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Indirect Effects of Elevated Body Mass Index on Memory Performance Through Altered Cerebral Metabolite Concentrations

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Abstract

Objective—Elevated body mass index (BMI) at midlife is associated with increased risk of cognitive decline in later life. The goal of the current study was to assess mechanisms of early brain vulnerability by examining if higher BMI at midlife has an effect on current cognitive performance through alterations in cerebral neurochemistry.

Methods—Fifty-five participants, aged 40–60 years, underwent neuropsychological testing, health screen, and proton magnetic resonance spectroscopy (^{1}H MRS) examining N-acetyl-aspartate (NAA), creatine (Cr), myo-inositol (mI), choline (Cho), and glutamate (Glu) concentrations in occipitoparietal grey matter. Concentrations of NAA, Cho, mI, and Glu were calculated as a ratio over Cr and examined in relation to BMI using multivariate regression analyses. Structural equation modeling was used to determine if BMI had an indirect effect on cognition through cerebral metabolite levels.

Results—Higher BMI was associated with elevations in mI/Cr ($F(5,45)= 3.843$, $p=0.006$, $\beta=0.444$, $p=0.002$), independent of age, sex, fasting glucose levels, and systolic blood pressure. Moreover, a chi-square difference test of the direct and indirect structural equation models revealed that BMI had an indirect effect on global cognitive performance ($\Delta X^2(df=2) =19.939$, $p<0.001$). Subsequent follow-up analyses revealed that this effect was specific to memory ($\Delta X^2(df=2) = 22.027$, $p<0.001$).

Conclusions—Higher BMI was associated with elevations in mI/Cr concentrations in the occipitoparietal grey matter and indirectly related to poorer memory performance through mI/Cr, potentially implicating plasma hypertonicity and neuroinflammation as mechanisms underlying obesity-related brain vulnerability.

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Conflict of Interest

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Keywords

BMI; obesity; proton magnetic resonance spectroscopy; myo-inositol

Introduction

Rates of overweight and obese status have increased dramatically over the past few decades with current estimates classifying over 50% of Europeans and Americans as overweight or obese based upon body mass index (BMI), the most commonly used measure of obesity (1,2). Through both direct and indirect pathways, obesity damages the heart, liver, and pancreas, increasing susceptibility to a myriad of chronic diseases such as hypertension, diabetes, and cancer (3). Additionally, obese BMI has been identified as a risk factor for cognitive decline (4,5), suggesting that the health consequences of obesity extend outside the periphery.

Midlife obesity has been associated with reduced grey matter volume in older age (6) and significantly increased risk for dementia (4). At younger ages, the effect of obesity on cognitive performance tends to be subtle ($r=-0.11$ to $r=-0.23$) (7); yet, significant disturbances in resting cerebral glucose metabolism have been detected in otherwise healthy young adults with high BMI (8), providing evidence of early brain vulnerability. Given the widespread prevalence of obesity, there is a pressing need to further investigate the physiological mechanisms linking increased body mass to brain vulnerability so that preventive measures can be developed to protect against obesity-related cognitive impairment. The small direct effects of obesity on cognition at midlife, however, can hinder studies of mediating mechanisms if traditional models requiring a significant direct effect are applied. When that is suspected to be the case, researchers have argued that direct and indirect effects should be examined independently of each other in order to prevent investigators from missing potentially interesting, important, or useful mechanisms by which an independent variable may exert an effect on a dependent variable (9). Thus, following the recommendations of MacKinnon et al (10), Shrout & Bolger (11), and Hayes (9), we set out to independently examine the direct and indirect effects of increased midlife BMI on current neuropsychological test performance through a hypothesized alteration in cerebral neurochemistry. Cerebral neurochemistry was specifically targeted as early changes in neurometabolite concentrations have been shown to predict cognitive decline in a variety of disorders including multiple sclerosis (12), Alzheimer's disease (13), and traumatic brain injury (14).

In this study, neurometabolite concentrations were examined through proton magnetic resonance spectroscopy (^{1}H MRS). ^{1}H MRS capitalizes on the unique precessional properties of atomic nuclei to measure concentrations of a variety of cerebral metabolites with neurobiological significance: N-aceytl-aspartate (NAA), a marker of neuronal viability; choline-containing compounds (free choline, phosphocholine and glycerophosphocholine, Cho), markers of membrane breakdown and turn over; creatine (Cr), a marker of energy metabolism; myo-inositol (mI), an organic osmolyte and glial marker; and glutamate (Glu), a marker of excitatory neurotransmission and synaptic integrity (15).

While we measured all spectroscopically visible metabolites, our hypotheses were centered on alterations in NAA and mI concentrations, both of which are accepted markers of prodromal cognitive impairment (16,17). We based our predictions on evidence that adipose tissue secretes proinflammatory adipocytokines capable of crossing the blood brain barrier (18). Within the central nervous system, adipocytokines can induce a local inflammatory response and activate microglia (19), which may result in oxidative damage. These

processes may be reflected in higher levels of the organic osmolyte and glial marker, mI, and lower levels of the neuronal marker NAA (15). Additionally, insulin dysregulation, an established consequence of obesity (20), may affect neuronal survival through disruption of cerebral glucose metabolism (21), also lowering NAA. Finally, obesity has been linked to alterations in osmotic regulation in the peripheral (22) and central nervous systems (23), which might induce cellular damage through shrinkage and swelling causing further changes in cerebral mI and NAA levels. Thus, we hypothesized that lower NAA concentrations could provide evidence of decreased neuronal integrity (24) and would be commensurate with the observations of reduced grey matter volume in association with higher BMI (6). Higher mI, on the other hand, would suggest microglial activation and osmotic regulation disturbance (25) as the potential mechanism relating higher BMI to lower cognitive function.

Materials and Methods

Participants

Adults between the ages of 40 and 60 years were recruited through flyers and newspaper advertisements. Individuals with a history of coronary artery disease, angina pectoris, myocardial infarctions, heart failure, and cardiac surgery were excluded in order to assess the impact of higher BMI on neurochemistry in otherwise healthy adults. Additional exclusion criteria included history of neurological disease (e.g., stroke, Parkinson's disease, clinically significant traumatic brain injury), major psychiatric illness (e.g. schizophrenia, bipolar disorder), substance abuse (i.e., diagnosed abuse and/or previous hospitalization for substance abuse), smoking (within the last two years), and MRI contraindications. Medical conditions, treatments, and other exclusion factors were assessed according to participants' self-report during telephone screening. Fifty-five participants completed the initial screen and were enrolled in the study after providing written consent. One participant was excluded from analyses due to poor quality MRS data (Cramer-Rao Lower Bounds for NAA/Cr, mI/Cr, Cho/Cr or Glu/Cr >12).

Procedures

The study was conducted in accordance with the Helsinki Declaration of 1975 and with approval from the local Institutional Review Board. All volunteers provided written informed consent before enrollment. Participants completed a medical history interview in which medical conditions and treatments were coded as either present or absent based on participants' self-report. Participants then underwent a full neuropsychological evaluation, brain imaging, and a general health assessment, including a fasting blood draw for lipid and glucose assay. Visits were conducted on separate days, and participants completed the study within one month. Data for the study was collected from January 2008 to July 2010.

Neuropsychological Assessment

Participants completed a two-hour assessment battery including standard clinical neuropsychological instruments with established reliability and validity (26). In effort to reduce multiple comparisons, a global cognitive domain score was created. Participants' raw test scores were converted into z-scores using the study sample mean and standard deviation. Timed test scores were multiplied by −1 so that higher scores indicate better performance. A composite global cognitive domain z-score was calculated for each participant by averaging the z-scores of all tests. The following test scores were included in the global cognitive domain: MMSE (27); WASI Vocabulary Subtest (28); WASI Matrix Reasoning Subtest (28); Category Fluency for Animals (29); RCF copy, immediate recall, delayed recall, and recognition discrimination (30); CVLT-II immediate recall, delayed recall, and recognition discrimination (31); Trail making A and B time to completion (32);

COWAT (33); WAIS-III Digit Span Subtest (34); and Grooved Pegboard-Dominant Hand time to completion (35).

In exploratory follow-up analyses, the global cognitive functioning score was split into five separate cognitive domains in order to more fully characterize the impact of higher BMI and altered cerebral neurochemistry. Neuropsychological measures were grouped as follows: 1) *general cognition*: MMSE (27) and WASI FSIQ (28); 2) *language*: WASI Vocabulary Subtest (28) and Category Fluency for Animals (29); 3) *visual-spatial*: RCF copy (30) and WASI Matrix Reasoning Subtest (28); 4) *memory*: CVLT-II immediate recall, delayed recall, and recognition discrimination (31), RCF immediate recall, delayed recall, and recognition discrimination (30); 5) *attention-executive-psychomotor functions*: Trail making A and B time to completion (32), COWAT (33), WAIS-III Digit Span Subtest (34), and Grooved Pegboard-Dominant Hand time to completion (35). All tests were administered and scored by a trained research assistant using standard administration and scoring criteria.

Imaging Protocols and MRS Data Processing

MRS data for each participant were acquired in a single session on a 3T GE Signa Excite MRI scanner equipped with a standard head coil. Imaging included single voxel proton MRS performed using the GE pulse sequence PROBE-P, an automated point resolved spectroscopy (PRESS) sequence with chemical shift selected (CHESS) water suppression. Each spectroscopic voxel was prescribed from 3D high-resolution Spoiled Gradient Echo (SPGR) sagittal images (256 × 256 matrix, FOV = 24 × 24 cm^2, 1 mm slice thickness, 0 gap) of the entire brain. ^{1}H-MRS parameters were as follows: echo time/repetition time (TE/TR) = 35/3000 ms, 128 excitations, 5000 Hz spectral width, volume ~6 cm^3 from the occipitoparietal gray matter including posterior cingulate gyrus (Fig. 1a). This region was selected because alterations in its neurochemical concentrations have well-documented associations with cognition (14,16,36). Commercially available software, LCModel, was used to quantify and separate the metabolite resonances from the macromolecule background (37) (Fig. 1b). The concentrations of NAA, Cho, mI, and Glu were reported as ratios relative to Cr in line with standard clinical protocols (38).

General Health Assessment

Participants abstained from caffeine and fasted for at least four hours prior to the assessment. Body mass in kilograms and height in centimeters were measured on a physician's balance scale for the subsequent calculations of BMI. BMI was calculated by dividing weight in kilograms by height in meters squared. Following 15 minutes of rest, participants sat upright while brachial blood pressure was measured using a semi-automated device. Approximately 3 milliliters of fasting blood was collected from the antecubital vein by venipucture. The plasma concentrations of glucose, triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol were measured using standard enzymatic technique (Cholestech LDX system, Cholestech Corporation, Hayward, CA).

Statistical Analyses

Descriptive statistics were calculated for demographics, medical variables, and raw cognitive test scores. The global cognitive domain score was assessed in relation to BMI and ^{1}H MRS markers (NAA/Cr, Glu/Cr, mI/Cr and Cho/Cr) using linear regression statistically adjusting for age and years of education.

Then two sets of analyses were conducted. First, the association between BMI and the ^{1}H MRS markers was analyzed using a single multivariate multiple linear regression model with all MRS parameters entered in at once, statistically adjusting for age, sex, fasting glucose levels, and systolic blood pressure. The above analysis was repeated using anti-

hypertensive medications, and hypoglycemic medications as additional covariates (0 = no, 1 = yes). A two-tailed alpha level of 0.05 was used as the criterion for statistical significance.

In the second set of analyses, the indirect effect of BMI on the global cognitive domain score through mI/Cr levels was assessed using structural equation modeling. In contrast to mediation, indirect effects do not require a significant direct association between the independent and dependent variables (39). The advantage of SEM over multiple regression is that the former can be used to estimate simultaneous multivariate associations (e.g., the effects of X on Y, X on Z, and Y on Z), which would have to be estimated independently using regression techniques. The indirect effect of BMI on the global cognitive domain score through mI/Cr was assessed by comparing the direct and indirect models. The indirect model included estimates of the direct effects of BMI on cognition and two additional paths, one from BMI to mI/Cr and another from mI/Cr to cognition. A successful indirect model requires that both the path from BMI to mI/Cr and the path from mI/Cr to cognition be significant. The direct effect of BMI to the global cognitive domain score was also assessed. Finally, the difference between direct and indirect models were compared using a chi-square test with a significance level of $p<0.05$. An additional assessment on the significance of the indirect model was conducted by calculating confidence intervals. Confidence intervals were obtained using Preacher and Hayes' SPSS macro (39), which utilizes as bootstrapping method for assessing indirect effects. In brief, this procedure involves taking one thousand random samples from the obtained data, sampling with replacement, and calculating the indirect effect for each sample. 95% confidence intervals are than calculated from the distributions of obtained scores over the samples correcting for bias due to the underlying distribution. In the current analysis, the global cognitive domain score was entered as the dependent variable, BMI was entered as the independent variable, and mI/Cr as the mediator. A 95% bias-corrected confidence interval that does not include 0 was considered as the criterion for significance. In subsequent exploratory analyses, the above procedures for detecting indirect effects were repeated for the five individual cognitive domain scores. Additionally, the indirect effect of BMI on global cognition through mI/Cr was re-assessed statistically adjusting for the memory domain in order to examine if the global cognitive domain score held predictive value over and above the contribution of the memory domain.

Structural equation modeling was accomplished using Mx 1.54a (40). All other statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL).

Results

Descriptive statistics

Selected demographic and medical characteristics of the subjects are reported in Table 1. Mean BMI was 29.4 kg/m^2 with a range from 19.0 to 42.8 kg/m^2. Fourteen participants (25.9%) had a normal BMI (18.5–24.9 kg/m^2), 15 participants (27.8%) had an overweight BMI (25.0–29.9 kg/m^2), and 25 participants (46.3%) had an obese BMI (≥30 kg/m^2). Eleven participants (20.4%) were currently being treated with anti-hypertensive medications, three participants (5.6%) with lipid lowering agents, four participants (7.1%) with hypoglycemics, three participants (5.6%) with biphosphonates, one participant (1.8%) with thyroid replacement therapy, and one participant (1.8%) with antidepressant medications. Based on their self-report, twenty-two participants (40.7%) were classified as Caucasian, twenty-five participants (46.3%) as Hispanic, four participants (7.4%) as African-American, and three (5.6%) as other. Table 2 displays the mean raw cognitive test scores. Descriptive statistical analyses revealed a cognitively normal, ethnically diverse, middle-aged sample, well representative of the population of the state of Texas based on year 2000 US census data.

BMI and cerebral metabolism in relation to global cognitive test performance

BMI was not significantly associated with the global cognitive domain score (β=−0.078, p=0.57) independent of age and years of education ($F(3,49)=1.473$, p=0.23). Consistent with the literature on mild cognitive impairment (38), higher levels of mI/Cr were associated with lower performance in the global cognitive domain (β=−0.290, p=0.03) independent of age and years of education ($F(3,49)=3.108$, p=0.04) (Fig. 2). No significant relation was found between NAA/Cr, Glu/Cr, and Cho/Cr and the global cognitive domain independent of age and years of education.

Cerebral metabolism in relation to BMI

The fully adjusted multivariate regression model successfully predicted the level of mI/Cr in occipitoparietal grey matter ($F(5,45)=3.843$, p=0.006), but not NAA/Cr ($F(5,45)=1.419$, p=0.24), Cho/Cr ($F(5,45)=1.314$, p=0.28) or Glu/Cr ($F(5,45)=1.166$, p=0.34). Higher BMI was significantly associated with higher mI/Cr (β=0.444, p=0.002), independent of age, sex, fasting glucose levels, and systolic blood pressure (Fig. 3). This relation remained unchanged when global cognitive function was included in the model ($F(6,44)=4.127$, p=0.002, β=0.417, p=0.003). Additionally, statistically adjusting for the use of anti-hypertensive ($F(6,44)=3.141$, p=0.01, β=0.434, p=0.004) or hypoglycemic medications ($F(6,44)=3.155$ p=0.01, β=0.455, p=0.002) did not alter the significance of the findings.

The indirect effect of BMI on global cognitive performance through mI/Cr levels

As expected, the direct effect of BMI on cognition in midlife was subtle and the path from BMI to global cognitive performance was non-significant (β=−0.11, 95% CI −0.38, 0.16) (Fig. 4a). For the indirect model (Fig. 4b), the paths from BMI to mI/Cr (β=0.50, 95% CI 0.26, 0.74) and from mI/Cr to global cognitive performance (β=−0.33, 95% CI −0.63, −0.03) were significant. The path from BMI directly to global cognitive performance was non-significant (β=0.06, 95% CI −0.24, 0.36). A chi-square difference test of the direct and indirect models revealed that the indirect model was a better fit for the data ($\Delta X^2(df=2) = 19.939$, p<0.001). The significance of the indirect effect was confirmed by the 95% confidence intervals (95% CI range −0.369 to −0.039) derived by Preacher and Hayes' (39) bootstrapping method for detecting indirect effects.

The indirect effect of BMI on individual cognitive domain scores through mI/Cr levels

Memory performance was the only individual cognitive domain score that BMI had an indirect effect on through mI/Cr. Consistent with our results for global cognition, the direct effect from BMI to memory performance was subtle and did not reach statistical significance (β=−0.02, 95% CI −0.29, 0.05) (Fig. 5a). For the indirect model (Fig. 5b), the paths from BMI to mI/Cr (β=0.50, 95% CI 0.26, 0.74) and from mI/Cr to memory performance (β=−0.40, 95% CI −0.70, −0.10) were significant. The path from BMI directly to memory performance was non-significant (β=0.18, 95% CI −0.12, 0.48). A chi-square difference test of the direct and indirect models indicated that the indirect model provided a better fit for the data ($\Delta X^2(df=2) = 22.027$, p<0.001). The significance of the indirect effect was also confirmed by 95% confidence intervals (95% CI −0.048, −0.006).

No significant indirect effects were found for the general cognition, language, visual-spatial, and attention-executive-psychomotor cognitive domains. The criteria for indirect effects include the provision that the path from the independent variable to the mediating variable be significant as well as the path from mediating variable to the dependent variable. In our case, only the path from the independent variable (BMI) to the mediating variable (mI/Cr) was significant (β=0.50, 95% CI 0.26, 0.74). The path from the mediating variable (mI/Cr) to the dependent variable (cognitive domain score) was non-significant for the general

cognition (β=−0.15, 95% CI −0.45, 0.15), language (β=−0.08, 95% CI −0.39, 0.20), visual-spatial (β=−0.29, 95% CI −0.60, 0.01), and attention-executive-psychomotor domain scores (β=−0.21, 95% CI −0.52, 0.10); thus preventing further exploration of any indirect effects of BMI on domain specific cognitive scores in those cases.

The indirect effect of BMI on global cognitive performance through mI/Cr levels controlling for the memory domain score

The indirect effect of BMI on global cognition was no longer detectable after statistically adjusting for memory performance. While the path from BMI to mI/Cr remained significant after adjustment for memory performance (β=0.50, 95% CI 0.26, 0.74), the path from mI/Cr to global cognition was no longer significant (β=0.01, 95% CI −0.15, 0.18); thus, violating criteria for further assessment of the indirect effect of BMI on global cognition in this case.

Discussion

Within our middle-aged sample, higher BMI was associated with elevations of mI/Cr in occipitoparietal grey matter. High levels of mI have been reported in patients with diabetes (41–43) and hypertension (44), conditions closely related to elevated BMI. In the current study, BMI was associated with elevations in mI/Cr even after statistically adjusting for systolic blood pressure, fasting glucose levels, and use of anti-hypertensive or hypoglycemic agents, suggesting that BMI independently accounts for variance in cerebral mI levels in otherwise healthy middle-aged adults. Consistent with the dementia literature (36,38), higher mI/Cr related to lower overall cognitive performance. More importantly, structural equation modeling determined that higher BMI had an indirect effect on poorer overall cognitive performance through the elevated cerebral mI levels. Exploratory follow-up analyses revealed that the memory score was only the individual cognitive domain score contributing to the effect. These results are similar to the findings by Kantarci et al. (2002) (38), which noted a significant correlation between elevated mI levels and verbal memory performance in individuals with MCI and Alzheimer's disease. Thus, elevated mI levels in association with higher BMI may provide preliminary evidence of a potential neurochemical mechanism that may underlie obesity-related cognitive vulnerability within the memory domain.

Elevations in mI were predicted in this study based on similar findings in other cognitively vulnerable populations (38,45). In contrast to our original hypothesis, however, the relation between higher BMI and lower NAA was not observed in the present study. NAA is a marker of neuronal viability, and reductions have typically been observed in disorders with cerebral atrophy (15). In contrast, mI is a simple sugar alcohol that serves as an osmotic regulator and a proposed marker of gliosis (38). Reductions in NAA frequently occur in conjunction with elevations in mI in cognitively vulnerable populations (38,46). Reductions in NAA and elevations in mI, however, do not necessarily correlate with one another (46), suggesting that they reflect different pathological processes. The current findings suggest that osmotic regulation and/or glial cell alterations may precede the neuronal loss associated with higher BMI.

In the present study, mI/Cr was the only neurometabolite to relate to cognitive performance. Our follow-up analysis revealed that this effect was specific to memory. The significance of mI/Cr for cognition in adults with memory disorders has been demonstrated by previous studies, which have found that mI/Cr levels can predict the degree of cognitive decline in individuals with mild cognitive impairment and Alzheimer's disease (36,38). As anticipated, BMI was not directly associated with cognitive performance in our sample. These results are not surprising given that the sample consisted of high-functioning middle-aged adults (mean FSIQ = 113.5). At midlife, obesity has small negative effects on cognition (r = −0.11 to r= −0.23) (7) that can be difficult to detect on pen and paper tests. Sensitive neurobiological

markers such as cerebral neurochemistry bear importance for prevention since they may provide evidence of cognitive vulnerability in individuals who are currently cognitively-intact. Given that higher mI/Cr levels were related to poorer memory performance, BMI-related elevations in mI/Cr may be a mechanism contributing to cognitive vulnerability within the memory domain. Longitudinal studies on BMI and cognition will be instrumental in determining if mI concentrations are predictive of future cognitive trajectories.

Given the detected associations of cerebral mI levels with BMI and memory performance, speculation about the physiological pathways via which elevated BMI may lead to increased cerebral mI and poorer memory performance is of interest. In the brain, mI servers an organic osmolyte (47), protecting cells from death or damage by shrinkage or swelling subsequent to large alterations in water diffusion. Chronic hypernatremia induces robust increases in cerebral mI concentrations and even small, persistent changes in plasma tonicity may increase mI concentrations in the brain (48). In the periphery, overweight and obese individuals have higher extracellular relative to intracellular body fluid (ECF/ICF) that is presumed to be due to higher plasma tonicity (22). Evidence for similar effects in the central nervous system is provided by a diffusion weighted imaging study demonstrating that obese individuals have higher apparent diffusion coefficient (ADC) values in numerous regions throughout the brain in comparison to lean controls (23). ADC values represent the diffusion of water molecules within a tissue and vasogenic edema, a condition of elevated cerebral extracellular water distribution, is related to higher ADC values (49). Similarly in obese individuals, higher ADC values may represent an increased ECF secondary to plasma hypertonicity (23). In such a state, cerebral concentrations of mI would be upregulated to maintain osmotic balance. Thus, plasma hypertonicity may be a mechanism linking higher BMI to elevations in cerebral mI. Over time alterations in osmotic regulation may cause cellular damage through shrinkage or swelling, ultimately resulting in cerebral atrophy and memory decline.

Alternatively, the association between mI and BMI may be explained by inflammation-related gliosis. Insult or injury in the brain induces astrocyte proliferation and the presence of activated microglia, which release inflammatory cytokines and oxidative radicals (50). MI resides primarily in glial cells so inflammatory-induced gliosis may increase its cerebral concentrations (38). Elevations in mI have been detected in several conditions associated with neuroinflammation such as multiple sclerosis (51), acquired immunodeficiency syndrome (52), and Alzheimer's disease (38). Inflammation is also a well-established symptom of obesity. Adipose tissue secretes numerous cytokines, some which are capable of crossing the blood brain barrier and initiating a local proinflammatory response (53). In rodents, high fat diets have been shown to increase astrocyte proliferation and microglial reactivity, resulting in poorer memory ability on a spatial navigation task (54). Obesity-related inflammation may therefore be a potential mechanism responsible for elevated mI concentrations and memory decline.

It is of note, however, that while the current study found a strong association between mI and BMI, the two other published ^{1}H MRS studies examining cerebral neurochemistry and BMI did not find significant association with mI levels (55,56). Examining middle-aged adults, Gadzinksi et al. (2008) (55) reported that greater BMI was associated with lower NAA/Cr levels in the frontal, parietal, and temporal white matter, lower NAA/Cr in the frontal grey matter, and lower Cho/Cr in the frontal white mater. In a similar study of older adults, Gazdzinski et al. (2010) (56) found that BMI was related with decreased NAA/Cr and Glu/Cr in the anterior cingulate. The prior studies reported no association between NAA/Cr and BMI in more posterior grey matter regions similar to the current findings. Yet, elevations in mI/Cr concentrations in occipitoparietal grey matter are a unique finding in this study. The variation in findings may be due to methodological differences. The study

conducted by Gazdzinski et al. (2008) (55) acquired data from a 1.5-Telsa scanner with an echo time/repetition time (TE/TR) = 25/1800 ms. In Gazdzinski et al. (2010) (56) a 4-Telsa scanner with a TE/TR = 15/2000 was utilized. The current study was conducted on a 3-Telsa scanner with a TE/TR = 35/3000 ms. Differences in magnetic field strength and TE/TR parameters can alter quantifiable precision due to changes in the signal-to-noise ratio and chemical shift dispersion (57). Another potential explanation for the discrepancy between the current study and previous ones may be explained by differences in the participant samples. In the current study, a relatively large proportion of participants were obese (47.2%) in comparison to the sparse representation of obese individuals in the other two studies, 10% (55) and 0% (56) respectively. It is possible that perturbations of mI/Cr in posterior areas do not emerge until BMI becomes sufficiently elevated.

While contemplating the results of the study, it is important to consider its strengths and limitations. A primary strength of the current study was the detailed characterization of the study participants in terms of cognitive function. Our comprehensive neuropsychological test battery provided a thorough assessment of participants' cognitive functioning across multiple domains, thus allowing us to rule out pre-existing undetected or under-reported clinically significant cognitive impairment as an alternative explanation of our results observations. The study also included objective assessment of physiological indices such as fasting glucose levels and systolic blood pressure. This enabled us to statistically adjust for common co-morbidities associated with obesity and cerebral alterations in order to determine BMI's independent impact on cerebral neurochemistry. A limitation of the study, on the other hand, was the relatively small and healthy sample, so the present findings must be considered preliminary. While the restriction of the sample to individuals without self-reported chronic diseases such as coronary artery disease, heart failure, and psychiatric illness allowed us to assess the impact of BMI on neurochemistry in and of itself, without the confounding effects of co-morbid medical conditions, the simultaneous examination of multiple cognitive risk factors will be important in future studies. Additionally, in the current analysis, BMI was the only measure of obesity assessed. Future studies would benefit from the inclusion of other indices such as waist circumference and waist-to-hip ratios in order to determine the definition of obesity that is most relevant to alterations in neurochemistry. Inclusion of physical fitness assessments would also be beneficial since a sedentary lifestyle is also a risk factor for cognitive deficits (58) and cerebral atrophy (59). Finally, our methods could be improved by inclusion of multiple spectroscopy voxels, tissue segmentation, and absolute quantification. The occipitoparietal region that was sampled in this study, though traditionally considered a grey matter region of interest, inherently includes some white matter. As neurometabolite concentrations have been noted to vary between grey and white matter tissue (24) and changes in grey/white matter composition have been reported in association with BMI (6), tissue segmentation may be a useful correction in the future. Inclusion of multiple voxels, on the other hand, would provide better characterization of BMI-related alterations across the cerebral cortex. Finally, absolute quantification methods would resolve potential problems with the use of Cr as a normalizing factor as they would remove the underlying assumption that Cr concentrations are constant across the full range of BMI. Last but not least, the cross-sectional nature of the study is a limitation. We are unable to determine if alterations in mI are pre-existing or develop as a result of higher BMI. It is also unclear as to whether mI elevations would reverse with successful weight loss. Longitudinal studies will be critical in determining whether the observed alterations in neurochemical concentrations are predictive of individual cognitive trajectories.

In conclusion, we found that higher BMI was associated with elevations in mI/Cr concentrations in the occipitoparietal grey matter and indirectly related to global cognitive performance through mI/Cr. Subsequent follow-up analyses revealed that this effect was

driven by memory performance. The findings potentially implicate plasma hypertonicity and neuroinflammation as mechanisms underlying obesity-related brain dysfunction. Longitudinal studies will be necessary to determine if the observed neurometabolic alterations are predictive of future memory decline. With validation and absolute quantification, studies of neurometabolites may help uncover the pathological the pathological mechanisms underlying the deleterious effects of elevated BMI on central nervous system functioning.

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Abbreviations

BMI	body mass index
^{1}H MRS	proton magnetic resonance spectroscopy
NAA	N-acetyl-aspartate
Cr	creatine
mI	myo-inositol
Cho	choline
Glu	glutamate
MRI	magnetic resonance imaging
TE/TR	echo time/repetition time
SPGR	spoiled gradient echo
LDL-cholesterol	low-density lipoprotein cholesterol
HDL-cholesterol	high-density lipoprotein cholesterol
ECF	extracellular fluid
ICF	intracellular fluid
ADC	apparent diffusion coefficient
MMSE	Mini Mental Status Exam
WASI	Wechsler Abbreviated Scale of Intelligence
FSIQ	full scale intelligence quotient
RCF	Rey Complex Figure Test
CVLT-II	California Verbal Learning Test-II
WAIS-III	Wechsler Adult Intelligence Scale-III
COWAT	Controlled Oral Word Association Test

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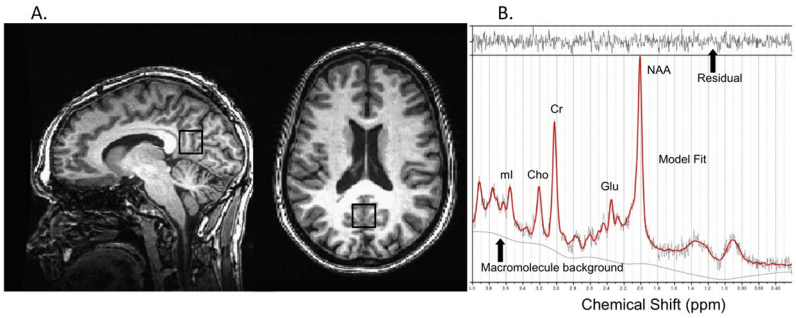

Figure 1.

Figure 1A. MRS voxel borders on high-resolution anatomy indicating volume of interest in the occipitoparietal junction

Figure 1B. Representative ^{1}H MRS spectrum. The narrow line width and small residual indicate excellent model fit. NAA = N-acetyl-aspartate; Glu = glutamate; Cr = creatine + phosphocreatine; Cho = choline + phosphocholine; mI = *myo*-inoitol

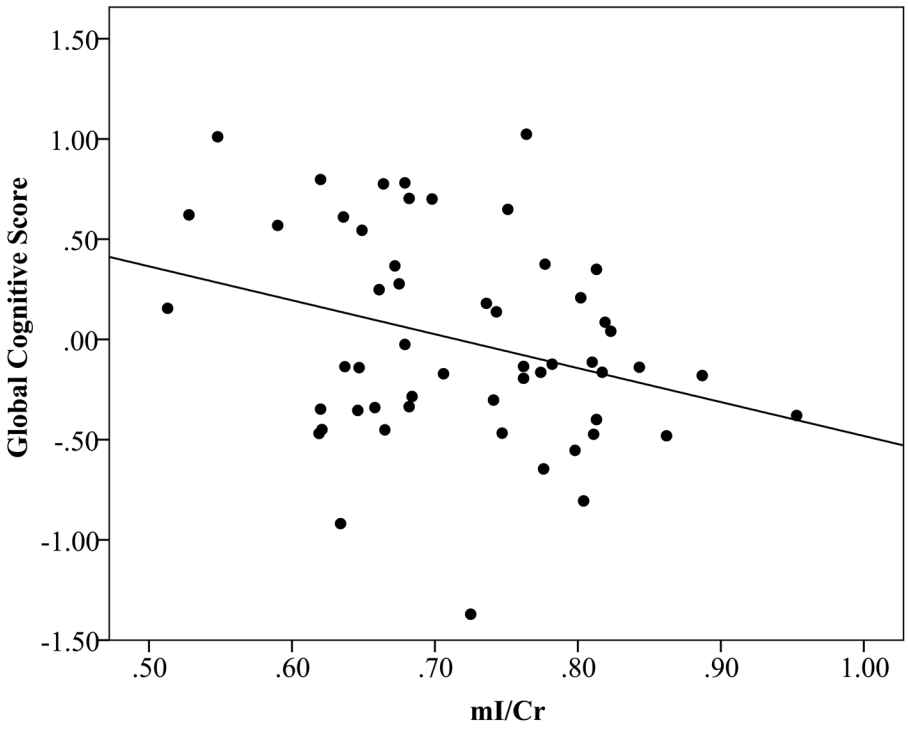

Figure 2.
Scatterplot displaying the relation between mI/Cr and the global cognitive domain score

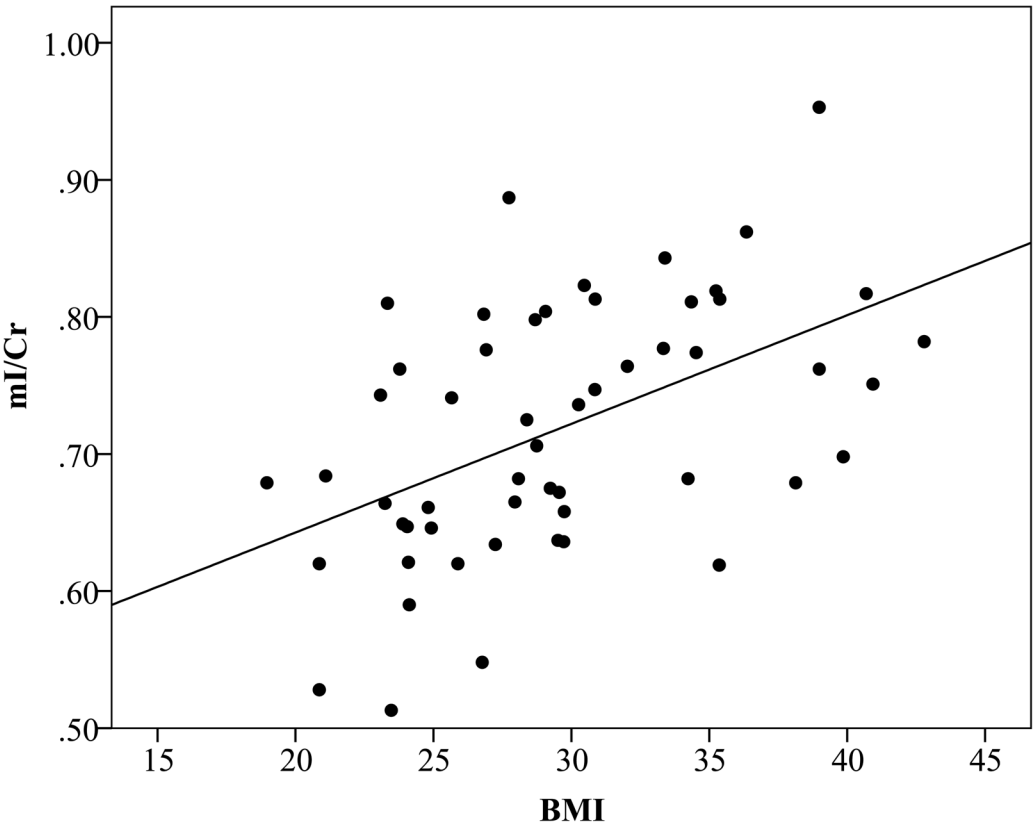

Figure 3.
Scatterplot displaying the relation between BMI and mI/Cr

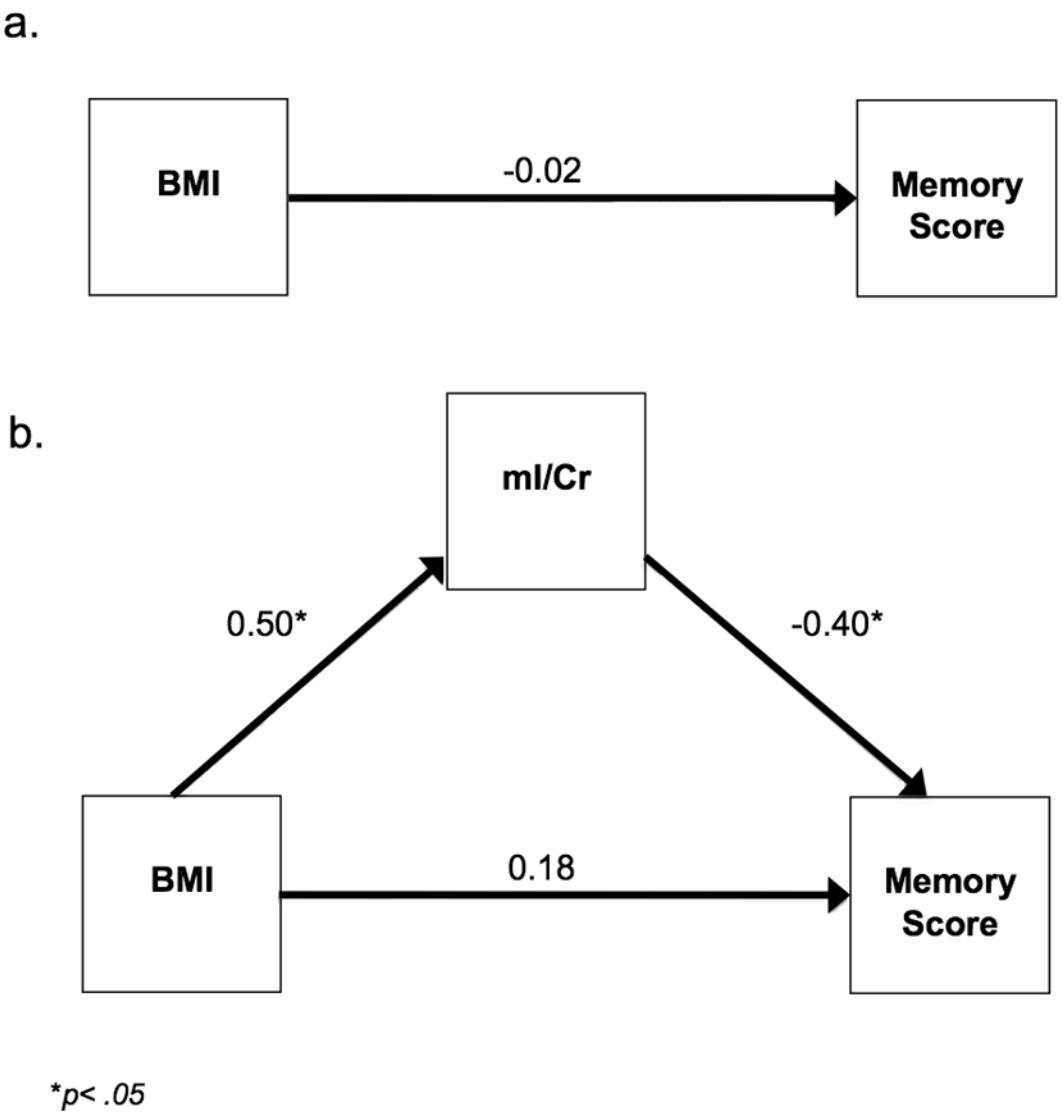

Figure 4.
Figure 4A. Direct structural equation model for the global cognitive domain score
Figure 4B. Indirect structural equation model for the global cognitive domain score

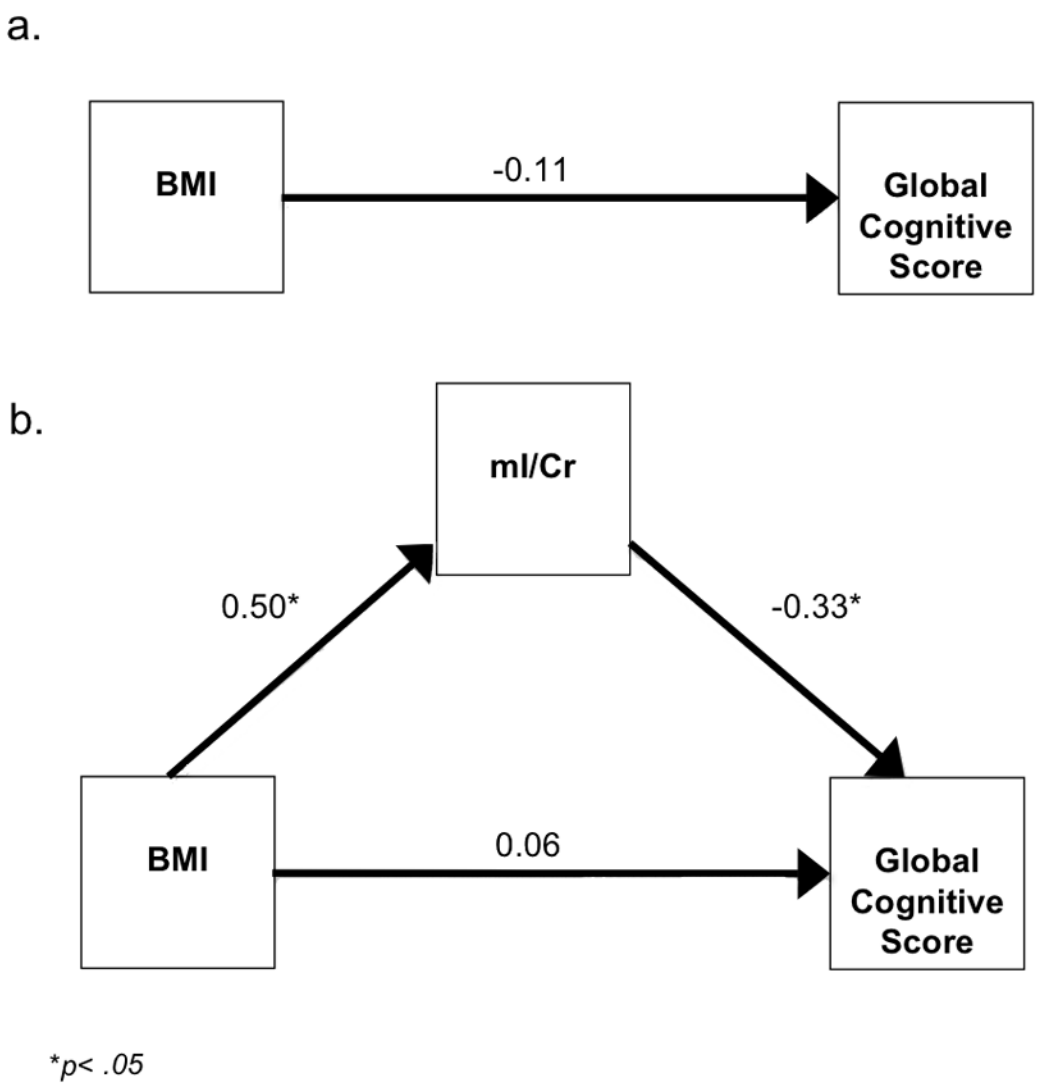

Figure 5.
Figure 5A. Direct structural equation model for the memory cognitive domain score
Figure 5B. Indirect structural equation model for the memory cognitive domain score

Table 1

Selected participant characteristics (n=54)

Characteristic	Mean±SD
Age, y	50.7±6.3
Sex (male/female)	24:30
Education, y	15.2±2.7
Body mass index, kg/m2	29.6±5.8
Systolic blood pressure, mmHg	126.3±16.7
Diastolic blood pressure, mmHg	75.9±9.4
LDL-Cholesterol, mg/dl	124.0±34.0
HDL-Cholesterol, mg/dl	49.0±16.7
Triglyceride, mg/dl	166.7±97.9
Glucose, mg/dl	105.5±31.1
mI/Cr	0.72±0.09
Glu/Cr	1.47±0.16
Cho/Cr	0.18±0.02
NAA/Cr	1.40±0.09

Table 2

Neuropsychological Test Results

Cognitive Domain	Mean±SD
General cognition	
Mini Mental Status Exam (MMSE)	28.4±1.3
Weschler Abbreviated Test of Intelligence (WASI)	
Full Scale IQ	113.5±11.9
Language	
WASI - Vocabulary Subtest	63.9±10.0
Category Fluency for Animals	23.4±5.7
Visual-Spatial	
WASI Matrix Reasoning Subtest	25.9±4.3
Rey Complex Figure Test (RCF) – Copy	30.3±3.9
Memory	
California Verbal Learning Test (CVLT)	
Immediate Recall	10.7±3.1
Delayed Recall	11.2±3.2
Recognition (Yes/No)	3.1±0.8
Rey Complex Figure Test (RCF)	
Immediate Recall	16.0±5.4
Delayed Recall	15.5±5.5
Recognition Discrimination	19.2±3.8
Attention-executive-psychomotor function	
Controlled Oral Word Association Test (COWAT)	37.3±10.8
Trail Making Test A, sec	32.0±10.7
Trail Making Test B, sec	80.0±31.0
Weschler Adult Intelligence Scale III (WAIS-III)	
Digit Span Subtest, total	16.1±4.1
Grooved Pegboard, Dominant Hand, sec	77.2±14.0