053
Lipid drug	Pillai's Trace	0.111	2.560 ^b	5.000	102.000	0.032	0.111
	Wilks' Lambda	0.889	2.560 ^b	5.000	102.000	0.032	0.111
	Hotelling's Trace	0.125	2.560 ^b	5.000	102.000	0.032	0.111
	Roy's Largest Root	0.125	2.560 ^b	5.000	102.000	0.032	0.111
HT drug	Pillai's Trace	0.040	0.851 ^b	5.000	102.000	0.517	0.040
	Wilks' Lambda	0.960	0.851 ^b	5.000	102.000	0.517	0.040
	Hotelling's Trace	0.042	0.851 ^b	5.000	102.000	0.517	0.040
	Roy's Largest Root	0.042	0.851 ^b	5.000	102.000	0.517	0.040
DM drug	Pillai's Trace	0.034	0.727 ^b	5.000	102.000	0.604	0.034
	Wilks' Lambda	0.966	0.727 ^b	5.000	102.000	0.604	0.034
	Hotelling's Trace	0.036	0.727 ^b	5.000	102.000	0.604	0.034
	Roy's Largest Root	0.036	0.727 ^b	5.000	102.000	0.604	0.034
Vitamins	Pillai's Trace	0.047	1.016 ^b	5.000	102.000	0.412	0.047
	Wilks' Lambda	0.953	1.016 ^b	5.000	102.000	0.412	0.047
	Hotelling's Trace	0.050	1.016 ^b	5.000	102.000	0.412	0.047
	Roy's Largest Root	0.050	1.016 ^b	5.000	102.000	0.412	0.047
Calcium	Pillai's Trace	0.076	1.682 ^b	5.000	102.000	0.146	0.076
	Wilks' Lambda	0.924	1.682 ^b	5.000	102.000	0.146	0.076
	Hotelling's Trace	0.082	1.682 ^b	5.000	102.000	0.146	0.076
	Roy's Largest Root	0.082	1.682 ^b	5.000	102.000	0.146	0.076
Fish oil	Pillai's Trace	0.045	0.961 ^b	5.000	102.000	0.445	0.045
	Wilks' Lambda	0.955	0.961 ^b	5.000	102.000	0.445	0.045
	Hotelling's Trace	0.047	0.961 ^b	5.000	102.000	0.445	0.045
	Roy's Largest Root	0.047	0.961 ^b	5.000	102.000	0.445	0.045

Lipid drugs: cholesterol lowering drugs, HT drugs: antihypertensive drugs, DM drugs: antidiabetic agents.

Tests of between-subjects effects

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.	Partial Eta squared
Corrected model	Folate	256.533a	8	32.067	2.310	0.025	0.148
	GSH	56.364b	8	7.045	0.568	0.802	0.041
	SH_THIOL	312.400c	8	39.050	3.035	0.004	0.186
	MDA	12967.632d	8	1620.954	0.677	0.711	0.049
	AOPP	7449.858e	8	931.232	0.996	0.443	0.070
Intercept	Folate	4.163	1	4.163	0.300	0.585	0.003
	GSH	22.906	1	22.906	1.847	0.177	0.017
	SH_THIOL	68.014	1	68.014	5.286	0.023	0.047
	MDA	24992.792	1	24992.792	10.440	0.002	0.090
	AOPP	2966.404	1	2966.404	3.173	0.078	0.029
Sex	Folate	102.426	1	102.426	7.377	0.008	0.065
	GSH	15.298	1	15.298	1.233	0.269	0.012
	SH_THIOL	35.493	1	35.493	2.758	0.100	0.025
	MDA	2544.073	1	2544.073	1.063	0.305	0.010
	AOPP	2763.073	1	2763.073	2.956	0.088	0.027
Age	Folate	33.342	1	33.342	2.401	0.124	0.022
	GSH	4.233	1	4.233	0.341	0.560	0.003
	SH_THIOL	0.720	1	0.720	0.056	0.813	0.001
	MDA	5004.701	1	5004.701	2.090	0.151	0.019
	AOPP	11.977	1	11.977	0.013	0.910	0.000
Lipid drug	Folate	27.675	1	27.675	1.993	0.161	0.018
	GSH	11.556	1	11.556	0.932	0.337	0.009
	SH_THIOL	94.834	1	94.834	7.371	0.008	0.065
	MDA	10.307	1	10.307	0.004	0.948	0.000
	AOPP	1666.822	1	1666.822	1.783	0.185	0.017
HT drug	Folate	7.186	1	7.186	0.518	0.473	0.005
	GSH	4.273	1	4.273	0.345	0.558	0.003
	SH_THIOL	19.037	1	19.037	1.480	0.227	0.014
	MDA	131.543	1	131.543	0.055	0.815	0.001
	AOPP	2046.088	1	2046.088	2.189	0.142	0.020
DM drug	Folate	29.513	1	29.513	2.126	0.148	0.020
	GSH	12.544	1	12.544	1.011	0.317	0.009
	SH_THIOL	0.581	1	0.581	0.045	0.832	0.000
	MDA	1030.454	1	1030.454	0.430	0.513	0.004
	AOPP	259.645	1	259.645	0.278	0.599	0.003
Vitamins	Folate	8.120	1	8.120	0.585	0.446	0.005
	GSH	0.466	1	0.466	0.038	0.847	0.000
	SH_THIOL	54.671	1	54.671	4.249	0.042	0.039
	MDA	542.471	1	542.471	0.227	0.635	0.002
	AOPP	19.389	1	19.389	0.021	0.886	0.000
Calcium	Folate	11.099	1	11.099	0.799	0.373	0.007
	GSH	6.570	1	6.570	0.530	0.468	0.005
	SH_THIOL	94.616	1	94.616	7.354	0.008	0.065
	MDA	643.855	1	643.855	0.269	0.605	0.003
	AOPP	93.414	1	93.414	0.100	0.753	0.001

Fish oil	Folate	58.425	1	58.425	4.208	0.043	0.038
	GSH	1.989	1	1.989	0.160	0.690	0.002
	SH_THIOL	6.489	1	6.489	0.504	0.479	0.005
	MDA	43.836	1	43.836	0.018	0.893	0.000
	AOPP	29.723	1	29.723	0.032	0.859	0.000
Error	Folate	1471.762	106	13.885			
	GSH	1314.752	106	12.403			
	SH_THIOL	1363.874	106	12.867			
	MDA	253766.942	106	2394.028			
	AOPP	99089.133	106	934.803			
Total	Folate	20401.568	115				
	GSH	4580.723	115				
	SH_THIOL	17282.591	115				
	MDA	2430801.202	115				
	AOPP	1093201.332	115				
Corrected total	Folate	1728.295	114				
	GSH	1371.115	114				
	SH_THIOL	1676.274	114				
	MDA	266734.573	114				
	AOPP	106538.991	114				

Estimates

95% Confidence Interval

Dependent variable	Lipid_Med	Mean	Std. error	Lower bound	Upper bound
Folate	No	13.348 ^a	0.552	12.254	14.441
	Yes	12.325 ^a	0.457	11.419	13.230
GSH	No	4.892 ^a	0.521	3.858	5.926
	Yes	5.553 ^a	0.432	4.698	6.409
SH_THIOL	No	12.769 ^a	0.531	11.716	13.822
	Yes	10.875 ^a	0.440	10.004	11.747
MDA	No	137.548 ^a	7.244	123.186	151.910
	Yes	136.923 ^a	5.995	125.038	148.809
AOPP	No	87.932 ^a	4.527	78.957	96.906
	Yes	95.871 ^a	3.746	88.444	103.299

No/Yes: use of antidiabetic drugs versus no drugs.

Supplementary Table 2. Effects of medications and supplements on lipid variables**Multivariate Tests 3****Partial Eta**

Effect		Value	F	Hypothesis df	Error df	Sig.	Squared
Intercept	Pillai's Trace	0.698	26.221 ^b	9.000	102.000	0.000	0.698
	Wilks' Lambda	0.302	26.221 ^b	9.000	102.000	0.000	0.698
	Hotelling's Trace	20.314	26.221 ^b	9.000	102.000	0.000	0.698
	Roy's Largest Root	20.314	26.221 ^b	9.000	102.000	0.000	0.698
Sex	Pillai's Trace	0.195	2.751 ^b	9.000	102.000	0.006	0.195
	Wilks' Lambda	0.805	2.751 ^b	9.000	102.000	0.006	0.195
	Hotelling's Trace	0.243	2.751 ^b	9.000	102.000	0.006	0.195
	Roy's Largest Root	0.243	2.751 ^b	9.000	102.000	0.006	0.195
Age	Pillai's Trace	0.066	0.806 ^b	9.000	102.000	0.612	0.066
	Wilks' Lambda	0.934	0.806 ^b	9.000	102.000	0.612	0.066
	Hotelling's Trace	0.071	0.806 ^b	9.000	102.000	0.612	0.066
	Roy's Largest Root	0.071	0.806 ^b	9.000	102.000	0.612	0.066
Lipid drug	Pillai's Trace	0.143	1.890 ^b	9.000	102.000	0.062	0.143
	Wilks' Lambda	0.857	1.890 ^b	9.000	102.000	0.062	0.143
	Hotelling's Trace	0.167	1.890 ^b	9.000	102.000	0.062	0.143
	Roy's Largest Root	0.167	1.890 ^b	9.000	102.000	0.062	0.143
HT drug	Pillai's Trace	0.084	1.039 ^b	9.000	102.000	0.415	0.084
	Wilks' Lambda	0.916	1.039 ^b	9.000	102.000	0.415	0.084
	Hotelling's Trace	0.092	1.039 ^b	9.000	102.000	0.415	0.084
	Roy's Largest Root	0.092	1.039 ^b	9.000	102.000	0.415	0.084
DM_drug	Pillai's Trace	0.132	1.724 ^b	9.000	102.000	0.093	0.132
	Wilks' Lambda	0.868	1.724 ^b	9.000	102.000	0.093	0.132
	Hotelling's Trace	0.152	1.724 ^b	9.000	102.000	0.093	0.132
	Roy's Largest Root	0.152	1.724 ^b	9.000	102.000	0.093	0.132
Vitamins	Pillai's Trace	0.055	0.654 ^b	9.000	102.000	0.748	0.055
	Wilks' Lambda	0.945	0.654 ^b	9.000	102.000	0.748	0.055
	Hotelling's Trace	0.058	0.654 ^b	9.000	102.000	0.748	0.055
	Roy's Largest Root	0.058	0.654 ^b	9.000	102.000	0.748	0.055
Calcium	Pillai's Trace	0.079	0.979 ^b	9.000	102.000	0.462	0.079
	Wilks' Lambda	0.921	0.979 ^b	9.000	102.000	0.462	0.079
	Hotelling's Trace	0.086	0.979 ^b	9.000	102.000	0.462	0.079
	Roy's Largest Root	0.086	0.979 ^b	9.000	102.000	0.462	0.079
Fish oil	Pillai's Trace	0.033	0.381 ^b	9.000	102.000	0.942	0.033
	Wilks' Lambda	0.967	0.381 ^b	9.000	102.000	0.942	0.033
	Hotelling's Trace	0.034	0.381 ^b	9.000	102.000	0.942	0.033
	Roy's Largest Root	0.034	0.381 ^b	9.000	102.000	0.942	0.033

Lipid drugs: cholesterol lowering drugs, HT drugs: antihypertensive drugs, DM drugs: antidiabetic agents.

Tests of between-subjects effects

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig	Partial Eta squared
Corrected model	Cholesterol	59415.532 ^a	8	7426.942	5.551	0.000	0.288
	HDL cholesterol	3713.932 ^b	8	464.242	2.366	0.022	0.147
	LDL cholesterol	37127.155 ^c	8	4640.894	4.164	0.000	0.232
	Triglycerides	46667.848 ^d	8	5833.481	0.894	0.524	0.061
	ApoA	13977.042 ^e	8	1747.130	2.945	0.005	0.176
	ApoB	9442.622 ^f	8	1180.328	2.867	0.006	0.173
	ApoB on ApoA	0.498 ^g	8	0.062	1.961	0.058	0.125
	FC	2692.486 ^h	8	336.561	4.720	0.000	0.256
	Zscore (LCAT) LCAT	4.596 ⁱ	8	0.575	0.548	0.818	0.038
Zscore (RCTreal)	12.980 ^j	8	1.623	1.670	0.114	0.108	
Intercept	Cholesterol	39374.294	1	39374.294	29.429	0.000	0.211
	HDL cholesterol	3033.239	1	3033.239	15.459	0.000	0.123
	LDL cholesterol	16233.902	1	16233.902	14.566	0.000	0.117
	Triglycerides	11941.227	1	11941.227	1.829	0.179	0.016
	ApoA	15037.746	1	15037.746	25.347	0.000	0.187
	ApoB	5705.556	1	5705.556	13.861	0.000	0.112
	ApoB on ApoA	0.172	1	0.172	5.399	0.022	0.047
	FC	1803.688	1	1803.688	25.295	0.000	0.187
	Zscore (LCAT) LCAT	0.437	1	0.437	0.416	0.520	0.004
Zscore (RCTreal)	0.637	1	0.637	0.656	0.420	0.006	
Sex	Cholesterol	12203.341	1	12203.341	9.121	0.003	0.077
	HDL cholesterol	1527.513	1	1527.513	7.785	0.006	0.066
	LDL cholesterol	2999.162	1	2999.162	2.691	0.104	0.024
	Triglycerides	335.577	1	335.577	0.051	0.821	0.000
	ApoA	6809.992	1	6809.992	11.479	0.001	0.094
	ApoB	779.411	1	779.411	1.893	0.172	0.017
	ApoB on ApoA	0.031	1	0.031	0.975	0.326	0.009
	FC	627.136	1	627.136	8.795	0.004	0.074
	Zscore (LCAT) LCAT	0.453	1	0.453	0.432	0.512	0.004
Zscore (RCTreal)	5.915	1	5.915	6.087	0.015	0.052	
Age	Cholesterol	3261.867	1	3261.867	2.438	0.121	0.022
	HDL cholesterol	262.455	1	262.455	1.338	0.250	0.012
	LDL cholesterol	1237.369	1	1237.369	1.110	0.294	0.010
	Triglycerides	3055.937	1	3055.937	0.468	0.495	0.004
	ApoA	696.196	1	696.196	1.173	0.281	0.011
	ApoB	216.660	1	216.660	0.526	0.470	0.005
	ApoB on ApoA	0.000	1	0.000	0.009	0.924	0.000
	FC	226.285	1	226.285	3.173	0.078	0.028
	Zscore (LCAT) LCAT	0.436	1	0.436	0.416	0.520	0.004
Zscore (RCTreal)	0.475	1	0.475	0.489	0.486	0.004	
Lipid drug	Cholesterol	2876.591	1	2876.591	2.150	0.145	0.019
	HDL cholesterol	117.625	1	117.625	0.599	0.440	0.005
	LDL cholesterol	5575.824	1	5575.824	5.003	0.027	0.044
	Triglycerides	14886.050	1	14886.050	2.281	0.134	0.020
	ApoA	244.372	1	244.372	0.412	0.522	0.004
	ApoB	557.760	1	557.760	1.355	0.247	0.012
	ApoB on ApoA	0.050	1	0.050	1.565	0.214	0.014
	FC	140.078	1	140.078	1.964	0.164	0.018
	Zscore (LCAT) LCAT	3.067E-5	1	3.067E-5	0.000	0.996	0.000
Zscore (RCTreal)	0.004	1	0.004	0.004	0.949	0.000	

HT drug	Cholesterol	7578.131	1	7578.131	5.664	0.019	0.049
	HDL cholesterol	521.555	1	521.555	2.658	0.106	0.024
	LDL cholesterol	4195.735	1	4195.735	3.765	0.055	0.033
	Triglycerides	21.663	1	21.663	0.003	0.954	0.000
	ApoA	828.789	1	828.789	1.397	0.240	0.013
	ApoB	853.410	1	853.410	2.073	0.153	0.018
	ApoB on ApoA	0.004	1	0.004	0.126	0.723	0.001
	FC	351.726	1	351.726	4.933	0.028	0.043
	Zscore (LCAT) LCAT	0.250	1	0.250	0.238	0.626	0.002
	Zscore (RCTreal)	1.041	1	1.041	1.071	0.303	0.010
DM drug	Cholesterol	14076.205	1	14076.205	10.521	0.002	0.087
	HDL cholesterol	42.582	1	42.582	0.217	0.642	0.002
	LDL cholesterol	9203.380	1	9203.380	8.258	0.005	0.070
	Triglycerides	3426.451	1	3426.451	0.525	0.470	0.005
	ApoA	222.565	1	222.565	0.375	0.541	0.003
	ApoB	2977.946	1	2977.946	7.234	0.008	0.062
	ApoB on ApoA	0.133	1	0.133	4.190	0.043	0.037
	FC	366.147	1	366.147	5.135	0.025	0.045
	Zscore (LCAT) LCAT	0.619	1	0.619	0.590	0.444	0.005
	Zscore (RCTreal)	0.715	1	0.715	0.735	0.393	0.007
Vitamins	Cholesterol	1141.546	1	1141.546	0.853	0.358	0.008
	HDL cholesterol	135.673	1	135.673	0.691	0.407	0.006
	LDL cholesterol	139.381	1	139.381	0.125	0.724	0.001
	Triglycerides	15018.092	1	15018.092	2.301	0.132	0.020
	ApoA	628.024	1	628.024	1.059	0.306	0.010
	ApoB	7.273	1	7.273	0.018	0.894	0.000
	ApoB on ApoA	0.004	1	0.004	0.116	0.734	0.001
	FC	162.136	1	162.136	2.274	0.134	0.020
	Zscore (LCAT) LCAT	1.717	1	1.717	1.636	0.204	0.015
	Zscore (RCTreal)	0.047	1	0.047	0.048	0.827	0.000
Calcium	Cholesterol	5639.655	1	5639.655	4.215	0.042	0.037
	HDL cholesterol	128.950	1	128.950	0.657	0.419	0.006
	LDL cholesterol	3817.332	1	3817.332	3.425	0.067	0.030
	Triglycerides	13968.389	1	13968.389	2.140	0.146	0.019
	ApoA	226.915	1	226.915	0.382	0.538	0.003
	ApoB	1950.028	1	1950.028	4.737	0.032	0.041
	ApoB on ApoA	0.128	1	0.128	4.014	0.048	0.035
	FC	337.310	1	337.310	4.731	0.032	0.041
	Zscore (LCAT) LCAT	0.531	1	0.531	0.506	0.479	0.005
	Zscore (RCTreal)	0.952	1	0.952	0.980	0.324	0.009
Fish oil	Cholesterol	604.847	1	604.847	0.452	0.503	0.004
	HDL cholesterol	12.638	1	12.638	0.064	0.800	0.001
	LDL cholesterol	2171.507	1	2171.507	1.948	0.166	0.017
	Triglycerides	2177.275	1	2177.275	0.334	0.565	0.003
	ApoA	200.646	1	200.646	0.338	0.562	0.003
	ApoB	476.342	1	476.342	1.157	0.284	0.010
	ApoB on ApoA	0.042	1	0.042	1.316	0.254	0.012
	FC	0.143	1	0.143	0.002	0.964	0.000
	Zscore (LCAT) LCAT	1.133	1	1.133	1.080	0.301	0.010
	Zscore (RCTreal)	0.016	1	0.016	0.016	0.899	0.000
Error	Cholesterol	147175.880	110	1337.963			
	HDL cholesterol	21582.639	110	196.206			
	LDL cholesterol	122597.484	110	1114.523			
	Triglycerides	717998.018	110	6527.255			
	ApoA	65260.068	110	593.273			
	ApoB	45280.487	110	411.641			
	ApoB on ApoA	3.495	110	0.032			
	FC	7843.553	110	71.305			
	Zscore (LCAT) LCAT	115.403	110	1.049			
	Zscore (RCTreal)	106.888	110	0.972			

Total	Cholesterol	5317221.000	119
	HDL cholesterol	451659.000	119
	LDL cholesterol	2084159.000	119
	Triglycerides	2010506.000	119
	ApoA	2927063.000	119
	ApoB	1003741.000	119
	ApoB on ApoA	46.204	119
	FC	201171.262	119
	Zscore (LCAT) LCAT	119.999	119
	Zscore (RCTreal)	119.870	119
Corrected total	Cholesterol	206591.412	118
	HDL cholesterol	25296.571	118
	LDL cholesterol	159724.639	118
	Triglycerides	764665.866	118
	ApoA	79237.109	118
	ApoB	54723.109	118
	ApoB on ApoA	3.994	118
	FC	10536.039	118
	Zscore (LCAT) LCAT	119.999	118
Zscore (RCTreal)	119.868	118	

- a. R Squared = 0.288 (Adjusted R Squared = 0.236)
- b. R Squared = 0.147 (Adjusted R Squared = 0.085)
- c. R Squared = 0.232 (Adjusted R Squared = 0.177)
- d. R Squared = 0.061 (Adjusted R Squared = -0.007)
- e. R Squared = 0.176 (Adjusted R Squared = 0.116)
- f. R Squared = 0.173 (Adjusted R Squared = 0.112)
- g. R Squared = 0.125 (Adjusted R Squared = 0.061)
- h. R Squared = 0.256 (Adjusted R Squared = 0.201)
- i. R Squared = 0.038 (Adjusted R Squared = -0.032)

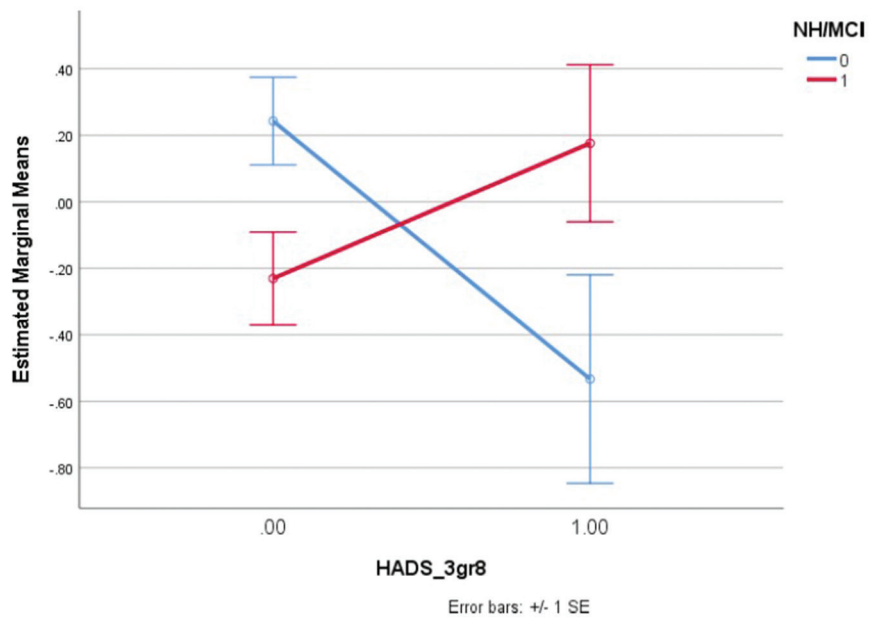
Estimates

95% Confidence Interval

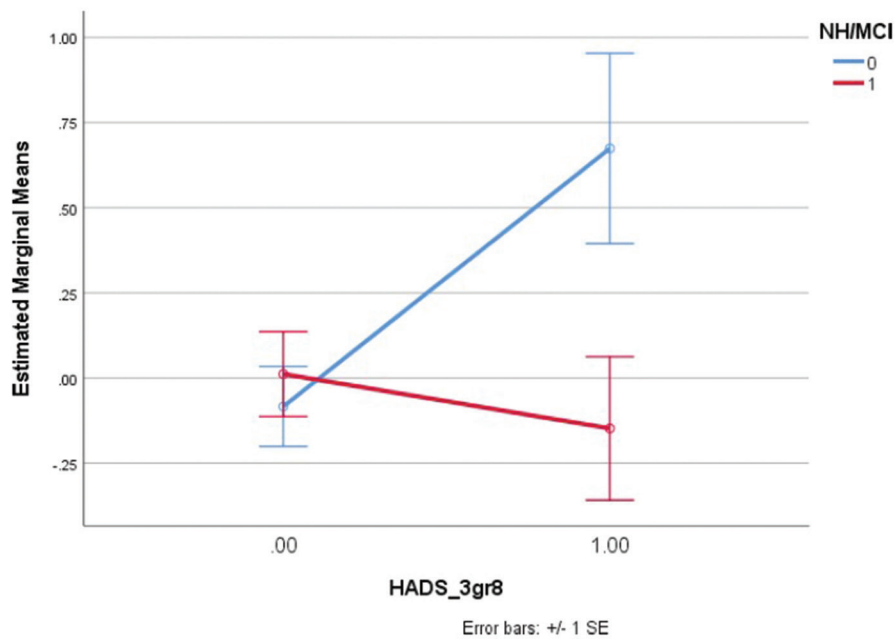
Dependent variable	Diabetes Med	Mean	Std. Error	Lower bound	Upper bound
Cholesterol	No	211.632 ^a	3.617	204.465	218.800
	Yes	178.928 ^a	9.349	160.401	197.456
HDL cholesterol	No	60.099 ^a	1.385	57.354	62.844
	Yes	58.300 ^a	3.580	51.205	65.395
LDL cholesterol	No	130.724 ^a	3.301	124.182	137.265
	Yes	104.279 ^a	8.533	87.369	121.189
Triglycerides	No	104.489 ^a	7.989	88.657	120.320
	Yes	88.353 ^a	20.650	47.430	129.276
ApoA	No	155.250 ^a	2.408	150.478	160.023
	Yes	151.138 ^a	6.226	138.801	163.476
ApoB	No	91.325 ^a	2.006	87.349	95.301
	Yes	76.283 ^a	5.186	66.006	86.559
ApoB on ApoA	No	0.609 ^a	0.018	0.574	0.644
	Yes	0.509 ^a	0.046	0.418	0.599
FC	No	40.734 ^a	0.835	39.079	42.389
	Yes	35.459 ^a	2.158	31.182	39.737
LCAT	No	80.682 ^a	0.266	80.154	81.209
	Yes	80.112 ^a	0.688	78.748	81.475
Zscore (RCTreal)	No	0.035 ^a	0.097	-0.158	0.228
	Yes	-0.198 ^a	0.252	-0.697	0.301

No/Yes: use of antidiabetic drugs versus no drugs.

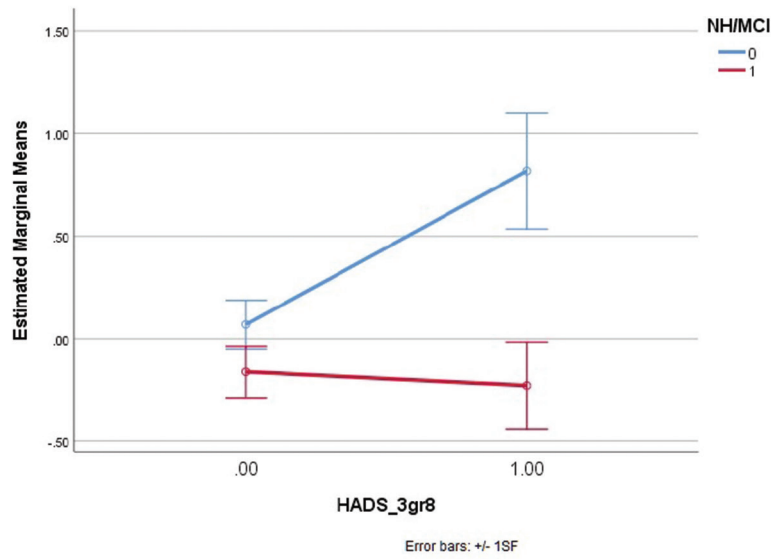
Supplementary figures



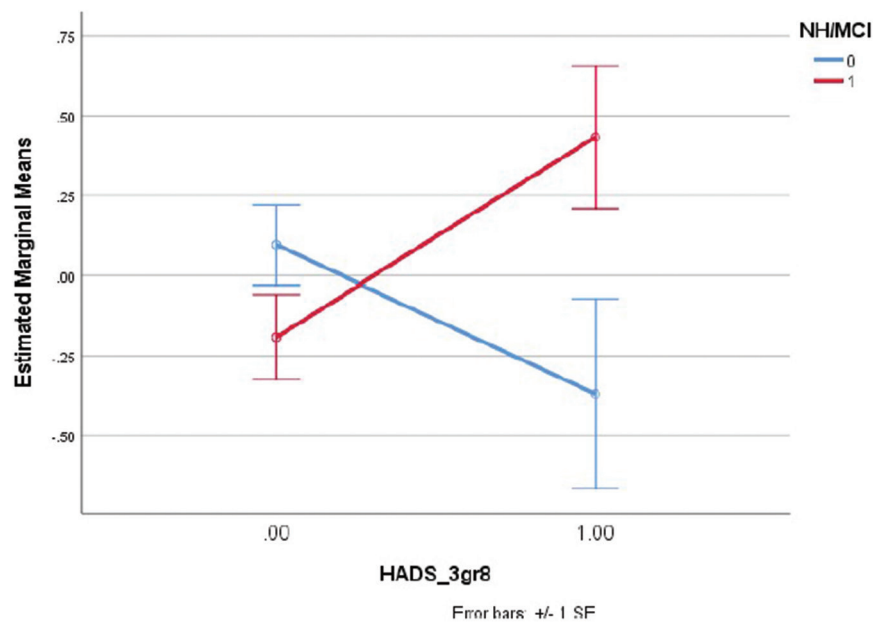
Supplementary Figure 1. Effects of the interaction between amnesic mild cognitive impairment (aMCI) and HADS-D groups (cut-off 8) on triglyceride levels [NH=normal controls (0) versus aMCI (1); the analysis is adjusted for age, sex, and body mass index and performed using the residualized values after regression on the drug state variables].



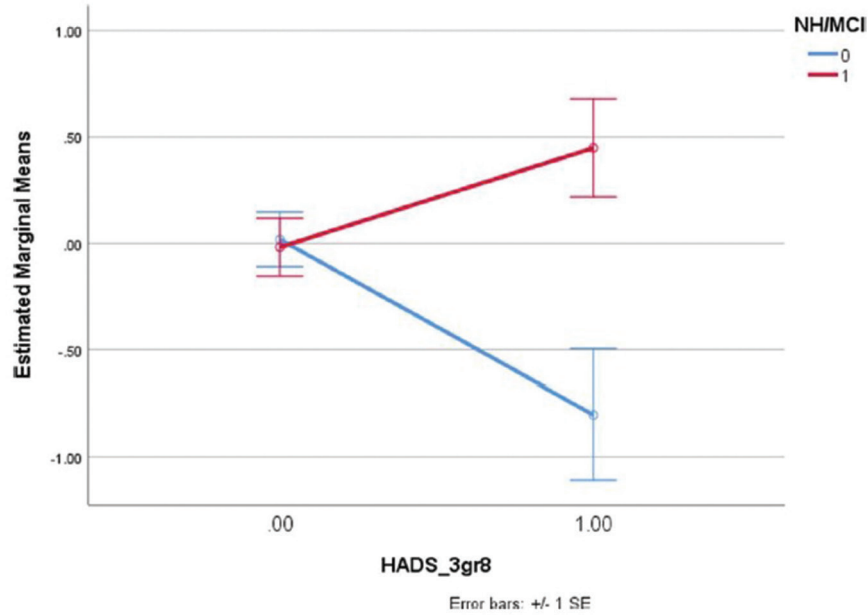
Supplementary Figure 2. Effects of the interaction between amnesic mild cognitive impairment (aMCI) and HADS-D groups (cut off of 8) on high-density lipoprotein. [NH=normal controls (0) versus aMCI (1); the analysis is adjusted for age, sex and body mass index and performed using the residualized values after regression on the drug state variables].



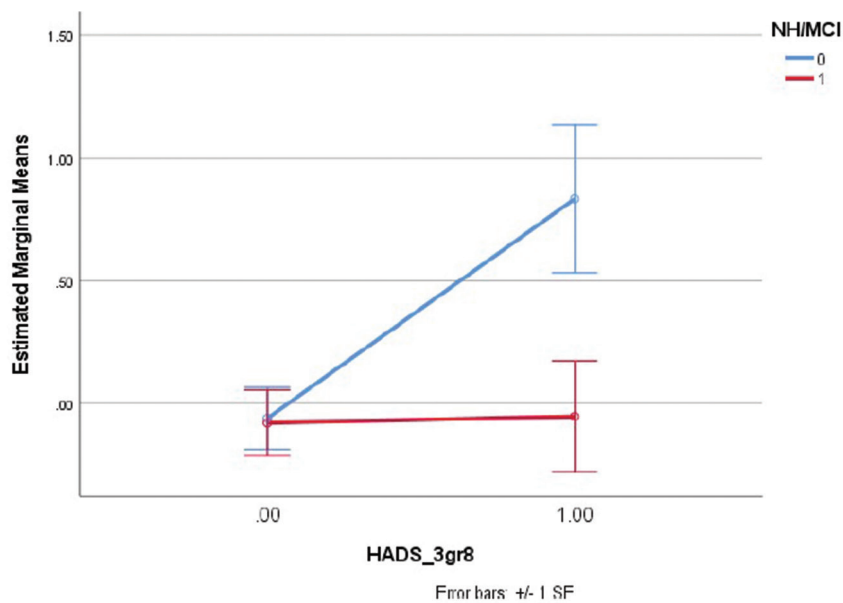
Supplementary Figures 3. Effects of the interaction between amnesic mild cognitive impairment (aMCI) and HADS-D (cut off of 8) groups on apolipoprotein A [NH=normal controls (0) versus aMCI (1); the analysis is adjusted for age, sex and body mass index and performed using the residualized values after regression on the drug state variables].



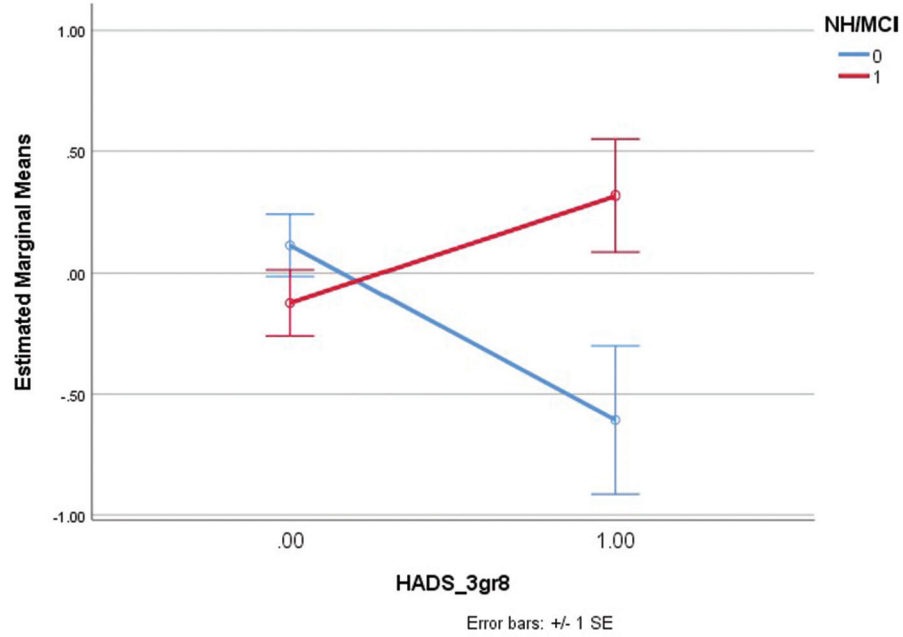
Supplementary Figure 4. Effects of the interaction between amnesic mild cognitive impairment (aMCI) and HADS-D groups (cut off of 8) on apolipoprotein B. [NH=normal controls (0) versus aMCI (1); the analysis is adjusted for age, sex and body mass index and performed using the residualized values after regression on the drug state variables].



Supplementary Figure 5. Effects of the interaction between amnesic mild cognitive impairment (aMCI) and HADS-D groups (cut off equals 8) on the apolipoprotein B (ApoB) / ApoB ratio [NH=normal controls (0) versus aMCI (1); the analysis is adjusted for age, sex and body mass index and performed using the residualized values after regression on the drug state variables].



Supplementary Figure 6. Effects of the interaction between amnesic mild cognitive impairment (aMCI) and HADS-D groups (cut off of 8) on the reverse cholesterol transport ratio [NH=normal controls (0) versus aMCI (1); the analysis is adjusted for age, sex and body mass index and performed using the residualized values after regression on the drug state variables].



Supplementary Figure 7. Effects of the interaction between amnesic mild cognitive impairment (aMCI) and HADS-D groups (cut off score of 8) on the Castelli risk index 1 [NH=normal controls (0) versus aMCI (1); the analysis is adjusted for age, sex and body mass index and performed using the residualized values after regression on the drug state variables].