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Original Article

Reduced Blood Choline in Obesity Is Associated with Metabolic and Alzheimer's Biomarkers

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ABSTRACT: Rising obesity rates pose significant concerns for aging and brain health. Insulin resistance (IR), prevalent in both obesity and Alzheimer's disease (AD), accelerates neurodegeneration. Adequate choline intake may help reduce obesity risk and IR, yet many individuals consume less than recommended—a deficiency associated with increased AD risk. Here, we examined circulating blood choline, metabolic dysfunction markers, inflammatory cytokines, and neurofilament light (NfL), a protein that is used as a prognostic marker for neuronal damage, in young-adult participants (mean age 33.6 years) with obesity (BMI > 30) versus healthy BMI (18.5–24.9) controls using a cross-sectional design. We also validated whether circulating choline levels correlate with NfL in a cohort of patients with mild cognitive impairment (MCI) with presence of either sparse or high neuritic plaque density and Braak stage and a second cohort with either moderate AD (moderate to frequent neuritic plaques, Braak stage = IV) or severe AD (frequent neuritic plaques, Braak stage = VI), compared to age-matched controls. We found that obese participants showed reduced circulating choline, correlating with higher %Body Fat, liver dysfunction markers, increased IR, and elevated inflammatory cytokines. NfL levels were elevated in obese participants and negatively correlated with circulating choline levels, findings consistent with that observed in MCI and AD cases. These findings reveal correlations between obesity, low choline, IR, systemic inflammation and NfL—key AD risk markers. Monitoring such markers in early adulthood may be useful for assessing future AD risk in individuals prone to obesity.

Keywords: Obesity, glucose, choline, Alzheimer's disease, neuroinflammation

INTRODUCTION

Obesity is a global pandemic whose prevalence continues to increase[1]. It is more common in women than in men, and this discrepancy increases with age [1]. Obesity is defined as a body mass index (BMI) of 30.0 kg/m² or higher and is associated with chronic health issues, including type 2 diabetes (T2D), chronic systemic inflammation, and neurodegenerative disorders [2]. A deeper understanding of how obesity contributes to these conditions and mechanisms is crucial, as early intervention could prevent or reduce the risk of developing more severe sequelae.

Insulin resistance (IR) is one of the most insidious metabolic dysfunctions that accompanies obesity. Obesity contributes to IR by increasing visceral fat which secretes pro-inflammatory cytokines, disrupting metabolism [3]. Insulin, secreted by pancreatic β-cells [4], promotes

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cellular glucose uptake by binding to insulin receptors and activating downstream signaling pathways such as PI3K–AKT [4, 5]. Insulin maintains glucose homeostasis by inhibiting hepatic glucose production and promoting glycogen, lipid, and protein synthesis [5]. IR occurs when cells become less responsive to insulin [3] and is associated with lipid accumulation in the liver and skeletal muscle, endoplasmic reticulum stress, and chronic inflammation, resulting in hyperglycemia, dyslipidemia, and hypertension [5]. A deeper understanding of the causes of IR could provide strategies to mitigate its adverse health impacts.

IR is a major risk factor for Alzheimer's Disease (AD), which currently affects 6.9 million adults aged 65 and older in the U.S. [6] AD is characterized by amyloid beta (Aβ) plaques, neurofibrillary tau tangles, and chronic systemic inflammation, resulting in memory loss and cognitive impairment [6]. Brain IR contributes to obesity by disrupting the brain's ability to regulate appetite and energy expenditure and contributes to cognitive decline by promoting Aβ plaque accumulation, tau hyperphosphorylation, and neurodegeneration—all hallmarks of AD [7]. In the brain, the PI3K/AKT/GSK-3β signaling pathway regulates tau phosphorylation, neuronal survival, and Aβ clearance [8], and is dysregulated by IR. Neurofilament light chain (NfL), a critical structural component of axons whose elevated circulating levels indicate axonal injury and can serve as a marker of neurodegeneration (deterioration and death of neurons), has been shown to be elevated in T2D [9]. Therefore, IR facilitates the progression of AD pathological features, and is a target for interventions to prevent AD.

Lifestyle factors contribute significantly to obesity. Dietary intake of choline has emerged as an area of interest due to its potential to mitigate IR, inflammation, and AD [10–18]. Choline plays critical roles across multiple biological systems, including lipid metabolism, neurotransmission (as a precursor of acetylcholine), and membrane phospholipid synthesis [11, 19]. While 30% of the required choline is produced endogenously by phosphatidylethanolamine N-methyltransferase (PEMT), the remainder must be acquired dietarily [19]. Alarmingly, 90% of Americans fail to meet the recommended daily intake of choline [20]. Recent studies highlight the association between higher dietary choline intake and a lower risk of developing IR [12] and T2D [13]. In healthy BMI adults, choline intake exceeding 310-mg/day is associated with reduced inflammatory markers—26% lower IL-6 and 6% lower TNF-α—which are implicated in metabolic dysfunction [18]. Similarly, higher levels of circulating choline are associated with reduced TNF-α in patients with mild cognitive impairment [10]. While dietary choline intake and circulating blood choline levels are often published independently, a recent double-blind randomized controlled choline feeding study in humans showed that choline concentrations are robust biomarkers of dietary choline intake [21]. In AD mice, a choline-deficient diet throughout adulthood led to elevated TNF-α [10], while dietary choline supplementation reduced inflammation and attenuated Aβ pathology and cognitive decline [16]. This highlights that adequate choline intake may offer a promising strategy to prevent or mitigate the interconnected pathologies of obesity, IR, chronic inflammation, and AD.

In this study, we explored the relationship between early to mid-life obesity, metabolic dysfunction, circulating choline, inflammation profiles, and a biomarker of neuronal axonal damage in blood plasma from individuals with obesity (BMI >30 kg/m^2) compared to age-matched individuals with healthy BMI (18.5-24.9 kg/m^2). We also validated whether circulating choline levels correlate with NfL in a cohort of patients with MCI and a cohort with either moderate or severe AD compared to age-matched controls. We hypothesized that obese participants would exhibit reduced circulating choline levels, which would correlate and be associated with markers of insulin resistance, elevated blood cytokines, proteins linked to liver dysfunction, and increased NfL levels—with the negative correlation between choline and NfL validated in individuals with MCI and AD.

MATERIALS AND METHODS

Human Participants

Healthy and obese participants were recruited through online and paper advertisements from the greater Phoenix metropolitan area in Arizona and assigned to the appropriate group based on their body mass index (BMI). Participants included individuals with BMI 18.5–24.9 kg/m^2 (healthy, n =15) and individuals with BMI > 30 kg/m^2 (obese, n = 15). The human studies were performed after obtaining approval from the Institutional Review Board at Mayo Clinic (IRB#: 20-003294). Written informed consent was obtained from each participant prior to any study procedures. Study participants were determined to be healthy based on medical history, routine physical examination, electrocardiogram, standard blood tests, and urinalysis. Those with diabetes, history of liver, renal, or heart disease were excluded from the study, as well as those who instead of that smoked, participated in a weight-loss regimen, took nutritional supplements, or used prescription or over-the-counter medications. Body composition, including percent body fat, was measured using bioelectrical impedance analysis (BIA) (BIA 310e, Biodynamics Corp., Shoreline, Washington). Blood

samples were collected from participants after a 12-hour fasting period and processed at the Mayo Clinic Laboratory, including measurements of hemoglobin A1C and triglycerides.

Human serum samples for patients with MCI and AD were obtained from the Arizona Study of Aging and Neurodegenerative Disorders/Brain and Body Donation Program [22]. Blood was collected at post-mortem via cardiac puncture and processed for serum. Pathological assessment of human cases was performed as previously described [10, 22]. MCI and AD cases were classified based on CERAD neuritic plaque density and Braak stage, a measure of tau, into healthy controls with Braak stage ≤ III (CON, n = 12 with APOE ε2/ε2 = 1, ε2/ε3 = 2, ε3/ε3 = 6, ε3/ε4 = 2, ε4/ε4 = 1), MCI with sparse pathology (MCI Sparse; sparse CERAD neuritic plaque density and Braak stage = II - III; n = 11 with APOE ε2/ε2 = 1, ε2/ε3 = 2, ε3/ε3 = 4, ε3/ε4 = 3, ε4/ε4 = 0, n = 1 not disclosed), MCI with high pathology (MCI High; frequent CERAD neuritic plaque density and Braak stage = IV - V; n = 12 with ε2/ε2 = 0, ε2/ε3 = 3, ε3/ε3 = 7, ε3/ε4 = 2, ε4/ε4 = 0), moderate AD (AD Mod; moderate to frequent CERAD neuritic plaque density and Braak stage = IV ; n = 12 with ε2/ε2 = 0, ε2/ε3 = 2, ε3/ε3 = 6, ε3/ε4 = 4 , ε4/ε4 = 0), and severe AD (AD Sev; frequent CERAD neuritic plaque density and Braak stage = VI; n = 12 with ε2/ε2 = 0, ε2/ε3 = 1, ε3/ε3 = 4, ε3/ε4 = 4, ε4/ε4 = 2, n = 1 not disclosed). MCI cases did not meet clinicopathological criteria for an AD diagnosis while AD Mod and AD Sev corresponded with National Institute on Aging-Regan Institute (NIA-RI) Intermediate and High classifications, respectively [10]. The cohort used in the present study has been previously described [10].

Multiplex Cytokine analysis

To assess the levels of cytokines in humans, we used a Bioplex human cytokine 11-plex kit (Bio-Rad, cat. no. #12003080) as previously described [23]. Briefly, plasma samples were diluted 1:10 in sample diluent, added in duplicate to a 96-well plate, and assayed according to the manufacturer's instructions. Measurements and data analysis were performed using the Bio-Plex Manager software (version 6.1). Standard curves were plotted using five-parameter logistic regression and concentrations were calculated accordingly. Cytokine levels that were not detected based on manufacture limits were not included in the analysis.

ELISA and Plate Assays

Plasma glucose concentration was measured using an automated glucose analyzer (YSI 2300, Yellow Springs, OH). Plasma insulin was measured using a commercial ELISA assay (Alpco Diagnostics Cat# 80-INSHU-E01.1) and used to calculate the Homeostatic Model Assessment for IR (HOMA-IR). Choline levels were measured with a commercially available colorimetric kit (Abcam, ab219944), following manufacturer guidelines, as previously described [10, 11]. Aldolase B activity was also assessed using a colorimetric assay kit from Millipore Sigma (MAK223). Commercially available ELISA kits were used to measure sorbitol dehydrogenase levels in human plasma samples (Abcam ab233613), human NfL levels (LS Bio LS-F6701-1) following the manufacturer's guidelines.

Statistical Analysis

Data were analyzed using GraphPad Prism (version 10.3.1). Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. Data that did not meet these assumptions were transformed using either square root or $\log_{10}$ transformations prior to analysis, as previously described [24, 25]. Statistical outliers were identified and excluded using the Robust Regression and Outlier Removal (ROUT) test in GraphPad Prism. One case from the obese BMI group did not have a detectable choline measure, and one case from the MCI Sparse group was excluded as an outlier based on choline levels. Group differences for the obese versus healthy BMI cohort were analyzed using two-way factorial ANOVA, followed by recommended post hoc comparisons when appropriate. For the CON, MCI, and AD groups, a one-way ANOVA was conducted, followed by post hoc pairwise comparisons with correction for multiple testing across six comparisons. Effect sizes for group differences were calculated using partial eta squared (η^2_p) and interpreted using the following guidelines: $\eta^2_p = 0.01$ indicates a small effect, $\eta^2_p = 0.06$ a medium effect, and $\eta^2_p = 0.14$ a large effect [26]. Linear correlations were calculated using Pearson's *r* coefficient, and associations were assessed using simple linear regression. Statistical significance was set at $p < 0.05$.

RESULTS

Obese participants exhibit metabolic disturbances, including IR

Subject profiles are detailed in Table 1. Participants were both age-matched and balanced for sex and included different racial and ethnic backgrounds (healthy males $n = 7$, $M_{age} = 35.86$ years, 1 African American and 6 Caucasians; healthy females $n = 8$, $M_{age} = 34.13$ years, 1 Hispanic and 7 Caucasians; obese males $n = 8$, $M_{age} = 35.14$ years, 3 Hispanics and 5 Caucasians; obese females $n = 7$, $M_{age} = 29.14$ years, 1 Hispanic, 1 African American

and 5 Caucasians). Obese participants had significantly higher body weight ($F_{(1,26)}$ = 52.54, p < 0.0001, η^2_p = 0.674 large effect) and BMI ($F_{(1,26)}$ = 141.6, p < 0.0001, η^2_p = 0.845 large effect) than healthy participants. Obese participants exhibited higher %Body Fat ($F_{(1,26)}$ = 76.11, p < 0.0001, η^2_p = 0.745 large effect) than healthy participants, and women had higher %Body Fat ($F_{(1,26)}$ = 83.78, p < 0.0001, η^2_p = 0.763 large effect) than men. There were also notable differences in common markers of metabolic health (Fig. 1A). Triglyceride levels were higher in obese ($F_{(1, 25)}$ = 5.543, p = 0.0267, η^2_p = 0.1814 large effect) than in healthy participants, and an interaction between BMI classification and sex ($F_{(1,25)}$ = 6.225, p = 0.0196, η^2_p = 0.199 large effect) showed that obese men had higher triglyceride levels than both healthy men (p = 0.0017) and obese women (p = 0.0081). Fasting glucose levels and A1C – a metric of average glucose levels over the last three months [27] – were higher in obese participants but did not reach statistical significance. Insulin levels ($F_{(1, 26)}$ = 23.13, p < 0.0001, η^2_p = 0.471 large effect) and HOMA-IR ($F_{(1, 26)}$ = 24.00, p < 0.0001, η^2_p = 0.480 large effect) – a metric of IR [28] – were both higher in obese participants compared to healthy participants, indicating IR in obese participants. These results highlight metabolic disturbances in the obese participants.

Table 1. Characteristics of participants classified as controls with a healthy BMI compared to those classified as obese BMI.

Characteristic	Healthy Male	Healthy Female	Obese Male	Obese Female	*BMI p* value
	n = 7	n = 8	n = 8	n = 7	-------
Age (years)	M = 35.86, SD = 11.50	M = 34.13, SD = 9.11	M = 35.14, SD = 7.54	M = 29.14, SD = 10.93	0.4481
Body weight (kg)	M = 72.76, SD = 11.55	M = 65.91, SD = 10.91	M = 102.96, SD = 10.34	M = 101.60, SD = 16.47	<0.0001****
BMI	M = 23.40, SD = 2.62	M = 23.66, SD = 2.33	M = 34.54, SD = 3.31	M = 36.77, SD = 2.76	<0.0001****
%Body Fat	M = 16.40, SD = 2.16	M = 29.59, SD = 3.79	M = 29.01, SD = 4.95	M = 40.36, SD = 2.84	0.0003***
Triglycerides	M = 85.43, SD = 65.67	M = 97.38, SD = 48.79	M = 190.63, SD = 82.73	M = 91.67, SD = 27.88	0.0267*
Fasting glucose	M = 83.62, SD = 6.82	M = 86.78, SD = 8.25	M = 91.08, SD = 13.55	M = 95.47, SD = 14.45	0.0632
A1C	M = 5.29, SD = 0.41	M = 5.20, SD = 0.26	M = 5.50, SD = 0.24	M = 5.56, SD = 0.62	0.0529
Insulin	M = 3.96, SD = 1.61	M = 4.19, SD = 1.83	M = 12.75, SD = 10.16	M = 10.48, SD = 4.21	<0.0001****
HOMA-IR	M = 0.82, SD = 0.38	M = 0.90, SD = 0.46	M = 2.94, SD = 2.37	M = 2.40, SD = 0.98	<0.0001****

Blood measures reported were taken after a 12-hour fasting period. *Abbreviations:* Body mass index (BMI), Homeostatic Model Assessment for Insulin Resistance (HOMA-IR).

Plasma choline is reduced in obese participants and correlates with markers of metabolic dysfunction

We next measured blood plasma choline levels and found that obese participants had lower choline levels than healthy participants (Fig. 1B; $F_{(1,25)}$ = 102.4, p < 0.0001, η^2_p = 0.804 large effect). Notably, we found lower choline levels in women than in men ($F_{(1, 25)}$ = 6.310, p = 0.0188, η^2_p = 0.202 large effect), consistent with reports highlighting that women consume less dietary choline than men [20]. Next, we conducted correlation analyses to examine the relationships between choline levels and various metabolic metrics and simple linear regression analyses to determine whether choline levels significantly predicted differences in the various dependent measures. Higher BMI ($r_{(27)}$ = -0.8293, p < 0.0001) and %Body Fat ($r_{(27)}$ = -0.6735, p < 0.0001) were correlated with lower plasma choline (Fig. 1C, D). A simple linear regression revealed significant relationships between choline and BMI $F_{(1,27)}$ = 59.47, p < 0.0001, R^2 = 0.6878 and %Body Fat $F_{(1,27)}$ = 22.41, p < 0.0001, R^2 = 0.4536, indicating that plasma choline explained approximately 48.80% and 45.36% of the variance in BMI and %Body Fat, respectively. There were no significant correlations nor regressions between choline levels and glucose or A1C (Fig. 1E, F). Insulin (Fig. 1G; $r_{(27)}$ = -0.4720, p = 0.0097) and HOMA-IR (Fig. 1H; $r_{(27)}$ = -0.4092, p = 0.0275) were negatively correlated with choline levels, highlighting that as choline levels go down, these metabolic measures increase. A simple linear regression revealed significant relationships between choline and insulin ($F_{(1,27)}$ = 7.740, p = 0.0097, R^2 = 0.2228) and HOMA-IR ($F_{(1,27)}$ = 5.431, p = 0.0275, R^2 = 0.1675), indicating that plasma choline explained approximately 22.28% and 16.75% of

the variance in these measures, respectively. Together, these results demonstrate that obesity and IR metrics correspond with lower circulating choline levels, which may be tied to either (1) a higher need of choline for proper metabolic function and/or (2) an indication of higher need of dietary choline intake.

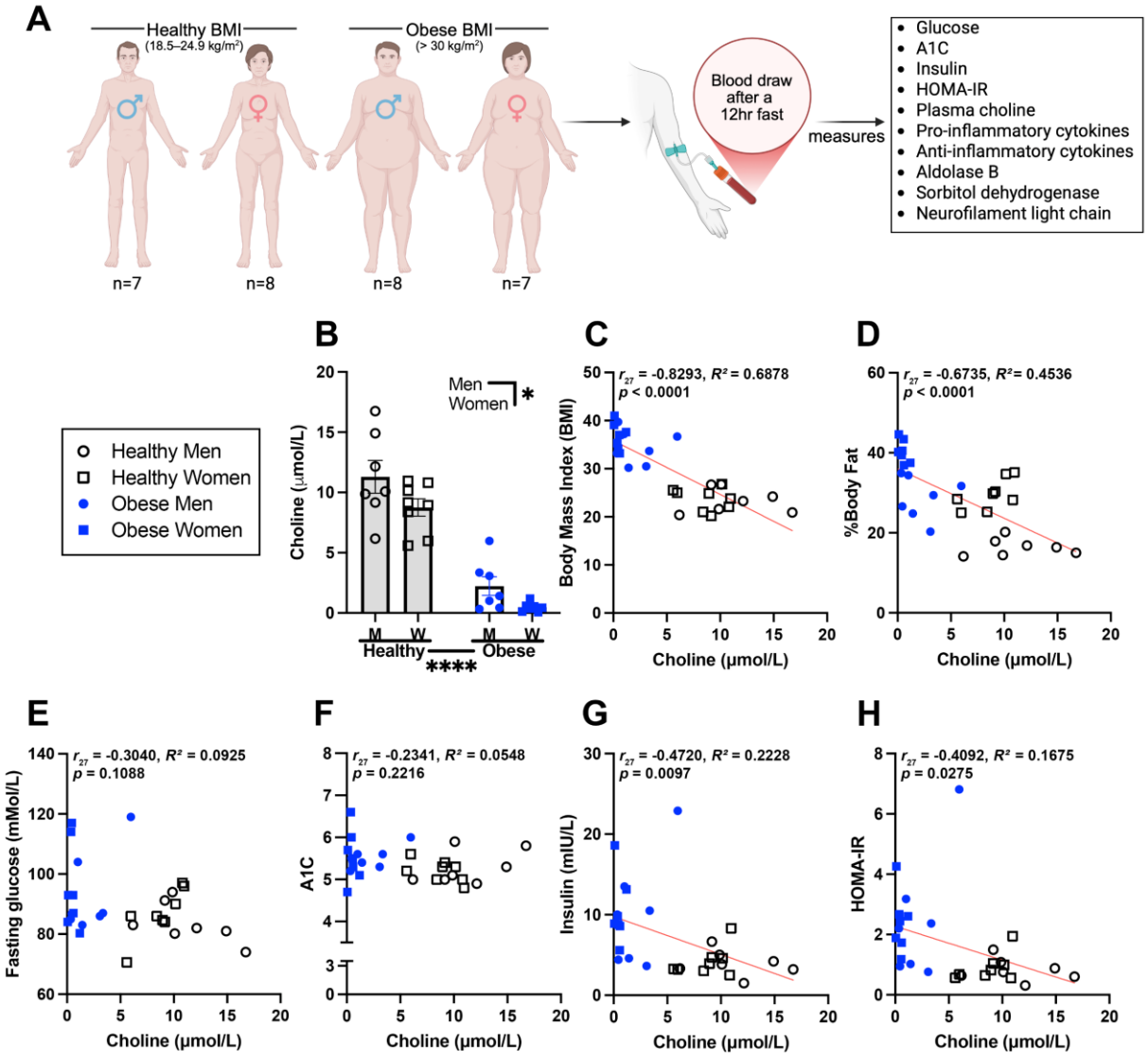


Figure 1. Plasma choline is reduced in obesity and negatively associates with key metabolic indicators. (A) Diagram depicting the healthy versus obese BMI study with endpoints collected. **(B)** Plasma choline levels were reduced in obese participants compared to healthy BMI participants and were lower in women than in men. The p-values were obtained using a two-way ANOVA, reporting significant main effects of BMI and sex with no significant interaction. **(C)** BMI and **(D)** %Body Fat were negatively correlated with plasma choline levels. Regression analyses confirmed significant negative associations for both measures. The p-values were derived from Pearson's r correlation and simple linear regression for this and all subsequent analyses in this panel. **(E)** Fasting glucose and **(F)** A1C were not significantly correlated with plasma choline levels. **(G)** Insulin and **(H)** HOMA-IR were negatively correlated with plasma choline. Regression analyses confirmed significant negative associations for both measures. Healthy BMI males n = 7, healthy BMI females n = 8, obese males n = 8, obese females n = 7. One case from the obese BMI group did not have a detectable choline measure and was not included in the analysis. Data are reported as means ± SEM. *p < 0.05, **P < 0.01, ***p<0.001, ****p < 0.0001.

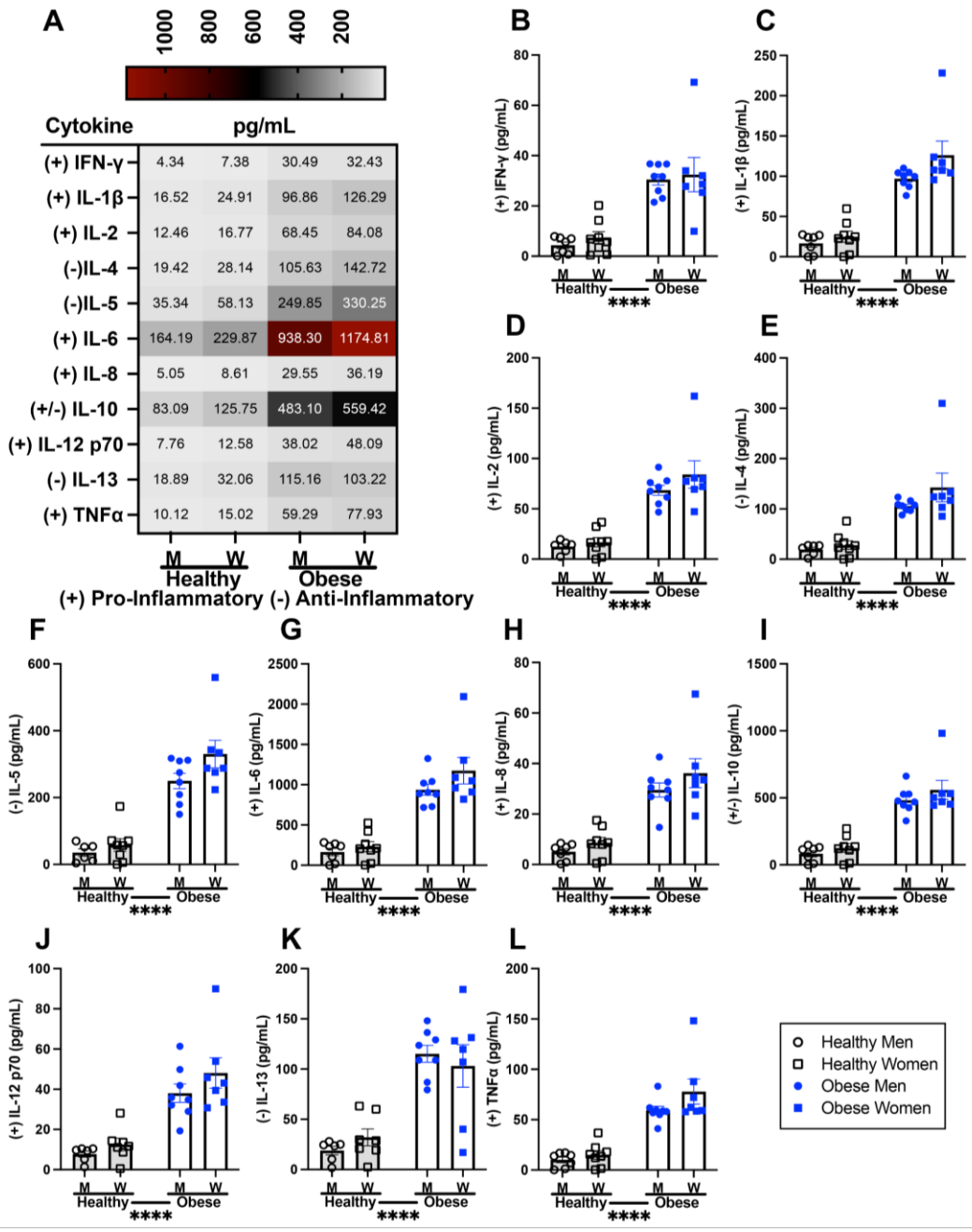


Figure 2. Eleven cytokines were elevated in obese compared to healthy participants. **(A)** A heat map of the cytokine panel illustrating the cytokines that were altered in obese participants. **(B)** IFN-γ, **(C)** IL-1β, **(D)** IL-2, **(E)** IL-4, **(F)** IL-5, **(G)** IL-6, **(H)** IL-8, **(I)** IL-10, **(J)** IL-12 p70, **(K)** IL-13, and **(L)** TNF-α were higher in obese participants than in healthy weight participants. The p-values were obtained using a two-way ANOVA, reporting significant main effect of BMI with no significant main effect of sex or interaction. Healthy BMI males n = 7, healthy BMI females n = 8, obese BMI males n = 8, obese BMI females n = 7. Cytokine levels that were not detected based on manufacture limits were not included in the analysis. Data are reported as means ± SEM. *p < 0.05, **p < 0.01, ***p<0.001, ****p < 0.0001.

Pro-and anti-inflammatory cytokine levels are elevated in obese participants

We next measured 11 cytokines that are known to be associated with IR and AD [3, 10, 23, 29–31] and found that all were significantly elevated in obese participants (Fig. 2A). The inflammatory cytokines IFN-γ (Fig. 2B; $F_{(1,26)} = 63.32$, $p < 0.0001$, $\eta^2_p = 0.709$ large effect), IL-1β (Fig. 2C; $F_{(1, 26)} = 90.28$, $p < 0.0001$, $\eta^2_p = 0.776$ large effect), and IL-2, (Fig. 2D; $F_{(1, 25)} = 24.21$, $p < 0.0001$, η^2_p

= 0.492 large effect) were significantly elevated in obese participants. Anti-inflammatory cytokines IL-4 (Fig. 2E; $F_{(1, 25)} = 73.98$, $p < 0.0001$, $\eta^2_p = 0.740$ large effect) and IL-5 (Fig. 2F; $F_{(1, 25)} = 94.75$, $p < 0.0001$, $\eta^2_p = 0.791$ large effect) were both significantly elevated in obese participants, indicating a potential compensatory mechanism attempting to restore homeostasis [3]. IR promoting cytokines IL-6 (Fig. 2G; $F_{(1, 26)} = 86.88$, $p < 0.0001$, $\eta^2_p = 0.770$ large effect) and IL-8 (Fig. 2H; $F_{(1, 26)} = 73.26$, $p < 0.0001$, $\eta^2_p = 0.737$ large effect) were significantly elevated in obese participants. IL-10, which, depending on the target tissue, can increase or decrease insulin sensitivity, (Fig. 2I; $F_{(1,26)} = 83.71$, $p < 0.0001$, $\eta^2_p = 0.763$ large effect) and IL-12 p70, the active form of IL-12, (Fig. 2J; $F_{(1, 24)} = 61.13$, $p < 0.0001$, $\eta^2_p = 0.718$ large effect) were significantly elevated in obese participants. The anti-inflammatory cytokine IL-13 (Fig. 2K; $F_{(1, 24)} = 44.39$, $p < 0.0001$, $\eta^2_p = 0.650$ large effect) was also significantly elevated in obese participants. Lastly, inflammatory cytokine TNF-α (Fig. 2L; $F_{(1, 26)} = 84.82$, $p < 0.0001$, $\eta^2_p = 0.765$ large effect) was significantly elevated in obese participants. To further investigate the relationship between circulating choline and cytokine levels, we ran correlations between circulating choline levels and cytokines. All cytokines were negatively correlated with choline (statistical outputs presented in Fig. 3A-K, $p < 0.0001$), indicating that as choline levels rise, such inflammatory molecules go down. Similarly, simple linear regression analyses revealed significant associations between plasma choline levels and cytokines, with choline accounting for 55.31% to 70.65% of the variance in all 11 cytokine levels measured ($p < 0.0001$). Together, these results highlight heightened inflammation in obese participants who also display IR and low circulating choline levels.

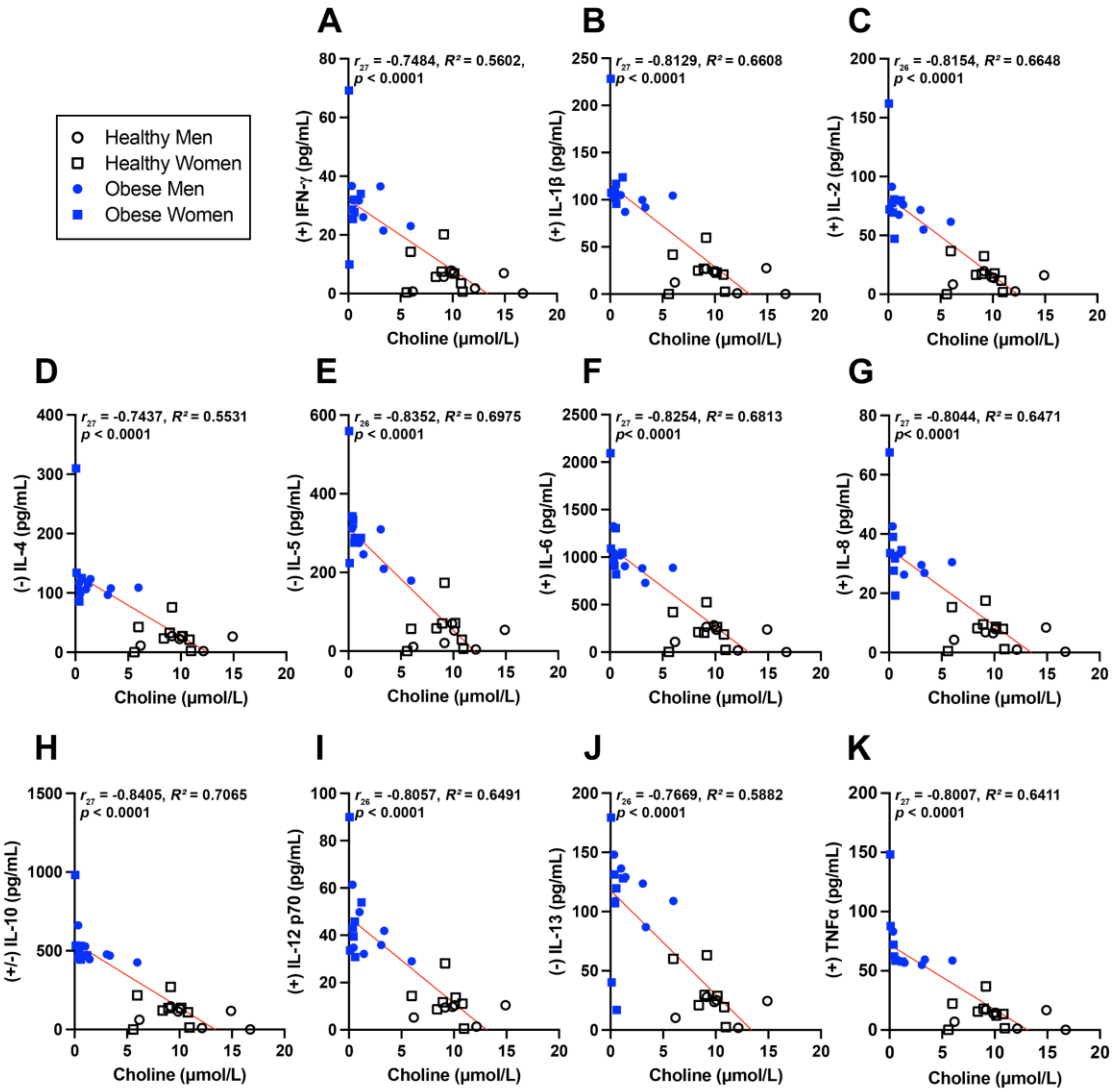


Figure 3. Correlations and simple linear regression analyses between 11 cytokines and choline levels in obese and healthy BMI participants. (A) IFN-γ, **(B)** IL-1β, **(C)** IL-2, **(D)** IL-4, **(E)** IL-5, **(F)** IL-6, **(G)** IL-8, **(H)** IL-10, **(I)** IL-12 p70, **(J)** IL-13, and **(K)** TNF-α were all negatively correlated with choline. Similarly, significant associations between plasma choline levels and cytokines were found, with choline accounting for 55.31% to 70.65% of the variance in all 11 cytokine levels measured. The p-values were derived from Pearson's r correlation and simple linear regression for all analyses in this panel. Healthy BMI males n = 7, healthy BMI females n = 8, obese males n = 8, obese females n = 7. One case from the obese BMI group did not have a detectable choline measure and was not included in the analysis. Cytokine levels that were not detected based on manufacture limits were not included in the analysis.

Enzymes that are indicative of liver dysfunction are elevated in the plasma of obese compared to healthy participants

We recently observed that enzymes aldolase B and sorbitol dehydrogenase were significantly elevated by a choline deficient diet in both NonTg (Log2 Fold Change = 2.25 for aldolase B; 1.62 for sorbitol dehydrogenase) and 3xTg-AD (Log2 Fold Change = 1.65 for aldolase B; 1.67 for sorbitol dehydrogenase) mice [11]. Aldolase B and sorbitol dehydrogenase are enzymes involved in carbohydrate metabolism and elevations in these enzymes in blood are indicative of liver injury and dysfunction [32, 33]. Aldolase B is expressed in the liver and contributes to the metabolism of fructose [33]. We found elevations in obese compared to healthy BMI participants (Fig. 4A; $F_{(1,26)} = 434.4$, $p < 0.0001$, $\eta^2_p = 0.944$ large effect). Moreover, aldolase B (Fig. 4B; $r_{(27)} = -0.8275$, $p < 0.0001$) negatively correlated with plasma choline. A simple linear regression revealed significant relationships between choline and aldolase B ($F_{(1,27)} = 56.63$, $p < 0.0001$, $R^2 = 0.6847$), indicating that plasma choline explained approximately 68.47% of the variance in aldolase B.

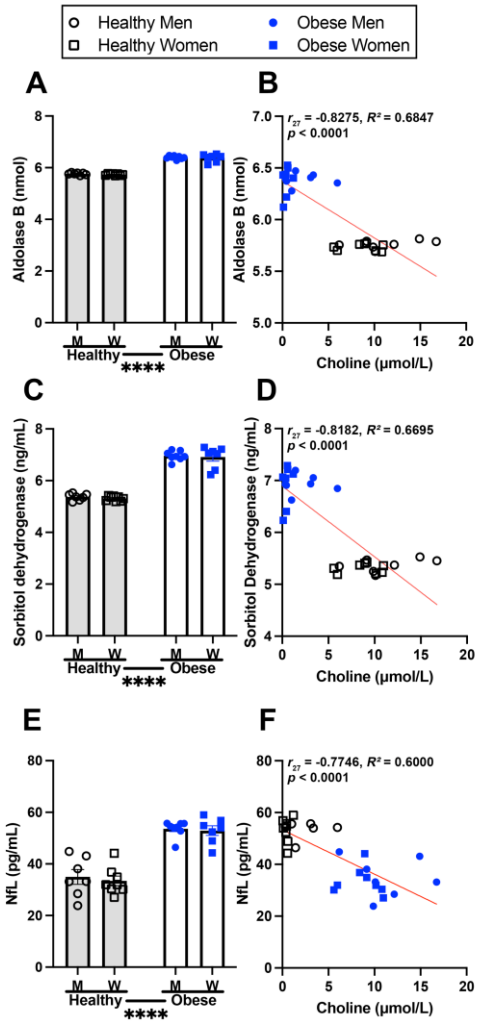
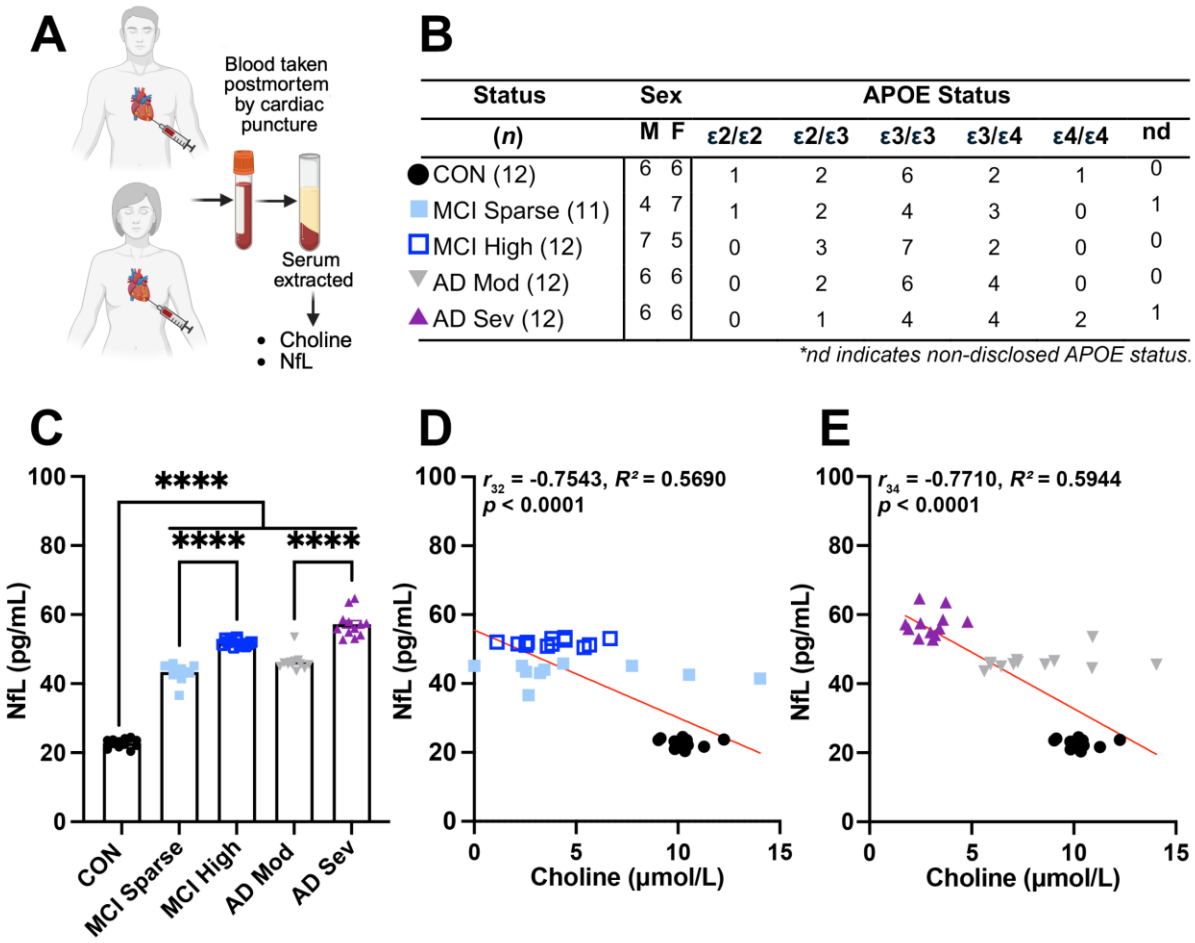


Figure 4. Liver enzymes, that indicate dysfunctional sugar metabolism, and a marker of neuronal damage are elevated in obese participants compared to healthy participants. (A) Aldolase B was elevated in obese compared to healthy participants and **(B)** negatively correlated with choline. **(C)** Sorbitol dehydrogenase was also elevated in obese and **(D)** negatively correlated with choline levels. **(E)** Neurofilament light chain (NfL), a marker of neuronal damage, was elevated in obese participants. **(F)** Choline levels were lower in cases with higher NfL. Similarly, significant associations were observed between plasma choline levels and aldolase B, sorbitol dehydrogenase, and NfL. For group comparisons, p-values were obtained using a two-way ANOVA, which revealed a significant main effect of BMI, with no significant effects of sex or interaction. For correlation and association analyses, p-values were derived from Pearson's r correlation and simple linear regression. Healthy BMI males n = 7, healthy BMI females n = 8, obese males n = 8, obese females n = 7. One case from the obese BMI group did not have a detectable choline measure and was not included in the analysis. Data are reported as means ± SEM. ****p < 0.0001.



Status	Sex		APOE Status					
(*n*)	M	F	ε2/ε2	ε2/ε3	ε3/ε3	ε3/ε4	ε4/ε4	nd
CON (12)	6	6	1	2	6	2	1	0
MCI Sparse (11)	4	7	1	2	4	3	0	1
MCI High (12)	7	5	0	3	7	2	0	0
AD Mod (12)	6	6	0	2	6	4	0	0
AD Sev (12)	6	6	0	1	4	4	2	1

*nd indicates non-disclosed APOE status.

Figure 5. Elevated blood serum NfL levels were significantly and negatively associated with choline concentrations in individuals with MCI and AD. (A) Diagram depicting the MCI, AD and aged-matched control (CON) endpoints derived from post-mortem blood serum samples from cardiac punctures. **(B)** Number of cases per condition, sex and APOE status for the choline and NfL analysis. **(C)** NfL levels were significantly elevated in MCI and AD cases. The p-values were obtained using a one-way ANOVA, followed by Bonferroni post hoc pairwise comparisons with correction for multiple testing across six comparisons. **(D, E)** Serum choline levels were lower in cases with higher NfL amongst the MCI, AD, and CON cases. For correlation and association analyses, p-values were derived from Pearson's r correlation and simple linear regression. Data are reported as means ± SEM. ****p < 0.0001.

Similarly, sorbitol dehydrogenase, which participates in the conversion of glucose into fructose [33], was also elevated in obese relative to healthy BMI participants (Fig. 4C; $F_{(1,26)}$ = 344.5, p < 0.0001, η^2_p = 0.930 large effect) and (Fig. 4D; $r_{(27)}$ = -0.8182, p < 0.0001) and negatively correlated with plasma choline. A simple linear regression revealed significant relationships between choline and sorbitol dehydrogenase ($F_{(1,27)}$ = 54.69, p < 0.0001, R^2 = 0.6695), indicating that plasma choline explained approximately 66.95% of the variance in sorbitol dehydrogenase. Together, this indicates IR and liver dysfunction in obese participants compared to healthy participants, and, since choline is important for maintaining liver health, suggests that low circulating choline levels may contribute to this dysfunction.

Obese participants show elevated NfL levels that negatively correlate with choline concentrations – a relationship validated in individuals with MCI and AD

Given that obesity and IR are risk factors for AD, we aimed to examine markers of neuronal axonal damage, specifically NfL, which is commonly used to track the progression of neurodegenerative disorders like AD—in relation to obesity, IR, and choline levels. We found significantly higher NfL in obese participants (Fig. 4E; $F_{(1,26)}$ = 93.70, p < 0.0001, η^2_p = 0.783 large effect), consistent with published reports [9]. Further, we found a strong negative correlation between NfL and choline levels in these participants (Fig. 4F; $r_{(27)}$ = -0.7746, p < 0.0001). A simple linear regression revealed significant relationships between choline and NfL within the obese

and healthy cases ($F_{(1,27)}$ = 40.50, p < 0.0001, R^2 = 0.6000), indicating that plasma choline explained approximately 60% of the variance in NfL. To validate whether such correlations exist in individuals with diagnosed neurological dysfunction, we assessed the relationship between NfL and plasma choline in a cohort of MCI and AD patients. We previously published work showing disease associated reductions in circulating choline levels that negatively correlated with disease severity in this cohort [10]. A one-way ANOVA revealed significant group differences (Fig. 5A, B; $F_{(4, 54)}$ = 356.1, p < 0.0001, η^2_p = 0.963 large effect), where CON had lower NfL levels than the MCI and AD groups (p < 0.0001). Additionally, NfL levels increased based on disease severity; NfL was higher in MCI High than MCI Sparse (p < 0.0001) and higher in AD Sev than in AD Mod (p < 0.0001). Consistent with our obese participant findings, elevated NfL correlated with lower choline levels in MCI (Fig 5C; $r_{(32)}$ = -0.7543, p < 0.0001) and AD (Fig 5D; $r_{(34)}$ = -0.7710, p < 0.0001) cases compared to CON, highlighting that NfL increases with disease severity and that higher neuronal damage corresponds with lower choline. A simple linear regression revealed significant relationships between choline and NfL within the MCI cases ($F_{(1,32)}$ = 42.25, p < 0.0001, R^2 = 0.5690) and AD cases ($F_{(1,34)}$ = 49.84, p < 0.0001, R^2 = 0.5944), indicating that serum choline explained approximately 56.90% and 59.44% of the variance in NfL, respectively. Taken together, these findings highlight that obesity is associated with elevations of a common marker of neuronal damage, detectable in early adulthood, which is inversely correlated with circulating choline levels. The combination of elevated blood NfL and low choline levels may serve as early biomarkers of metabolic dysfunction and increased risk for AD, potentially detectable decades before clinical onset.

DISCUSSION

Here, we demonstrate a relationship between early-life obesity, IR, circulating choline, and inflammation, emphasizing their potential as risk factors for disorders such as AD. Choline levels were lower in obese participants compared to those with a healthy BMI. Importantly, several metabolic indicators were elevated in the obese group, and body composition markers (BMI and %Body Fat) and insulin sensitivity markers (insulin levels and HOMA-IR) were negatively correlated as well as associated with choline levels. Obese participants also exhibited dysregulated inflammatory profiles; 11 cytokines were elevated. Additionally, levels of aldolase B and sorbitol dehydrogenase — liver enzymes involved in sugar metabolism — were higher in obese individuals and negatively correlated with choline levels, paralleling our previous findings in AD mice on a choline-deficient diet [11]. Evidence of neuronal axonal damage was observed in obese participants; as plasma NfL was elevated and inversely correlated with choline levels, this relationship was validated in independent MCI and AD cohorts. Of note, blood samples in the obese versus healthy BMI study were collected antemortem, while in the MCI and AD cohort they were obtained post-mortem via cardiac puncture; thus, comparisons of choline and NfL levels between these cohorts should be interpreted cautiously reflecting directional trends rather than absolute values. Collectively, these findings support the idea that low circulating choline levels may contribute to the metabolic and inflammatory dysfunctions associated with obesity and may increase the risk of neurodegenerative diseases.

IR in obese participants was correlated with reduced circulating choline levels, further emphasizing the link between metabolic dysfunction and choline deficiency. IR disrupts glucose metabolism and can lead to pancreatic β-cell dysfunction, hampering the pancreas's maintenance of normal glucose levels [4]. IR is prevalent in prediabetes, T2D, and AD, and is integral to the pathology of each condition by disrupting cellular metabolism and promoting inflammation [34, 35]. Obesity increases the risk of developing T2D by seven-fold [2]. Obesity further contributes to IR through increased adiposity, which secretes pro-inflammatory cytokines and fatty acids that disrupt normal metabolic processes [3]. This chronic inflammation induces IR through peripheral tissues and the brain [2, 36]. Historically, the relationship between cognitive impairment and metabolic diseases was underappreciated, but emerging evidence highlights a significant association between them [37]. Indeed, IR, chronic low-grade inflammation, and disrupted brain insulin signaling may serve as shared pathogenic mechanisms between obesity, T2D, and AD [37, 38]. T2D is associated with a 10-year reduction in life expectancy and increased risks of cardiovascular disease, kidney failure, blindness, and limb amputations [39]. Additionally, it raises the risk of AD by promoting IR in the brain, which disrupts tau phosphorylation and impairs Aβ clearance [8]. We found evidence that young adults with obesity and IR exhibit elevated NfL levels, a marker of neuronal axonal damage. These findings suggest that targeting common pathways—such as insulin signaling, inflammation, and metabolic dysregulation—could offer valuable therapeutic opportunities for T2D and AD, potentially slowing disease progression and improving the quality of life for individuals affected by these conditions.

Choline deficiency is widespread; on average, males consume only 402mg/day of choline and females consume 278mg/day, significantly below the recommended daily intake of 550 and 425mg/day,

respectively [40]. Reduced choline has been associated with a higher prevalence of AD, with a recent report showing that greater than 350mg/day is protective against AD risk [41]. Circulating choline levels were lower in obese individuals compared to those with a healthy BMI. Women also showed lower levels than men, despite estrogen's activation of the PEMT enzyme, which enhances endogenous choline synthesis and offers some protection against deficiency [42]. However, because women typically consume less dietary choline than men [40], endogenous production may not fully offset this deficit. This is noteworthy given the relatively young average age of women in our study (31.65 years), well before the average onset of menopause (~51 years; [43]). After menopause, the decline in estrogen further reduces PEMT activity [44], which may compound the effects of low dietary intake and potentially contributing to lower circulating choline, impaired metabolic health, and elevating AD risk. Further, strong correlations were observed between reduced plasma choline levels and metrics of obesity and IR. Our female participants had higher %Body Fat, consistent with research suggesting that women experience more pronounced metabolic and inflammatory effects from obesity due to higher %Body Fat [3]. Additionally, previous work has shown that decreased choline is associated with higher pathological and clinical markers of AD, including elevated neuritic plaque density and Braak stage [10, 17], lower Mini-Mental State Examination (MMSE) scores, and decreased brain weight [10]. Here, we found that higher NfL levels in obese participants correlated with lower choline levels, suggesting early signs of neuronal axonal damage in insulin-resistant, non-diabetic individuals. We validated such changes in independent cohorts of MCI and AD cases, highlighting that such measures are dysregulated in cases with confirmed neurological dysfunction. Taken together, these findings indicate an association between lower choline levels and IR in adulthood, which may be related to elevated AD risk. Lastly, the lower choline levels observed in women may contribute to their increased risk of developing AD [6].

Obesity and IR are also characterized by inflammation that further complicates metabolic health [3]. Pro-inflammatory and anti-inflammatory cytokines are elevated in individuals with obesity, contributing to chronic inflammation [3, 18], which plays a central role in the development of IR, β-cell dysfunction, and ultimately T2D and AD [2]. Adipose tissue inflammation impairs insulin receptor function or insulin secretion, which alters glucose sensitivity and drives IR [2]. Moreover, inflammation exacerbates AD pathogenesis through increased apoptosis and reduced synaptic activity [36]. Inflammation disrupts insulin signaling and downstream pathways [8]. IR in the brain promotes the release of pro-inflammatory cytokines [36]. Eleven cytokines were significantly elevated in obese participants compared to healthy controls, with pro-inflammatory cytokines, such as INF-γ, IL-1β, and TNF-α being notably increased. Interestingly, anti-inflammatory cytokines like IL-4, IL-5, and IL-13—which counteract inflammation in adipose tissue and enhance insulin sensitivity—were also elevated [3]. This suggests that the body may attempt to counterbalance the inflammatory response. However, the imbalance between pro- and anti-inflammatory signals only further exacerbates the chronic inflammatory environment [45]. Notably, elevated levels of several of these cytokines—especially IL-6, IFN-γ, and TNF-α—have been proposed as links between IR and dementia, with higher levels associated with increased AD pathological burden [3].

These findings add to the growing body of data that adequate choline levels are vital to prevent chronic inflammation and metabolic disorders. Data from both humans and rodents suggest higher dietary choline intake reduces the risk of IR [12], T2D [13], impaired glucose tolerance, and alterations in insulin-degrading proteins that increase the likelihood of IR [11]. However, other evidence points to a more nuanced relationship between choline and IR. While choline levels decrease in non-obese individuals as they transition from insulin sensitivity to IR, higher levels of choline phospholipids are observed during the progression to IR, suggesting that choline metabolism dysregulation may contribute to IR development [14]. Moreover, studies highlighting the increased risk of obesity, IR, and other health issues focus on the choline metabolite trimethylamine N-oxide (TMAO), which is produced by certain gut microbiome species which are more prevalent in individuals with diets high in red meat and low in fiber [46]. While our study indicates that higher circulating choline levels are associated with positive metabolic outcomes, when considered alongside the whole of the literature, choline intake should complement – not replace – a balanced diet. To that end, intervention studies demonstrate that consumption of products high in dietary choline can reduce risk of AD. The Mediterranean diet, which is rich in choline-containing foods like eggs, fish, legumes, and leafy greens, has been associated with a significantly lower risk of AD—by up to 30% in some studies [47], and frequent egg consumption (more than once per week) has been linked to a 39% reduced risk of AD, with dietary choline intake accounting for a substantial part of this protective effect [48].

Previously, we identified the liver enzymes aldolase B and sorbitol dehydrogenase as dysregulated by a choline deficient diet [11]. Here, we found that these enzymes are increased in obese participants compared to healthy controls, and that circulating choline levels

negatively correlate with rises in such enzymes. These enzymes play a crucial role in sugar metabolism: aldolase B metabolizes fructose, while sorbitol dehydrogenase metabolizes glucose [33]. Elevated levels of both enzymes in the blood can indicate liver dysfunction [49]. Furthermore, increased aldolase B is associated with reduced insulin production [50], and raised sorbitol dehydrogenase levels are found in T2D, contributing to oxidative stress and vascular damage [51]. Weight loss can reverse the increase in aldolase B [52], suggesting potential restoration of liver health. Given choline's role in supporting liver and metabolic health, elevated levels of aldolase B and sorbitol dehydrogenase may reflect a mechanism by which reduced choline contributes to the metabolic dysfunction observed in T2D, potentially elevating the risk for AD.

While we observed lower circulating choline levels in obese participants, the root cause remains unclear. Although we did not assess dietary choline intake, research has shown that obese individuals report lower dietary choline intake than healthy BMI counterparts [12]. Additionally, low circulating choline levels may result from choline being redirected to fat cells to form phospholipids, a major component of cell membranes, due to the higher body fat percentage in obese individuals. Indeed, as waist circumference increases, certain types of choline-derived phospholipids increase [53]. Redistribution of choline could reduce its availability to other organs, such as the brain and liver, impairing their function. Additionally, a high-fat diet can significantly increase the body's demand for choline [54], potentially lowering blood choline levels, which may be the case for obese individuals. Notably, a recent double-blind randomized control choline feeding study in humans showed that choline concentrations are robust biomarkers of dietary choline intake [21]. Regardless of the underlying cause of reduced circulating choline levels, existing literature suggests that increasing choline intake could help mitigate various metabolic issues [12, 55].

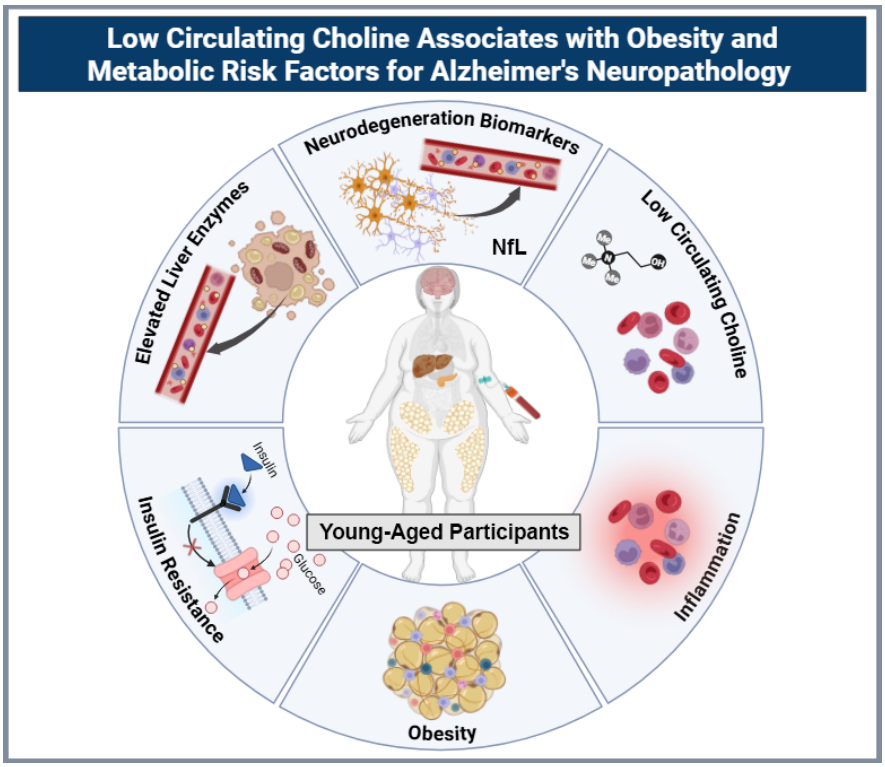


Figure 6. Schematic summarizing key findings from the obese versus healthy BMI cohort, illustrating correlations between obesity, low choline, IR, systemic inflammation and NfL.

Several limitations of the study should be noted. We did not assess dietary choline intake, which limits our ability to determine whether lower circulating choline levels reflect reduced intake, altered metabolism, or sequestration into adipose tissue. However, prior studies suggest that obese individuals may consume less choline [12, 56], and that increased adiposity may shift choline toward phospholipid synthesis, thereby lowering circulating levels [52]. The study's sample size was modest, and a larger, more diverse cohort may have captured broader population trends [1]. Our study did not include cognitive assessments, though elevated NfL was

observed [9] and has been associated with cognitive decline in non-demented adults [57]. Our previously published report demonstrates that serum choline correlates with performance on the MMSE, which assesses multiple cognitive domains including orientation, registration, attention, calculation, short-term memory, and language [10]. Furthermore, a prior study has shown that low serum choline concentrations are associated with poorer cognitive performance in individuals with metabolic syndrome, particularly in verbal fluency and the Trail Making Test (TMT), which evaluate attention, processing speed, mental flexibility, and executive function [58]. Moreover, limited blood availability prevented measurement of choline metabolites such as TMAO, derived from gut microbes [45]; these will be included in future studies given the relevance to obesity and cognitive decline. Our future work will also incorporate dietary intake assessments alongside blood biomarkers to better determine the relationship between choline consumption, status, and metabolism. Longitudinal and interventional studies are also needed to assess whether restoring choline levels can reduce metabolic dysfunction, inflammation, and/or NfL levels. Previous animal studies have demonstrated that inadequate dietary intake increases AD pathology [10, 11], while chronic choline supplementation may mitigate AD-like pathology and associated cognitive deficits [16]. These findings highlight the importance of examining choline's role across the lifespan, particularly in metabolically vulnerable populations. Investigating choline's relationship with hepatic markers such as aldolase B and sorbitol dehydrogenase may help clarify how liver dysfunction intersects with systemic and neuronal changes observed in obesity and IR [11, 31, 32, 49, 50]. Together, these future directions will help clarify the role of choline in bridging metabolic and neurodegenerative disease risk, and inform the development of early, nutrient-based interventions.

Our findings underscore circulating choline as a key indicator of obesity-related metabolic disturbances and a potential biomarker—alongside NfL—for future AD risk (Fig. 6). Reduced choline in obese individuals corresponded with metabolic, inflammatory, and neuronal axonal damage, reinforcing the shared metabolic dysfunction between IR and AD. Low choline may thus represent a modifiable factor in the prevention of IR, T2D, and AD. Considering sex differences and identifying low choline early could guide dietary interventions to reduce metabolic and neurodegenerative risk.

Availability of data and materials

Data will be made available upon reasonable request.

Competing interests

Dr. Ramon Velazquez is a research advisor for Performance Lab, LLC. No funds from Performance lab were used for the study. Drs. Beach and Serrano perform contracted research for Lantheus Holdings, Inc. and Dr. Beach has been a consultant for Biogen. All other authors have no conflict of interest.

Ethics Approval and Consent to Participate

All human subjects provided informed consent and approved by the Institutional Review Board at Mayo Clinic (IRB #: 20-003294).

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Author Contributions

WW-Conceptualization, Data Curation, Formal Analysis, Methodology, Writing original draft. JMJ-Data Curation, Formal Analysis, Methodology, Writing original draft. ST-Data Curation, Investigation, Writing–reviewing editing. GES-Funding, Data Curation, Formal Analysis, Writing–reviewing editing. TGB-Funding, Methodology, Data Curation, Writing–reviewing editing. LRR-Methodology, Data Curation, Writing–reviewing editing. ED-Data Curation, Methodology, Writing–reviewing editing. BB-Methodology, Data Curation, Writing–reviewing editing. CK-Conceptualization, Methodology, Data Curation, Writing–reviewing editing. RV-Funding, Conceptualization, Formal Analysis, Methodology, Writing original draft.

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