Changes in brain glutathione in patients with mild and major neurocognitive disorders

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Exit Seminar, August 30, 2023

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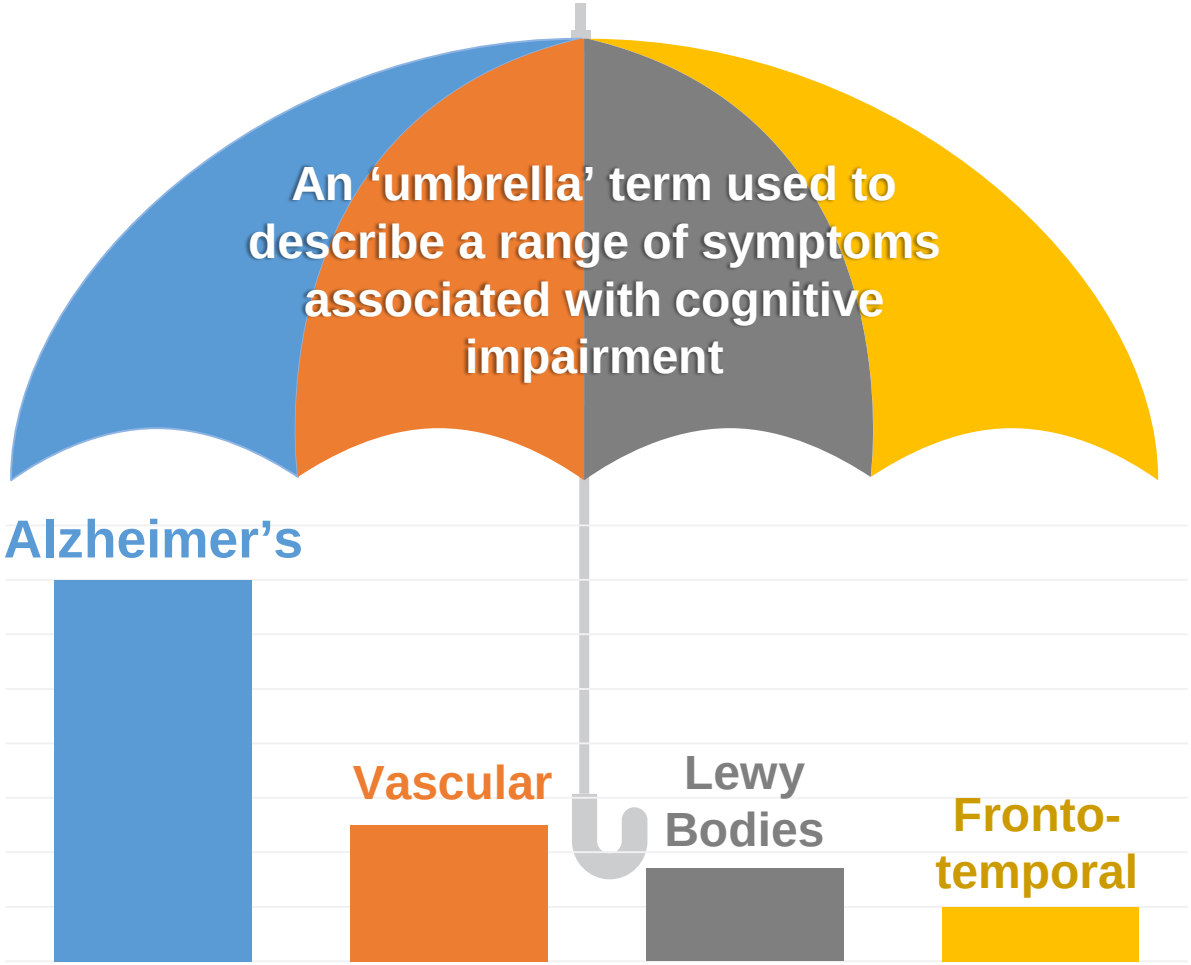
Neurocognitive Disorders (NCDs)

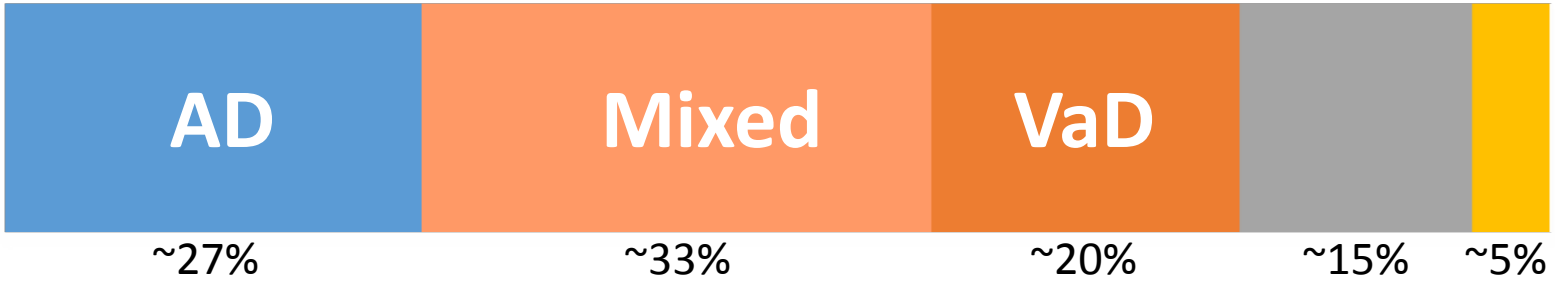


Neurocognitive Disorders

- Dementia has an overwhelming impact on the health care system and caregivers
- **A**lzheimer's **D**isease (AD) and **Va**scular **D**ementia (VaD) are the two most prevalent

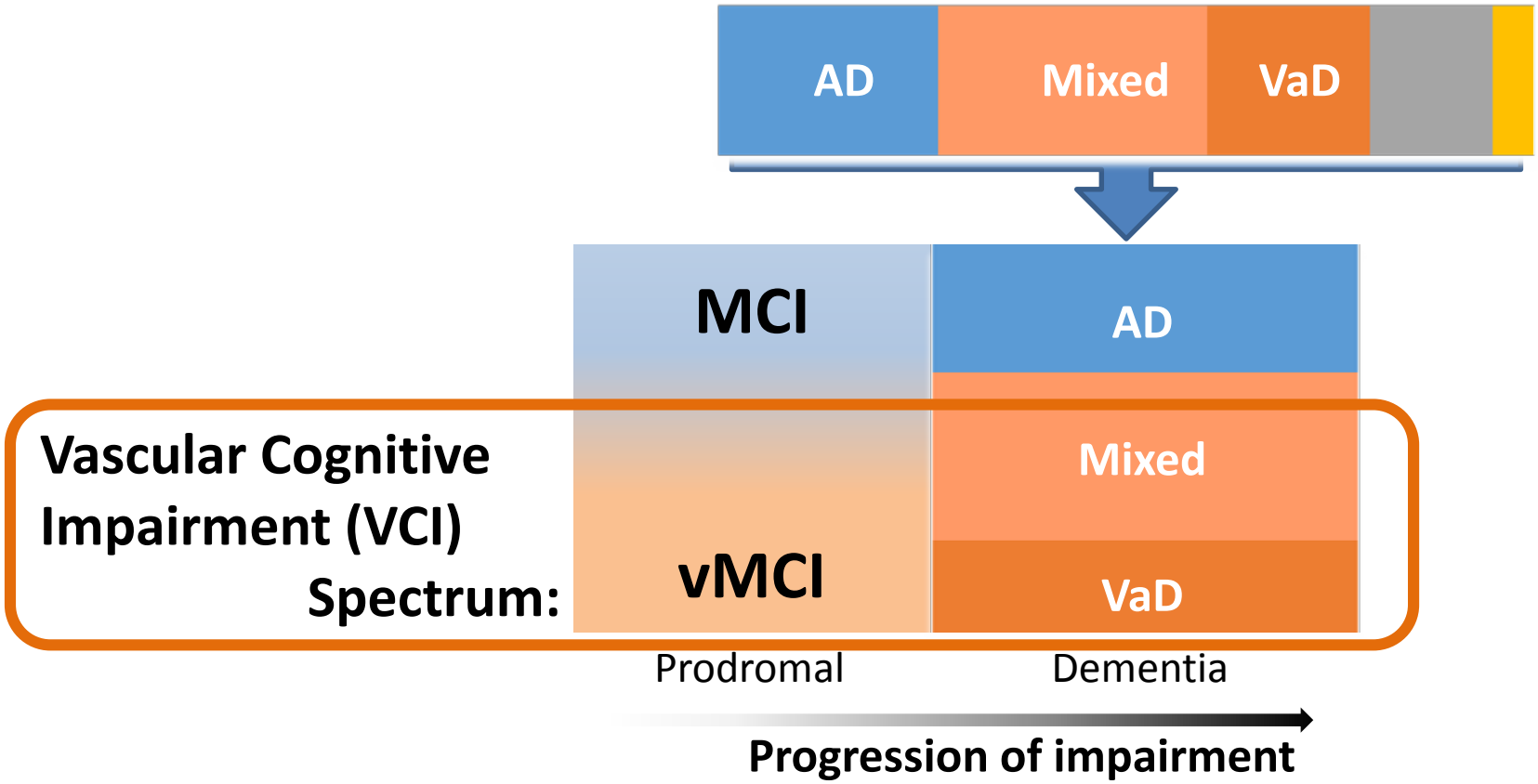
Adapted from Niedowicz, D. M., et al. (2011). "Alzheimer's disease: pathological mechanisms and recent insights." Current neuropharmacology **9**(4): 674-684.

Introduction: Dementia



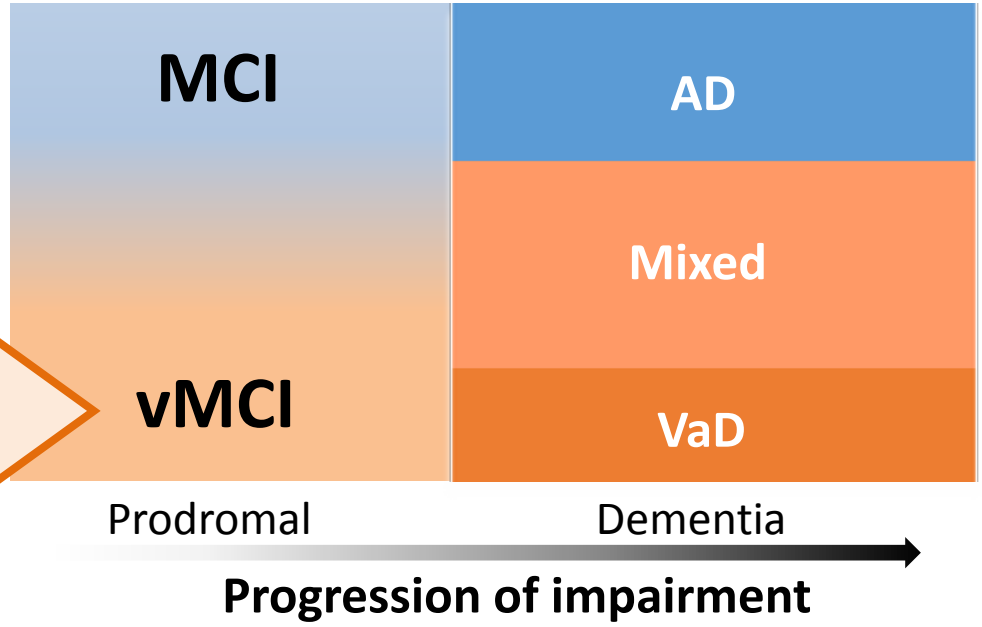
- Dementia has an overwhelming impact on the health care system and caregivers
- **A**lzheimer's **D**isease (AD) and **Va**scular **D**ementia (VaD) are the two most prevalent
- Cerebral vascular changes seen in 46-70% of clinically-diagnosed AD patients
- Vascular contributions plays an important role in dementia

Vascular Cognitive Impairment (VCI)



Vascular Mild Cognitive Impairment (vMCI)

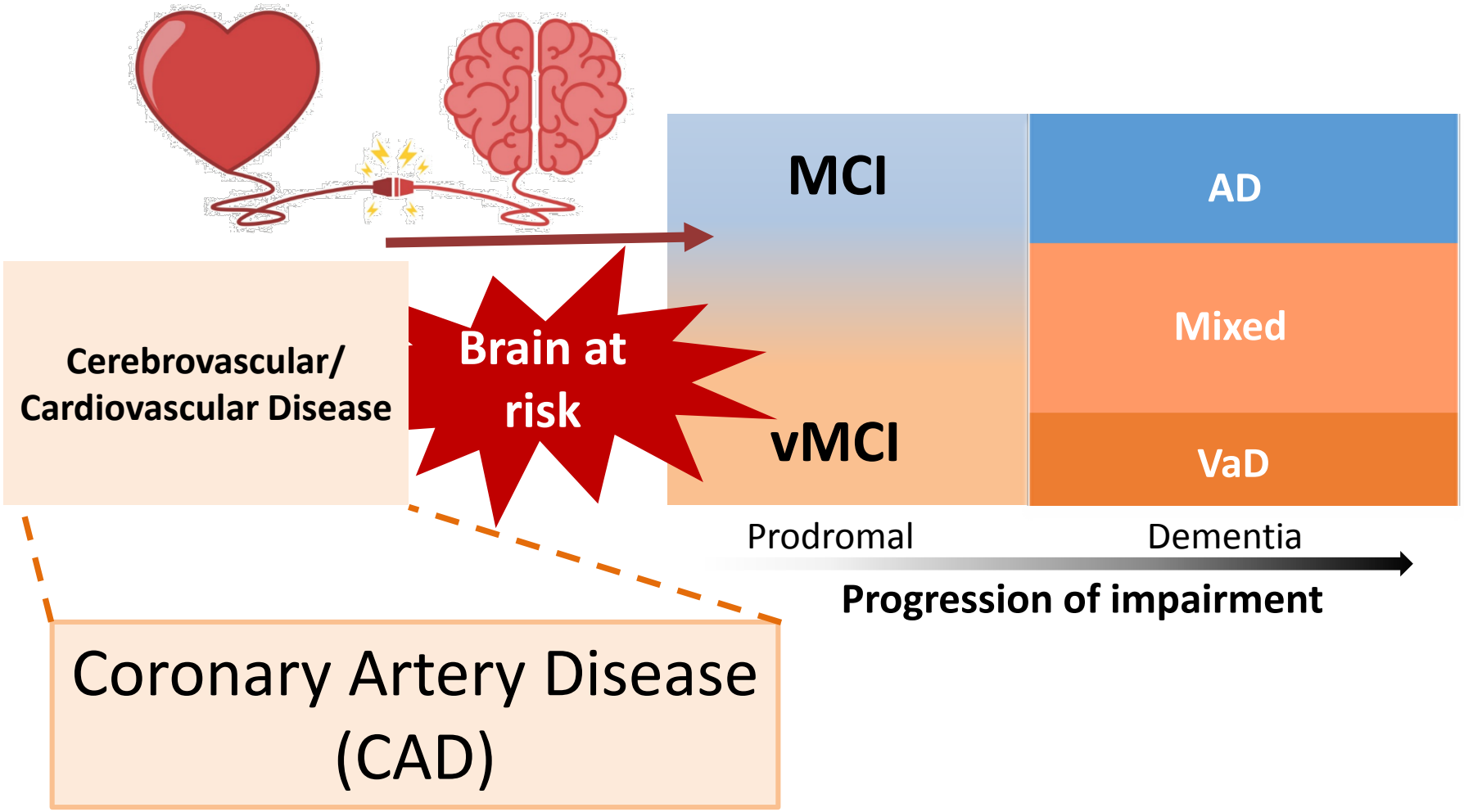
- Most prevalent form of VCI (age 64-84)
- Early cognitive deficits
- Risk of progression
- **Window of opportunity**



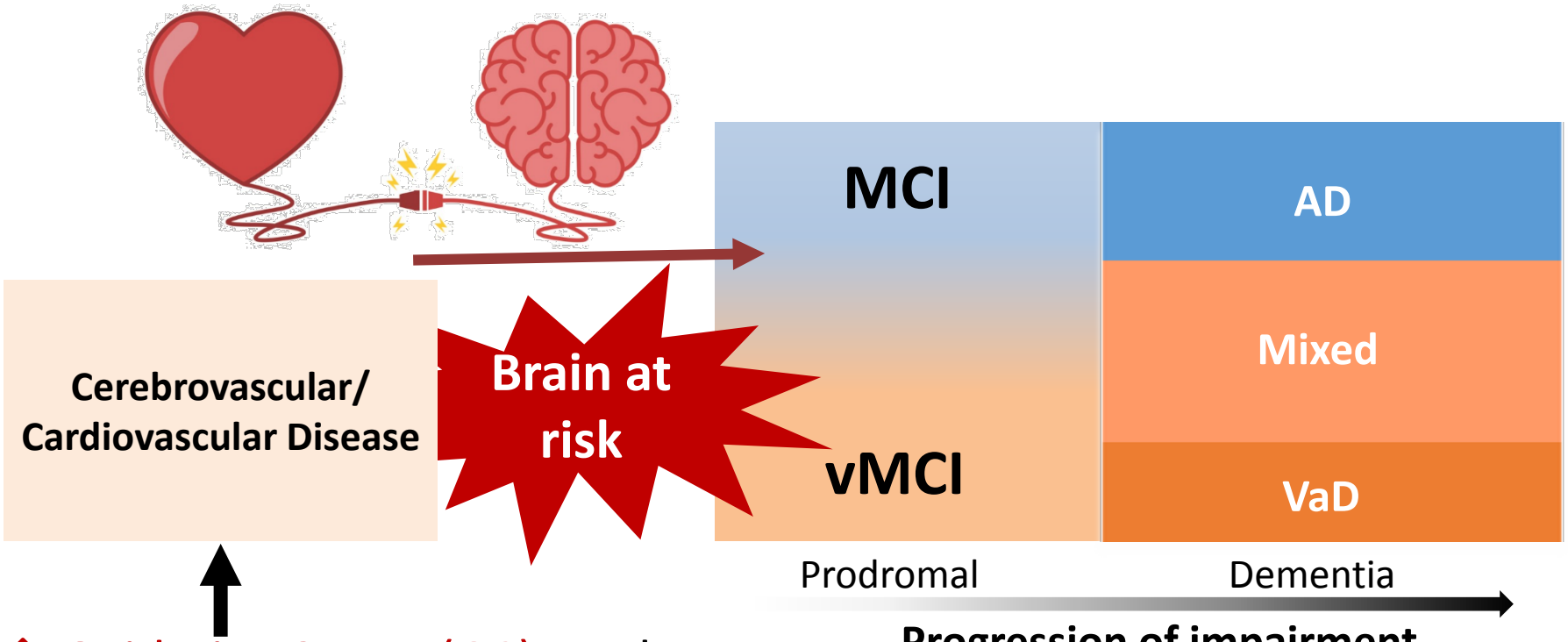
Current treatments for NCDs

- Only approved for mild to moderate AD dementia, modest efficacy
- Adacanumab, donanemab, lacenumab approved recently (USA)
  - Controversial
  - $$$$$
  - Cerebral edema and micro-haemorrhages
- Currently not available in Canada

vMCI and Coronary Artery Disease



vMCI and Oxidative Stress (OS)

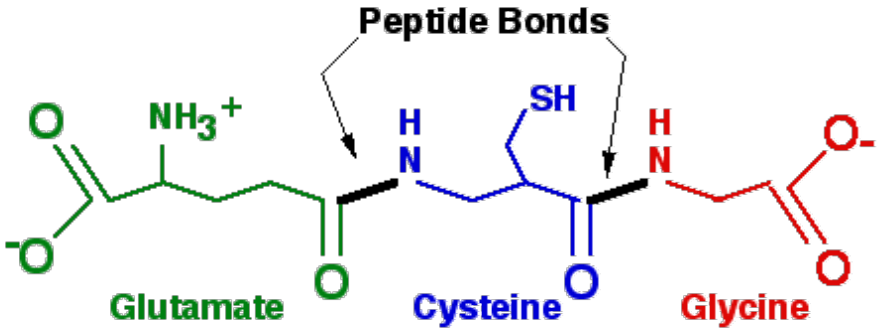


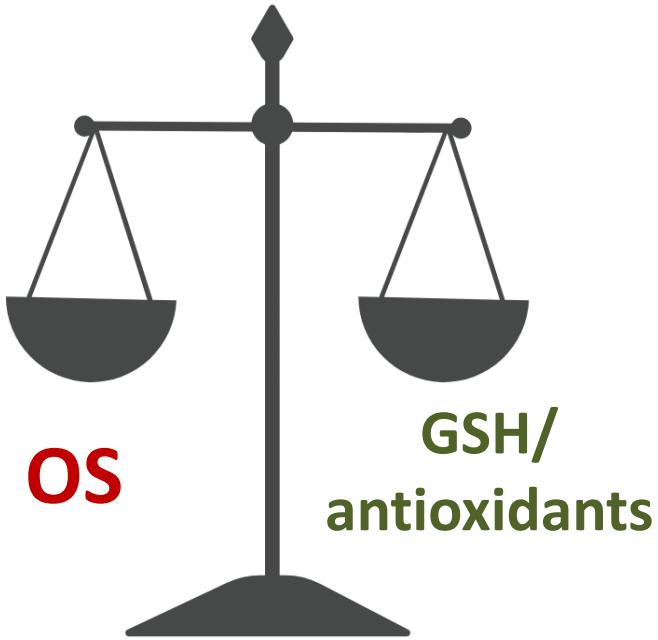
↑ **O**xidative **S**tress (OS) markers associated with ↓ **improvement** in cognitive performance after 6 months of cardiac rehabilitation

Suridjan et al., 2017. "Lipid Peroxidation Markers in Coronary Artery Disease Patients with Possible Vascular Mild Cognitive Impairment". J Alzheimers Dis;58(3):885-896.

Glutathione (GSH)

- Tripeptide antioxidant
- Primary antioxidant molecule in the brain
- Level in brain cells depends strongly on precursors' availability
- Depletion may be important in neuronal death

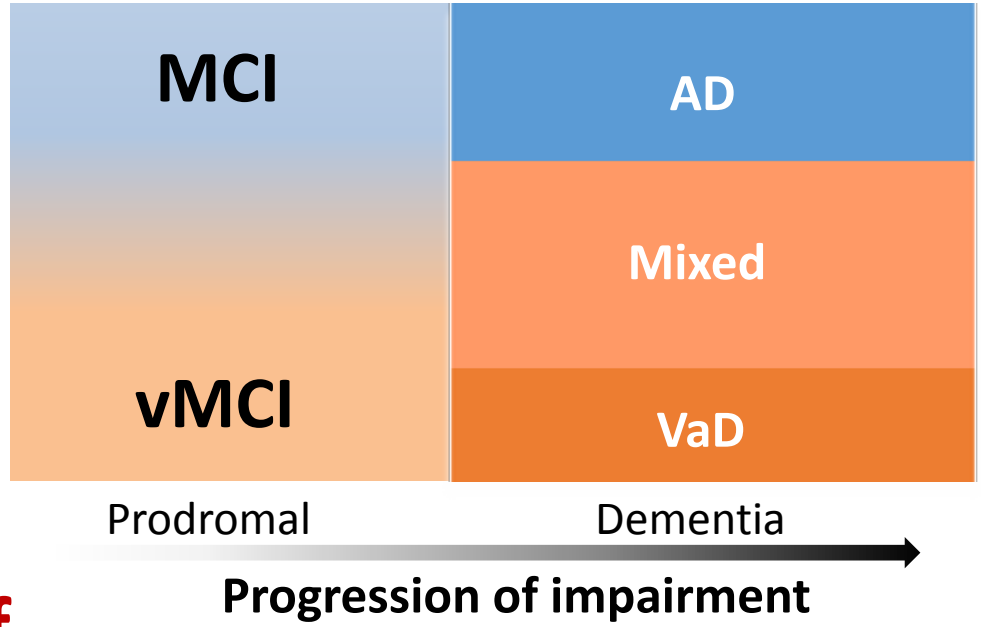


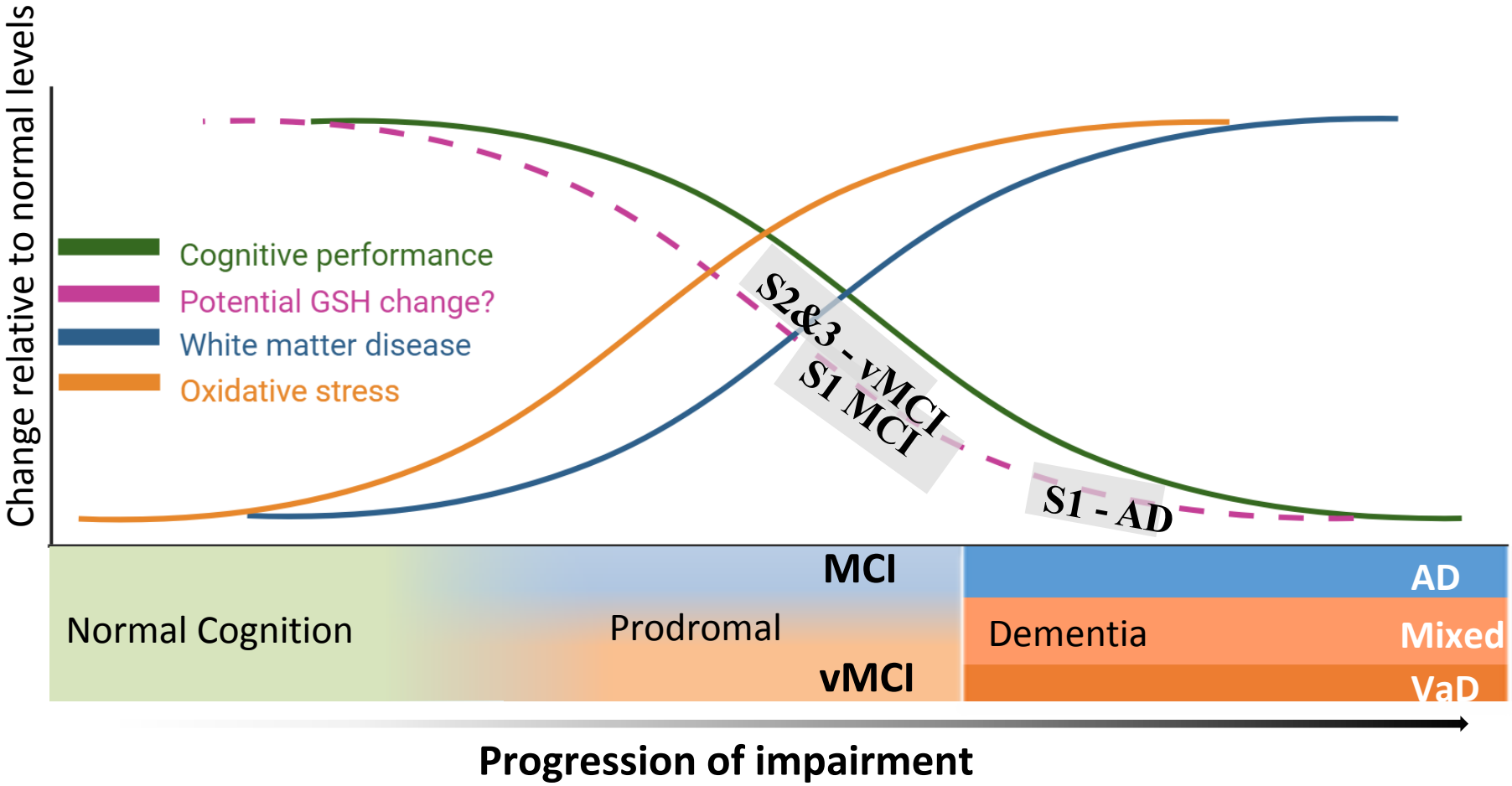


Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th edition. New York: W H Freeman; 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21154/

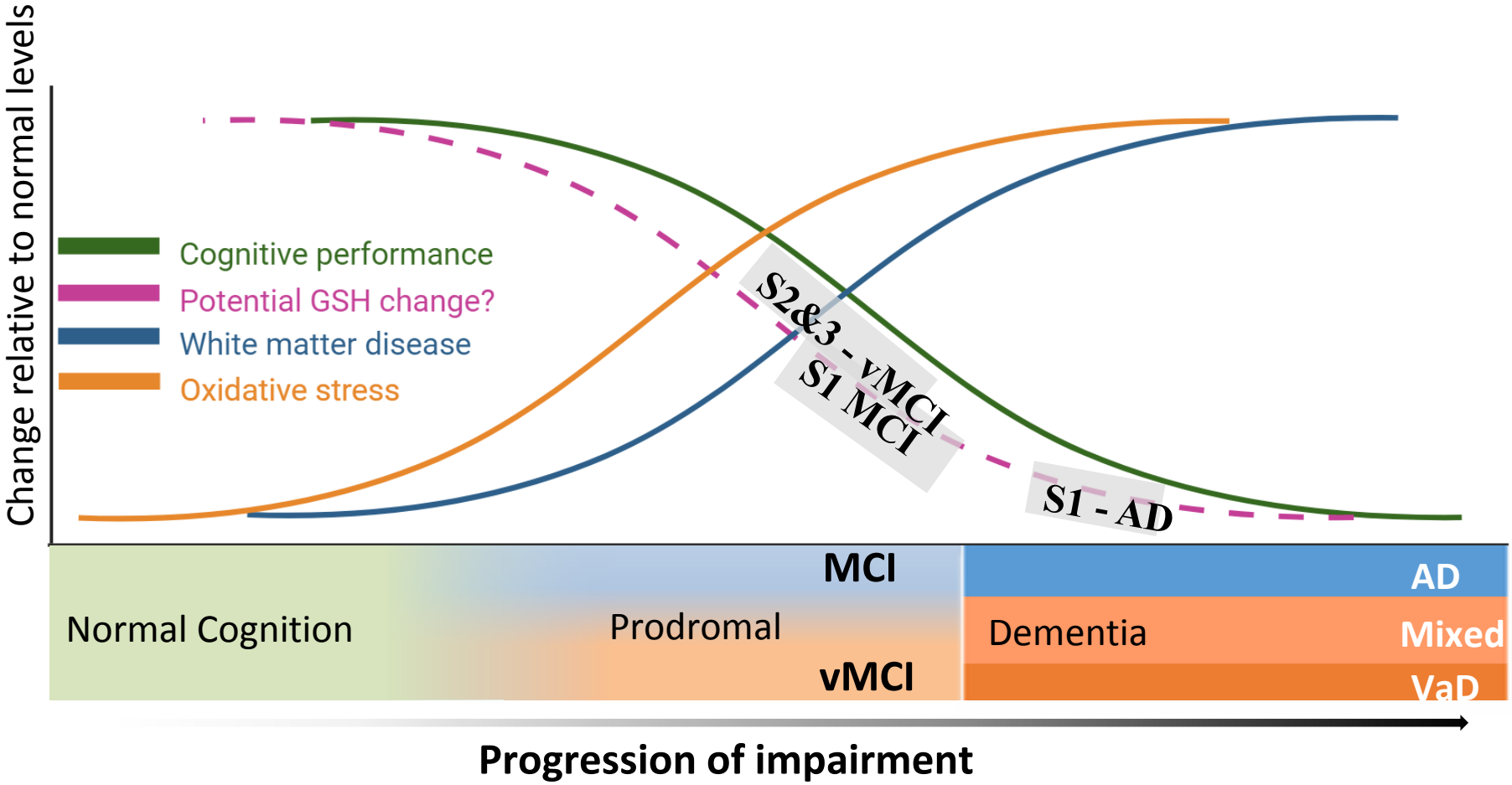
What are the changes in GSH?

↑ OS implicated the progression of MCI to AD





- S1 – meta analysis of GSH in AD & MCI vs. controls
- S2 – cross-section differences of GSH in vMCI vs. controls
- S3 – longitudinal GSH changes in vMCI following intervention



- **S1 – meta analysis of GSH in AD & MCI vs. controls**
- S2 – cross-section differences of GSH in vMCI vs. controls
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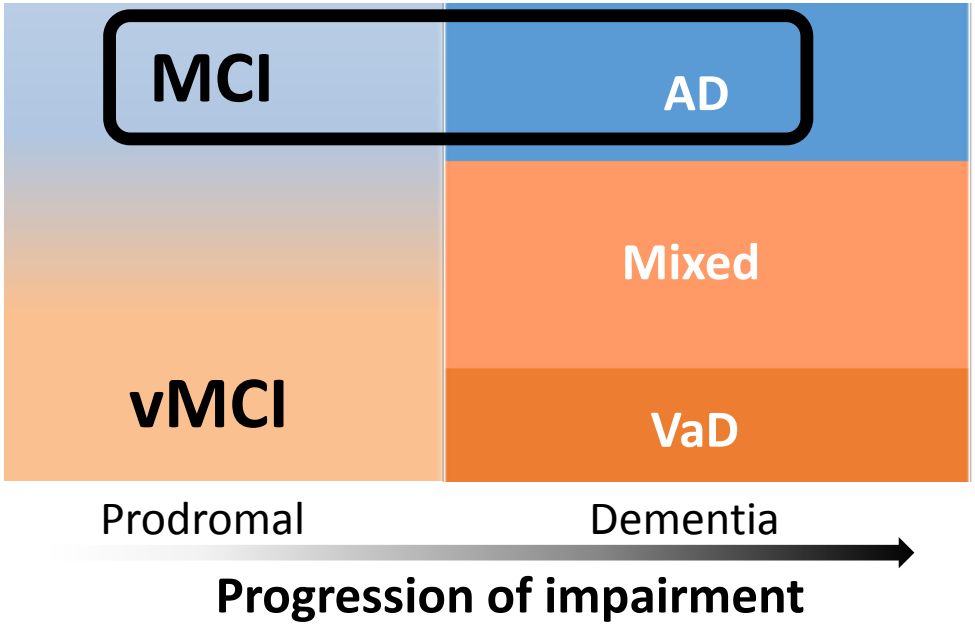
Study 1 – GSH in AD and MCI

? GSH in MCI and AD



Study 1 – GSH in AD and MCI

- **Aim 1:** Consolidate and quantitatively review the body literature of *in vivo* GSH (brain and blood) using meta-analytic methods in AD and mild cognitive impairment (MCI) patients

Hypothesis: GSH will be lower in MCI and AD compared to controls, due to increased oxidative stress and depletion of GSH

Study 1 – GSH in AD and MCI

Chen *et al. Alzheimer's Research & Therapy* (2022) 14:23
https://doi.org/10.1186/s13195-022-00961-5

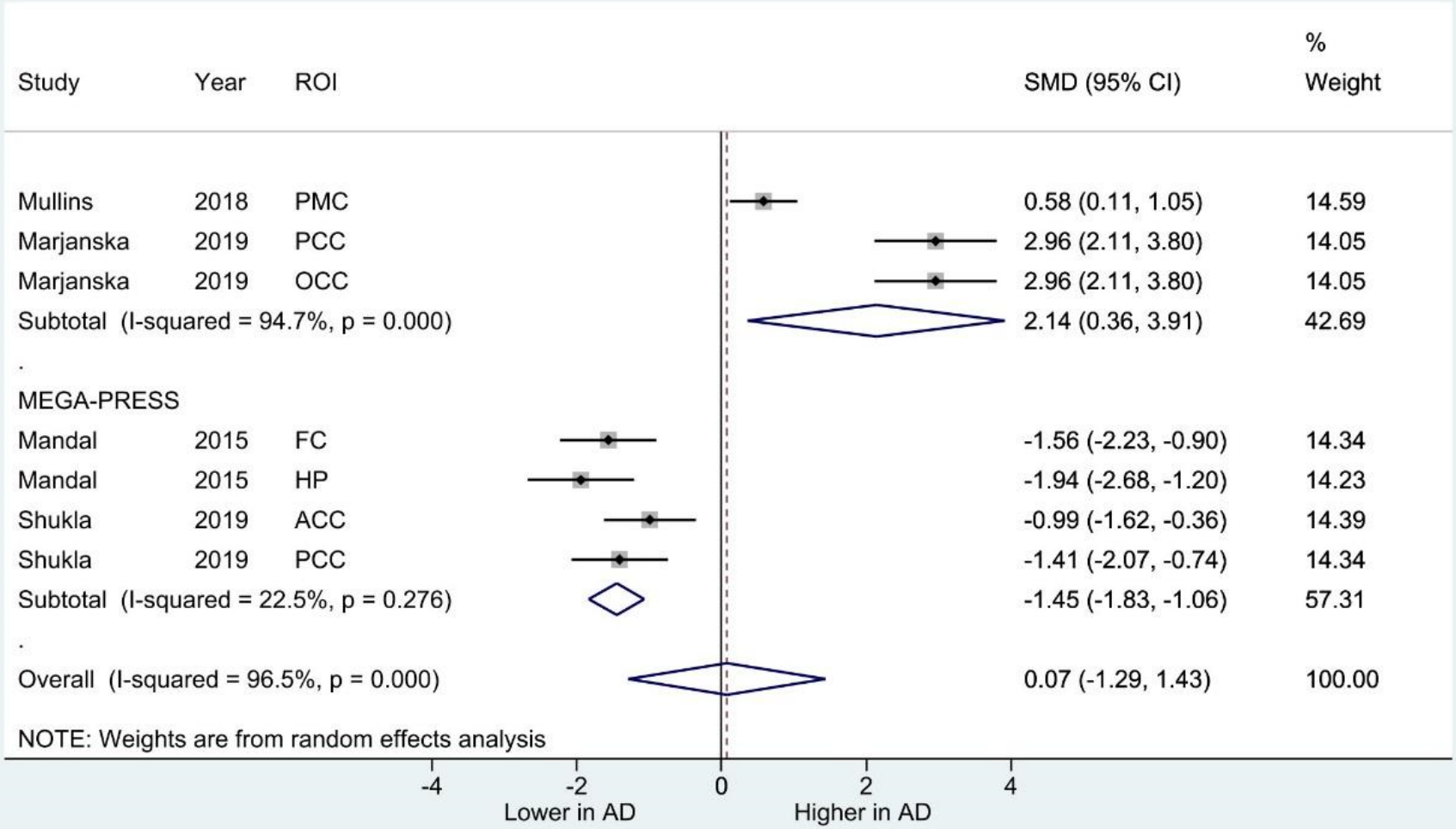
Alzheimer's Research & Therapy

RESEARCH Open Access

Altered central and blood glutathione in Alzheimer's disease and mild cognitive impairment: a meta-analysis

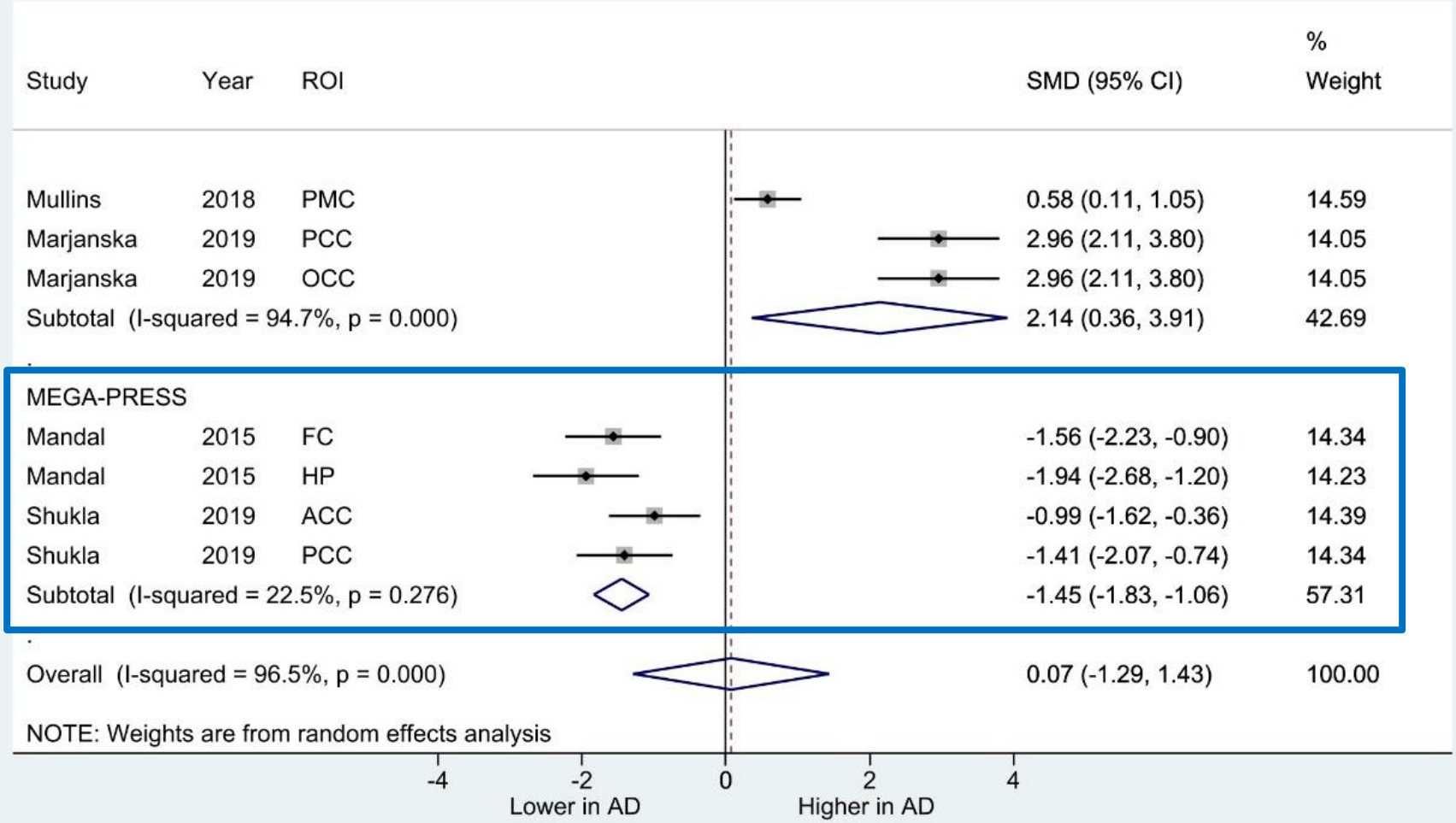
Jinghan Jenny Chen[1,2], Mathura Thiyagarajah[1,2], Jianmeng Song[1], Clara Chen[1], Nathan Herrmann[1,3,4], Damien Gallagher[3,4], Mark J. Rapoport[3,4], Sandra E. Black[5,6], Joel Ramirez[5], Ana C. Andreazza[2], Paul Oh[6], Susan Marzolini[6], Simon J. Graham[7] and Krista L. Lanctôt[1,2,3,4,5*]

Results – Brain GSH (AD)



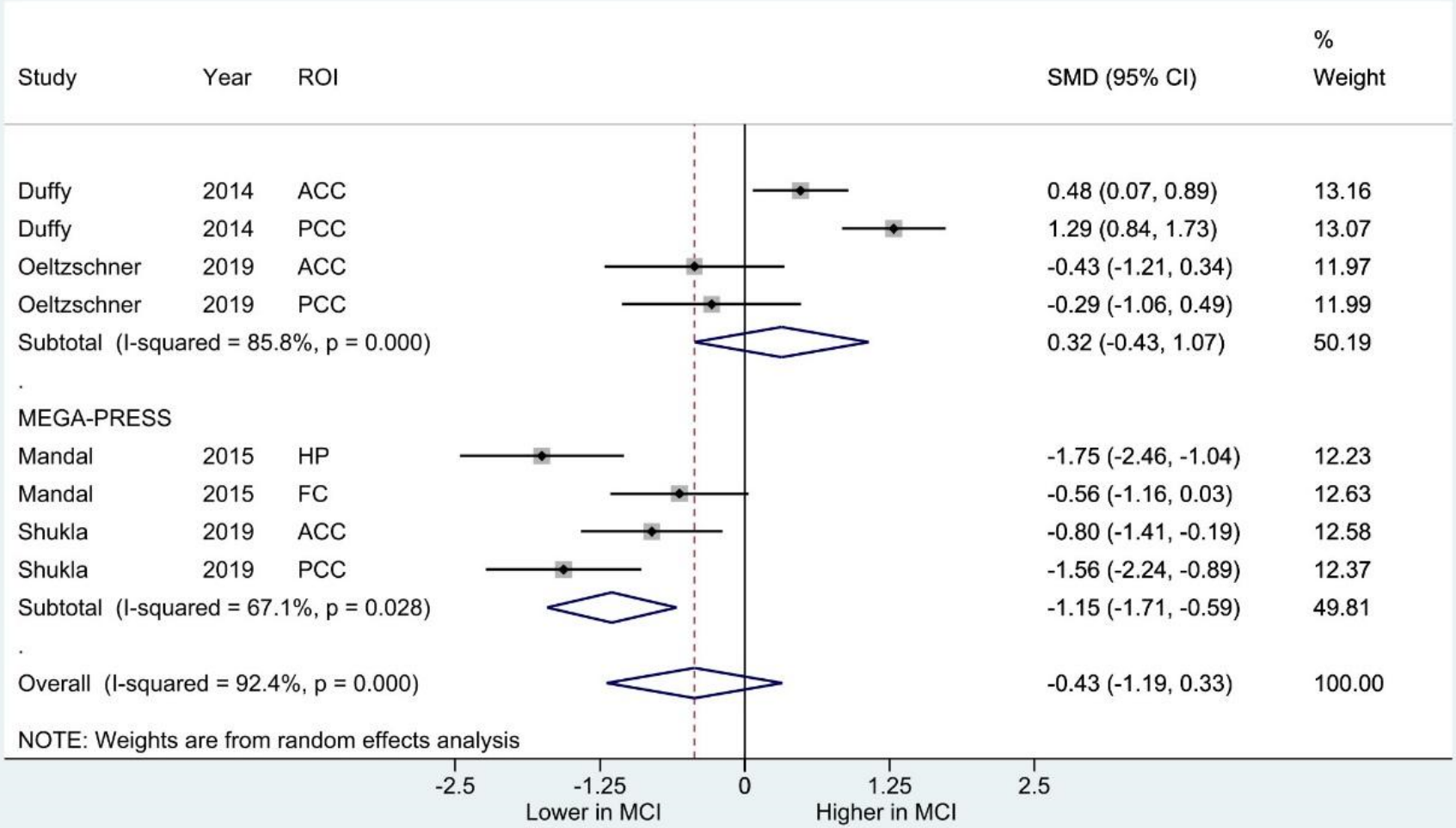
Study 1: Meta Analysis of GSH in AD/MCI

Results – Brain GSH (AD)



Study 1: Meta Analysis of GSH in AD/MCI

Results – Brain GSH (MCI)



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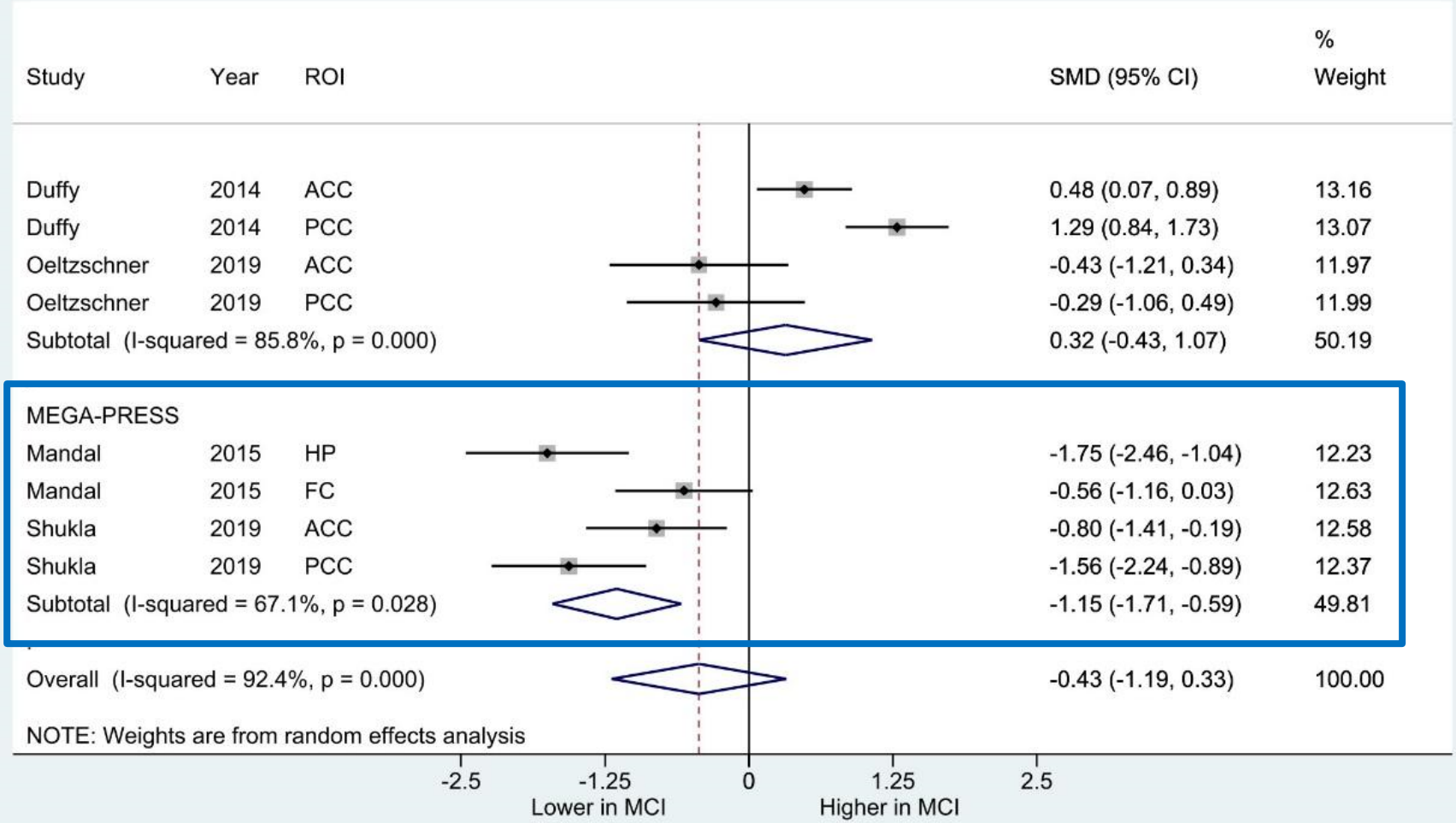
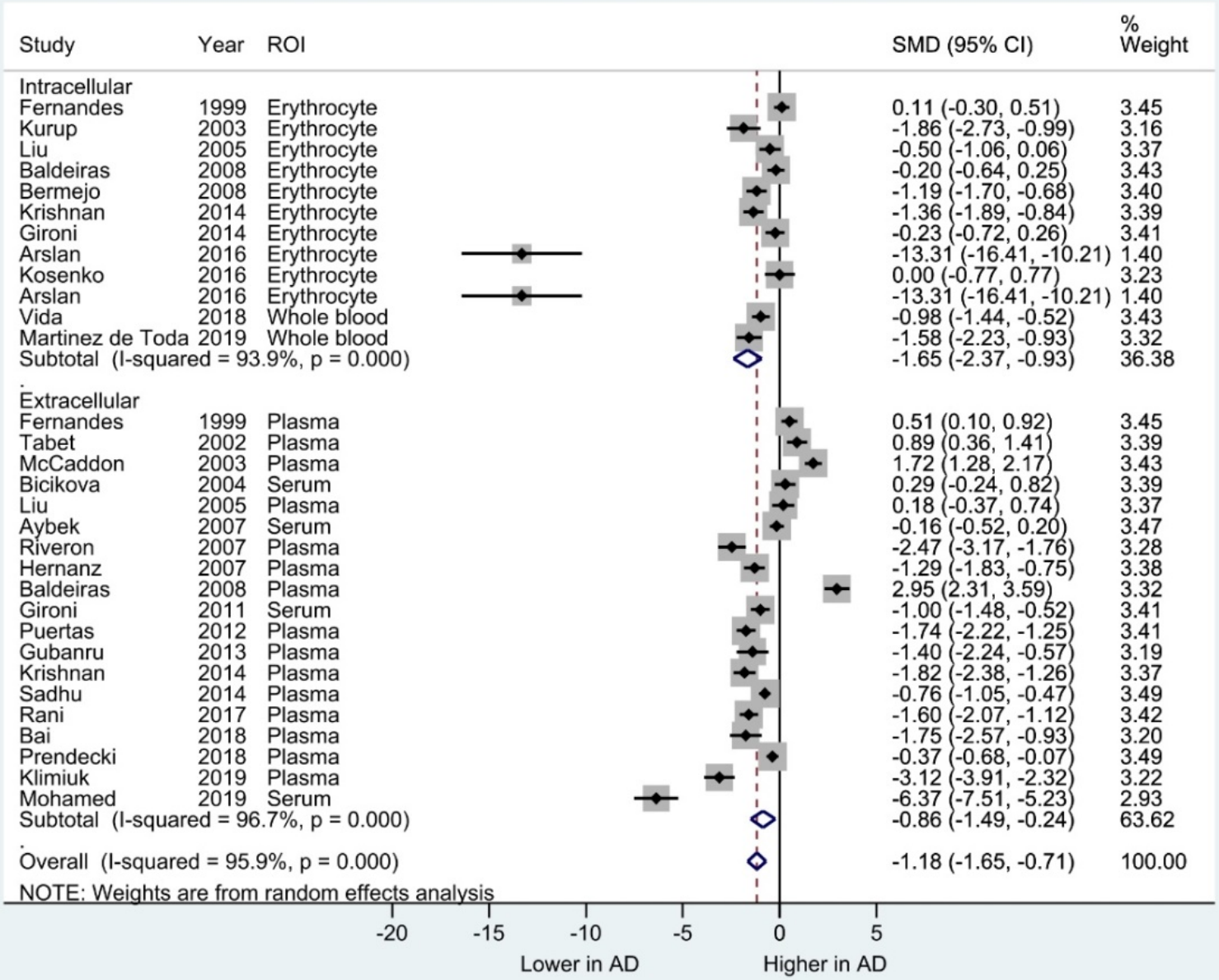


Results – Blood GSH (AD)



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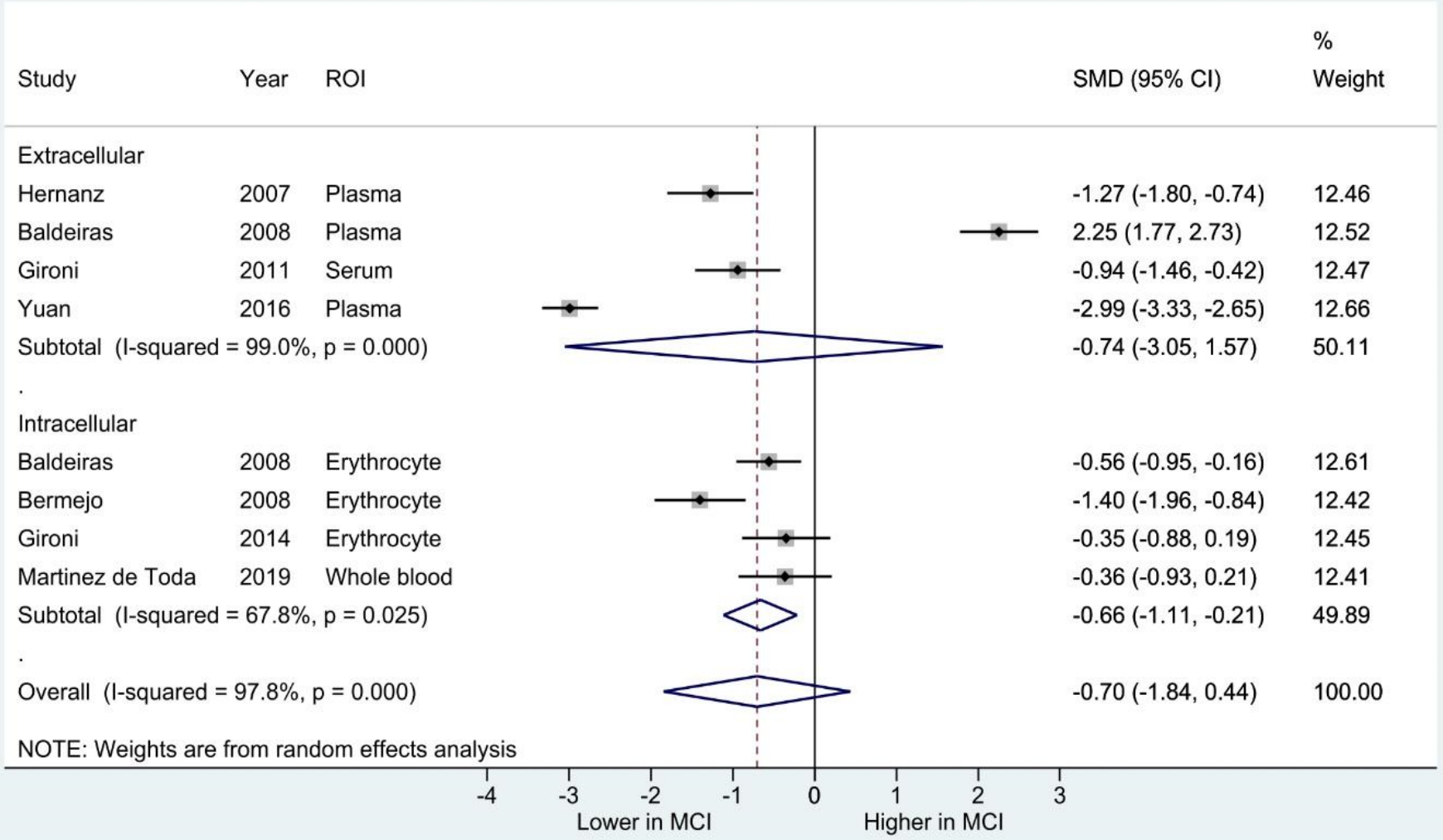

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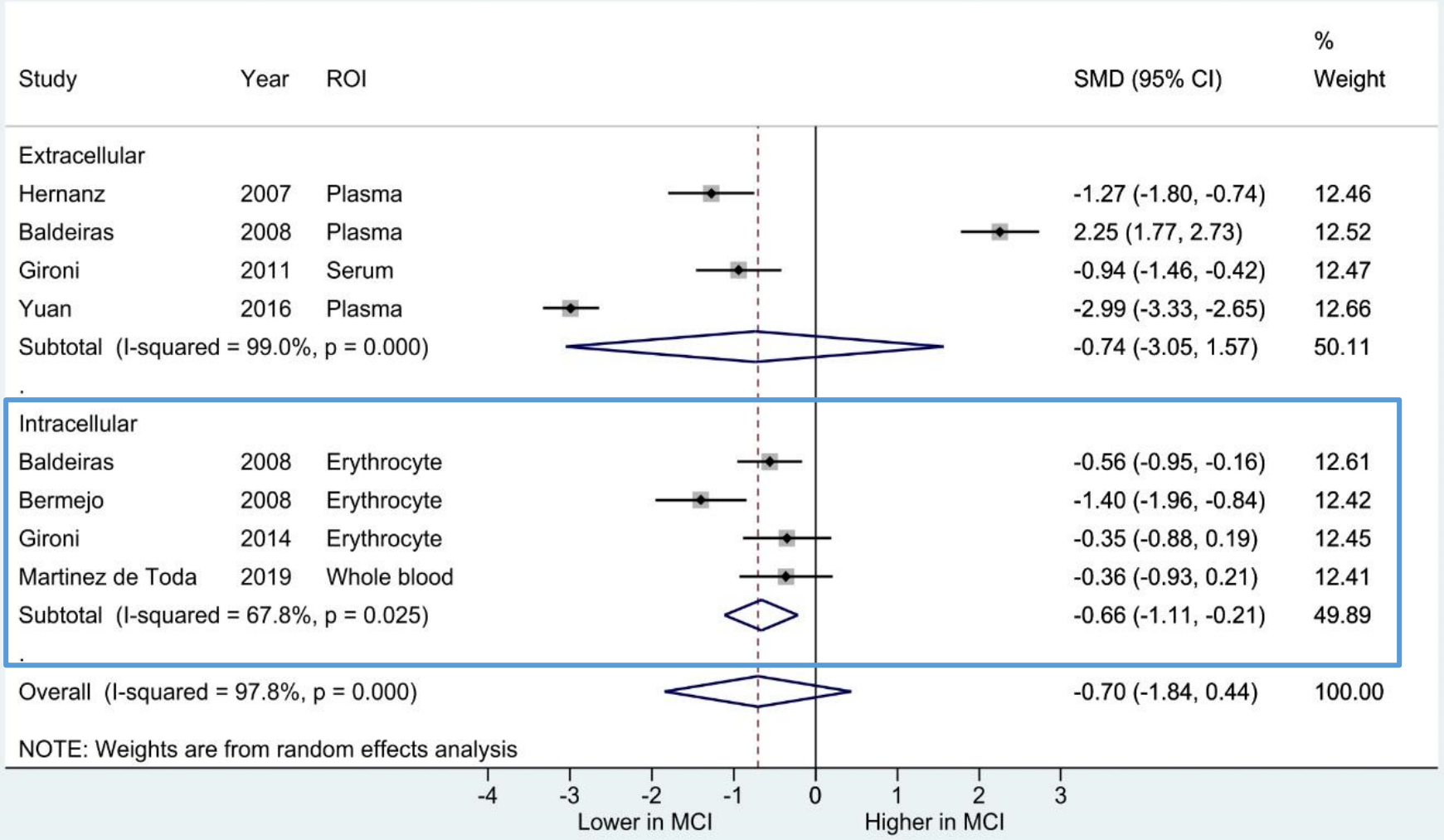


Study 1: Summary

- ↓ blood GSH in AD and intracellular blood GSH in MCI
- Overall lack of main effect of *in vivo* brain GSH
  - Wide range of Magnetic Resonance Spectroscopy sequences available to measure *in vivo* brain GSH
  - **Subgroup: MEGA-PRESS sequence studies have significantly lower GSH in MCI and AD**

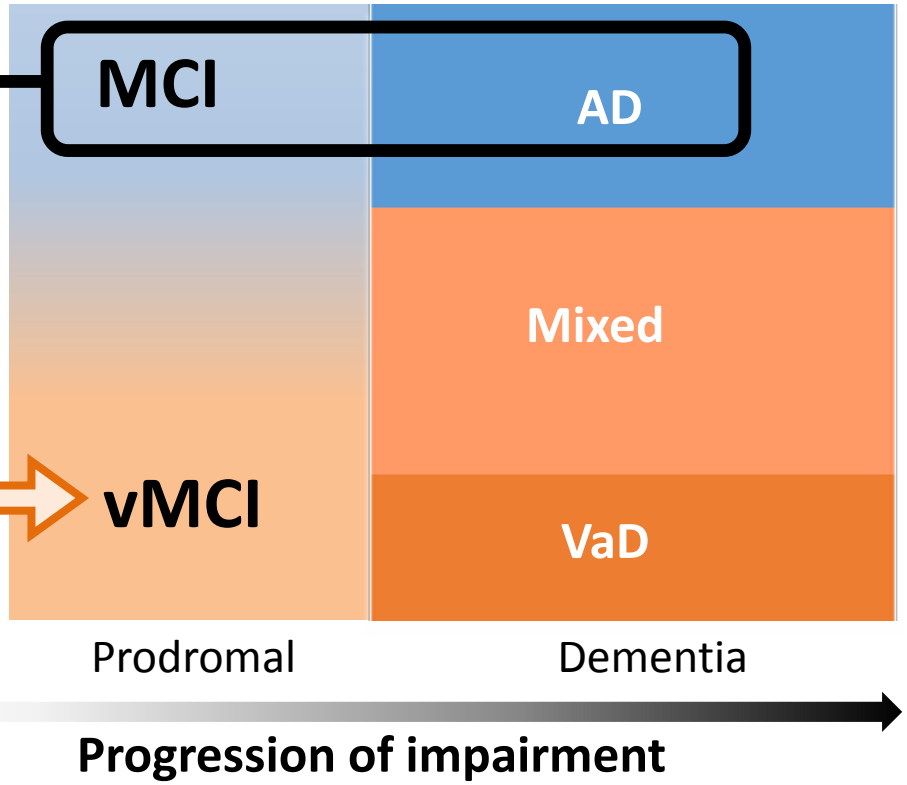
Limitations

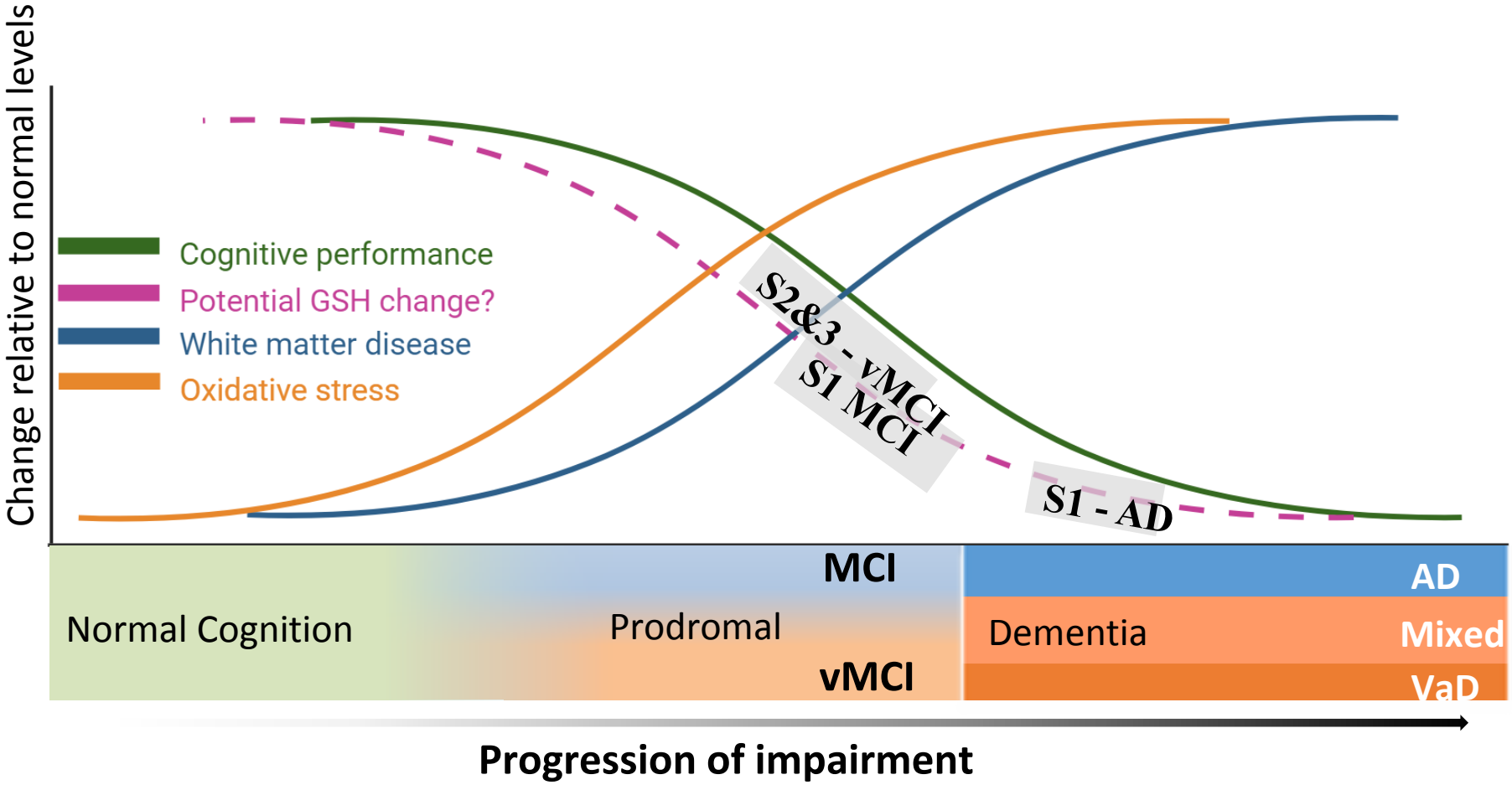
- Additional sources of heterogeneity
  - Disease severity
  - Concomitant illnesses and medications
- Small number of studies in MCI and AD patient reporting GSH in the brain
- Each brain region of interest was treated as an individual study

Summary so far…

Study 1:
Blood GSH : ↓ in AD & MCI
Brain GSH : ↓ in AD & MCI
(MEGA-PRESS only)

Study 2: brain GSH (using MEGA-PRESS)





- S1 – meta analysis of GSH in AD & MCI vs. controls
- **S2 – cross-section differences of GSH in vMCI vs. controls**
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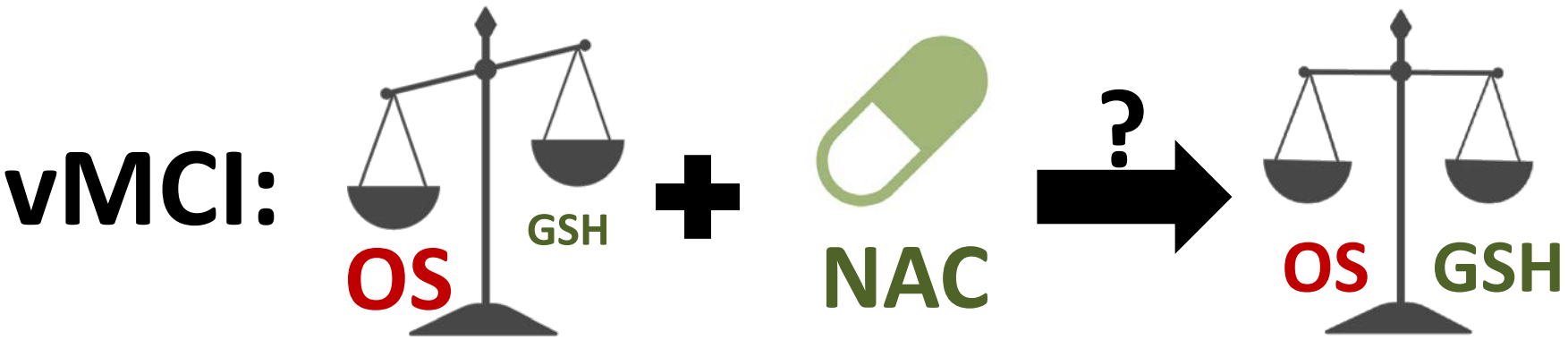
Study 2

- **Aim:** Assess brain GSH as a diagnostic marker of vMCI
- A cross-sectional analysis of brain GSH in vMCI and matched controls will be completed

Hypothesis: brain GSH will be lower in vMCI compared to cognitive-normal controls with CAD

N-acetylcysteine (NAC)

- ↑ availability of cysteine → ↑ GSH
  - ↓ lipid peroxidation
- Accumulate in the brain (animal studies)
- Positive effects but variable across trials
  - (MCI, mild to moderate AD)



Study 3

- **Aim (longitudinal):** Does NAC treatment have target engagement in the brain?
- Repeated measures general linear model of change in brain GSH over 6 months and NAC treatment

Hypothesis: NAC treatment will result in greater increase in GSH over time compared to placebo

Study Design

Inclusion	Exclusion

Possible vMCI:
- Males/females 55-85 years old
- **Mo**ntreal **C**ognitive **A**ssessment (MoCA) <28
- NINDS-CSN 60 minute battery: ≤-1.0 SD below norm in these domains:
  - Executive function
  - Verbal & visuospatial memory
  - Working memory
  - Processing speed
Probable vMCI:
- Extensive white matter disease (Fazekas ≥ 2 AND/OR ≥ 2 lacunes) on MRI
	- History of stroke, epilepsy, traumatic brain injury
	- Presence of severely impaired organ function
	- Current major psychiatric or neurological condition
	- Contraindication to MRI/MRS scan

Cognitively-healthy CAD control: - MoCA ≥28 OR screen failed on NINDS-CSN 60 min	

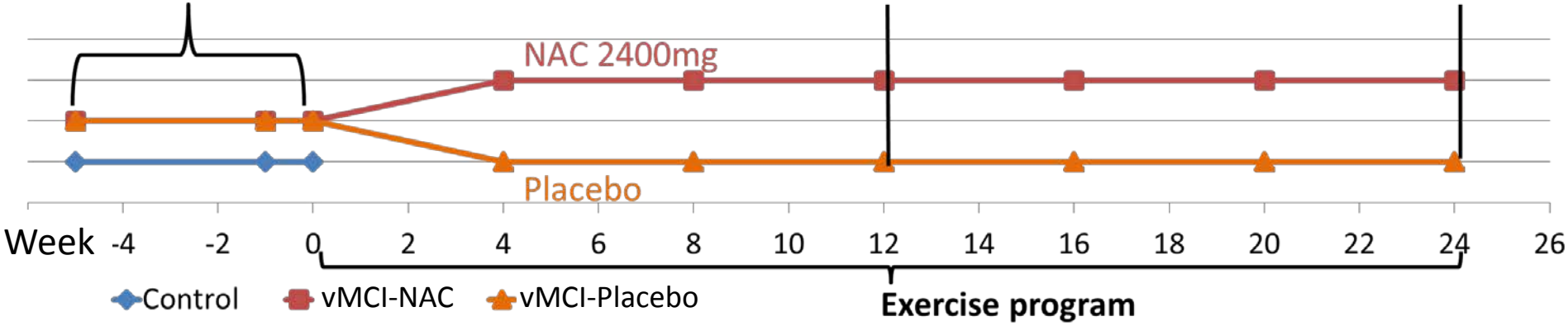
Gorelick et al. "Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American heart association/American stroke association". *Stroke*. 2011 Sep; 42(9): 2672-713.

Study Schedule

Screening + Baseline:
Eligibility, MoCA/more cognitive tests (vMCI), MRI/MRS scan, blood markers

Midpoint (vMCI only)
MoCA and cognitive tests, blood markers

Endpoint (vMCI only)
MoCA and cognitive tests, MRI/MRS scan, blood markers



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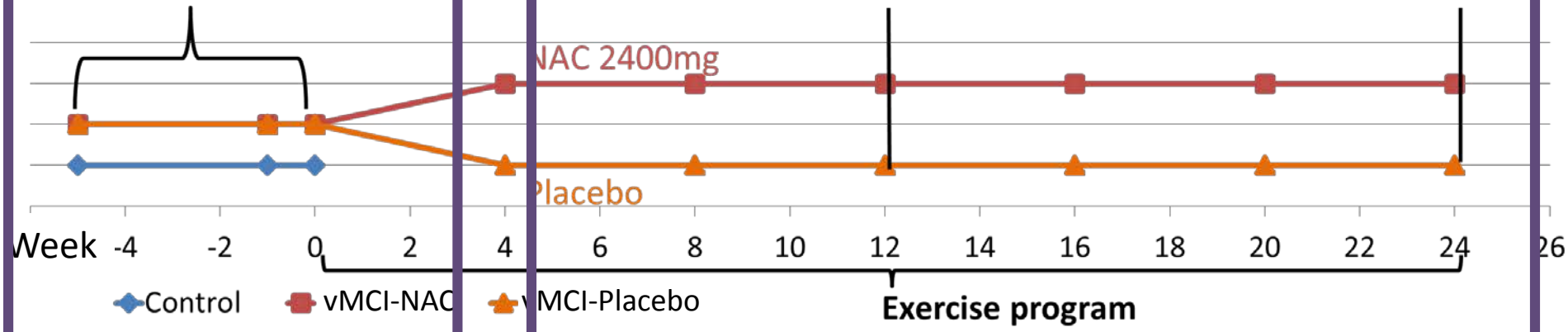


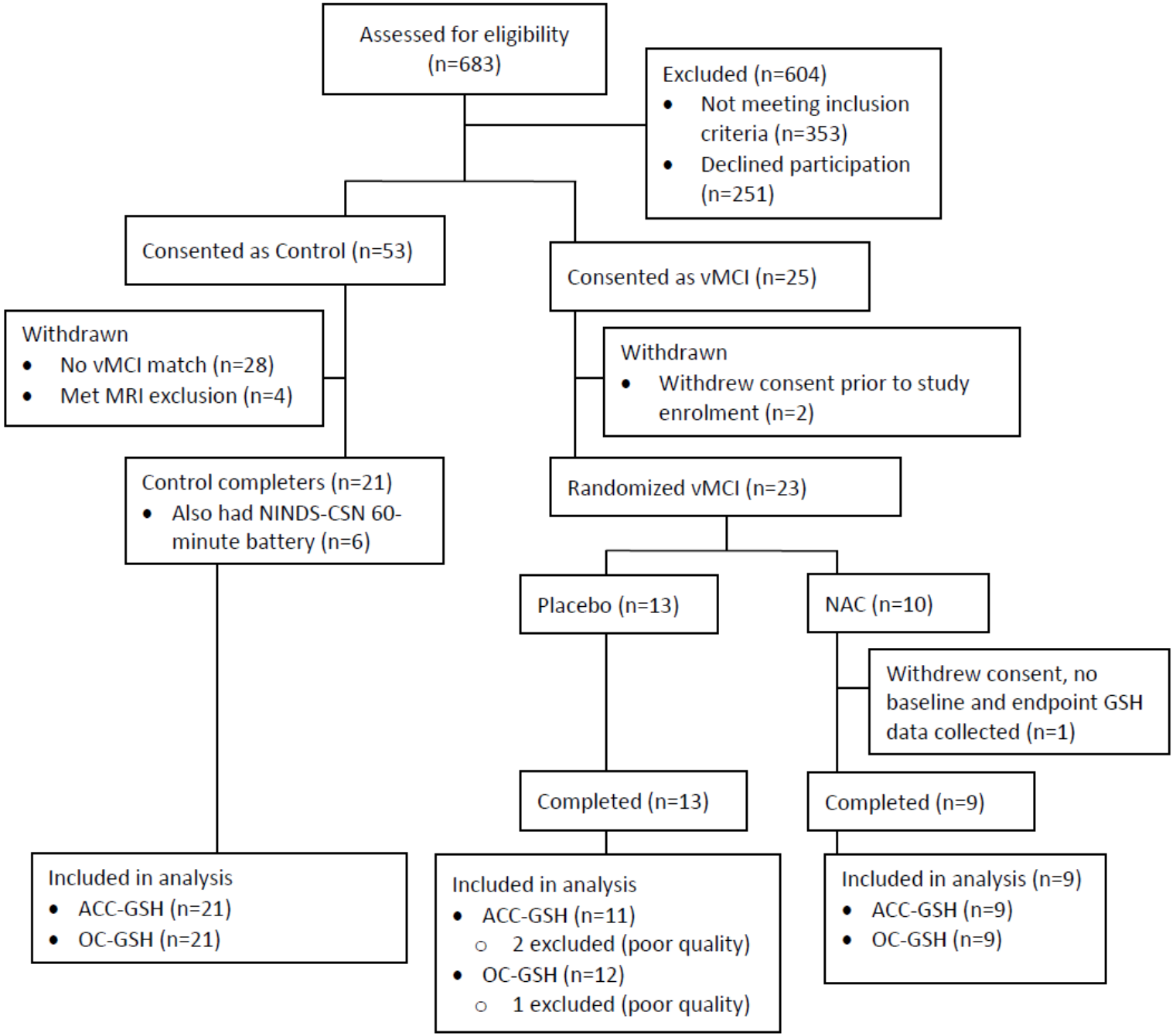
Power Calculation

Study 2

- Mandal et al., 2015 reported reduced brain GSH in MCI (n=22) vs. controls with a very large effect size. **Based on this, a sample size of 18 (9 per group)** achieves 81.4% power for a an ANCOVA with a two-sided α=0.05.

Study 3

- NAC's effect on brain GSH has not been investigated in cognitively-impaired populations. An estimate that **a sample size of 30 (15 placebo vs. 15 NAC) will achieve 80% power** with a two-sided α=0.05 to detect a medium to large effect size.



Study 2 & 3: GSH in vMCI and NAC intervention

Study 2: Clinical/Demographic Characteristics

	Control (n=21)	vMCI (n=22)	Significance
Age, mean (SD)	66.7 (7.8)	67.4 (7.3)	n.s.
Male, %	86%	73%	n.s.
Caucasian, %	67%	55%	n.s.
Drinks per week, mean (SD)	2.5 (2.9)	2.1 (4.2)	n.s.
History of smoking, %	8 (42%)	6 (27%)	n.s.
Years of education, years (SD)	18.3 (2.9)	15.3 (3.4)	**$F_{(1,40.3)}$=9.2, p=.004**
Mean Arterial Pressure, mmHg (SD)	87.5 (9.60)	96.3 (9.08)	**$F_{(1,41)}$=9.5, p=.004**
Hypertension, %	33%	73%	**X^2 (1, n=43) = 6.7, p=.01**
Diabetes, %	5%	27%	n.s.
Dyslipidemia, %	43%	68%	n.s.
BMI>=30, %	29%	9%	n.s.
Current smoker, %	0%	5%	n.s.
Number of vascular risk factors (SD)	1.14 (1.24)	1.82 (1.30)	n.s.

Concomitant Medications

Drug category, n (%)	Control (n=19)	vMCI (n=22)
Aspirin	12 (63%)	19 (86%)
Other antiplatelet medications	14 (74%)	11 (50%)
Statins	17 (90%)	21 (96%)
Thiazide diuretics	1 (5%)	1 (5%)
Calcium channel blockers	2 (11%)	5 (23%)
Systemic beta-blockers	9 (47%)	15 (68%)
ACE inhibitors	8 (42%)	8 (36%)
Thyroid hormone	3 (16%)	1 (5%)
SSRI, SNRI, other antidepressants	1 (5%)	2 (9%)
Vitamins, minerals and supplements	7 (37%)	9 (41%)

n.s. between control and vMCI for all major drug categories

Cognition

(mean ± SD)	Control	vMCI	Significance
Global cognition	(n=21)	(n=22)	
MoCA	27.7 (1.20)	23.0 (1.95)	$F_{(1, 35.1)}$=90, p<.001
Domain composite z-scores	(n=6)**	(n=22)	
Executive function	0.78 (0.50)	-0.39 (0.50)	$F_{(1, 26)}$=25.9, p=<.001
Verbal memory	0.56 (0.69)	-0.87 (0.92)	$F_{(1, 26)}$=12.5, p=.002
Visual memory	0.31 (0.87)	-0.17 (0.84)	n.s.
Working memory	0.48 (0.59)	-0.21 (0.63)	$F_{(1, 26)}$=5.9, p=.02
Processing speed	0.24 (0.14)	-0.07 (0.66)	$F_{(1, 25.6)}$=4.2, p=.05

***15 control participants scored 28 or higher the MoCA and subsequently did not complete the NINDS-CSN 60-minute battery.*

Whole brain volumetrics

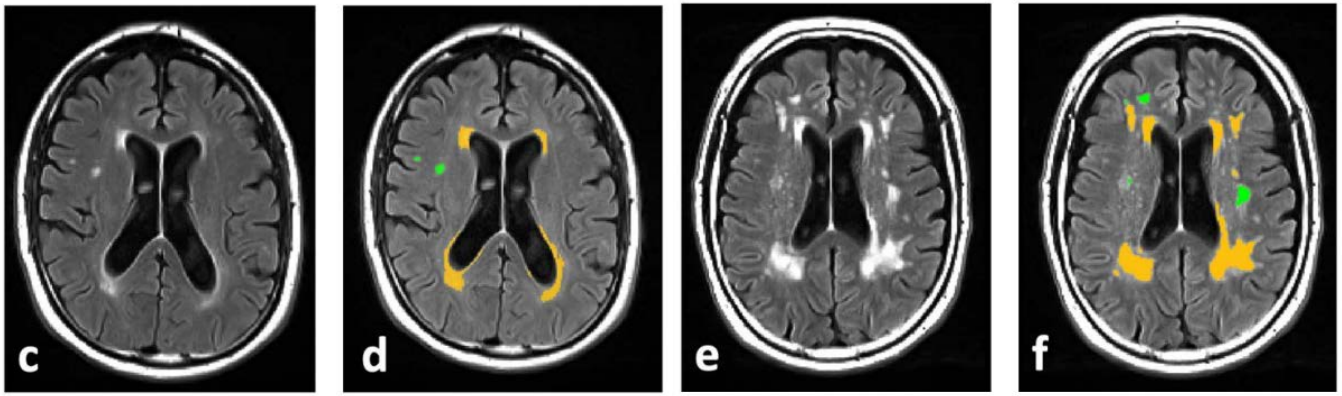
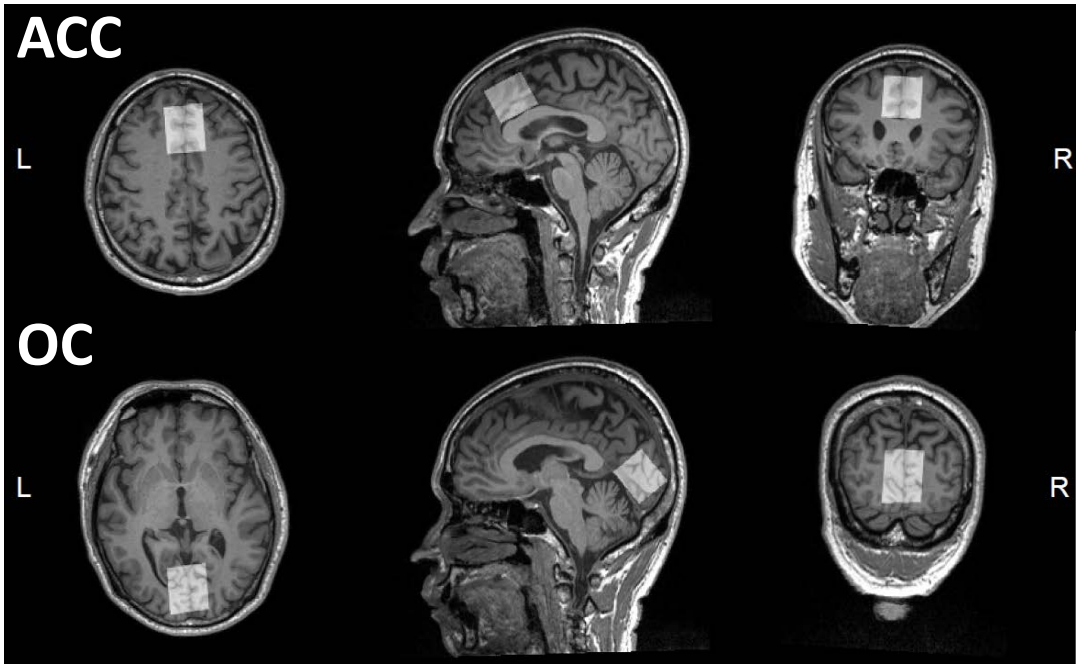


Image from Ramirez J, Scott CJ, Black SE. A short-term scan-rescan reliability test measuring brain tissue and subcortical hyperintensity volumetrics obtained using the lesion explorer structural MRI processing pipeline. Brain Topogr. 2013 Jan;26(1):35-8. doi: 10.1007/s10548-012-0228-z.

Whole brain volumetrics, cm^3 (SD)	Control (n=21)	vMCI (n=22)	Significance
Normal-Appearing White Matter	422.0 (64.2)	426.0 (79.4)	$F_{(1,41)}$=.03, p=.88
Normal-Appearing Grey Matter	553.0 (49.2)	551.0 (65.8)	$F_{(1,41)}$=.01, p=.92
Sulcal CSF	237.0 (52.8)	232.0 (64.1)	$F_{(1,40.2)}$=.08, p=.77
Ventral CSF	43.2 (25.3)	151.0 (226.0)	**$F_{(1,21.6)}$=4.9, p=.04**
Periventricular WMH	8.84 (13.70)	50.60 (82.60)	**$F_{(1,22.2)}$= 5.4, p=.03**
Deep WMH	0.58 (0.92)	10.50 (23.40)	$F_{(1,21)}$=3.9, p=.06

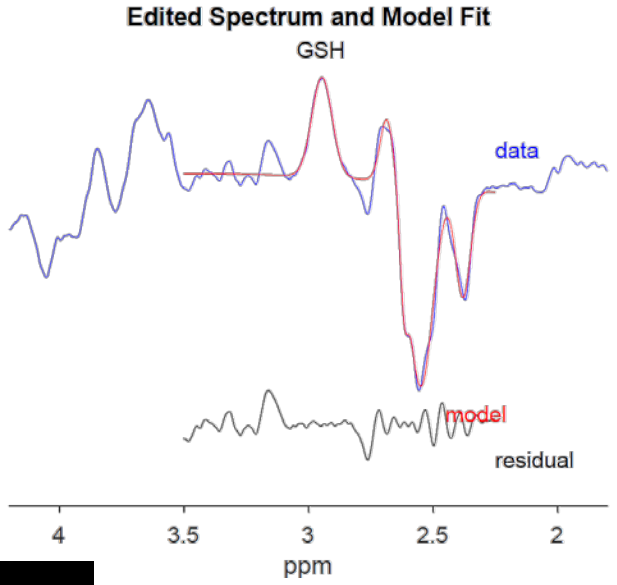
in vivo **Brain GSH - M**agnetic **R**esonance **S**pectroscopy (MRS)

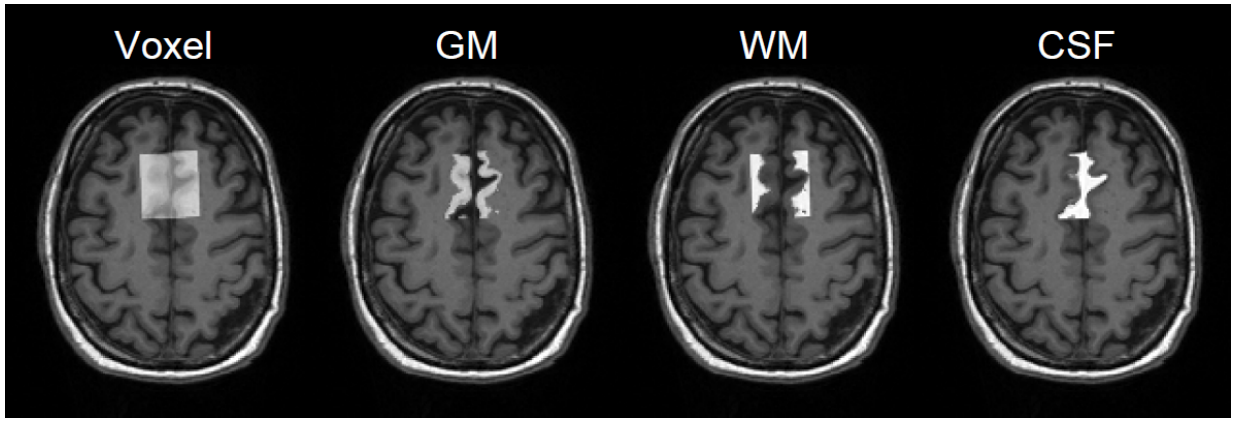
- Anterior cingulate cortex (ACC)
- Occipital cortex (OC)
- **Me**scher-**Ga**rwood **P**oint **Re**solved **S**pectroscopy (MEGA-PRESS) pulse sequence at 3T
- 3x3x3cm voxel size
- TE/TR = 68/2600ms



in vivo Brain GSH - Magnetic Resonance Spectroscopy (MRS)

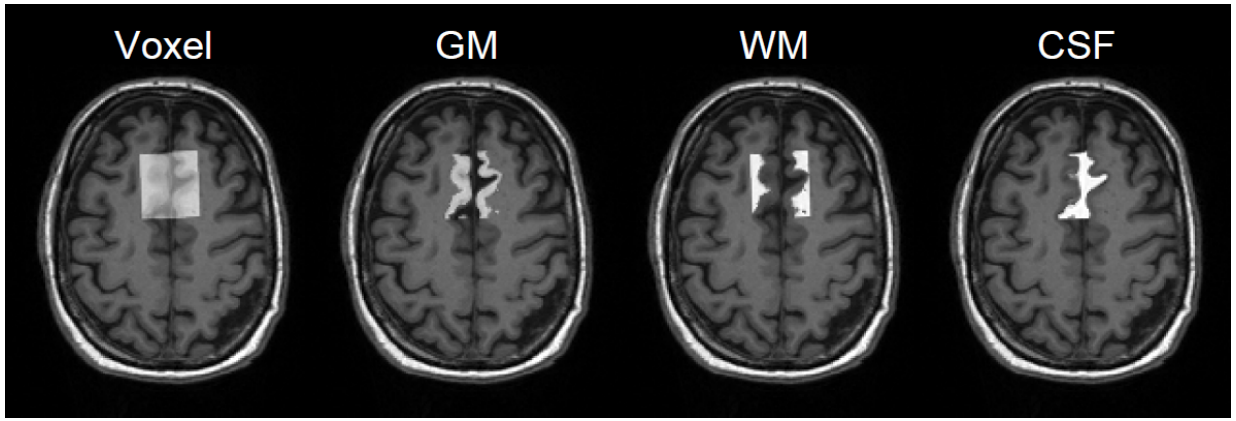
- Concentration relative to water calculated using Gannet (v 3.1)
- Excluded
  - 2 ACC-GSH baseline scans
  - 1 OC-GSH baseline scan





- Cerebrospinal Fluid (CSF)-corrected levels obtained using T1-weighted images and SPM12

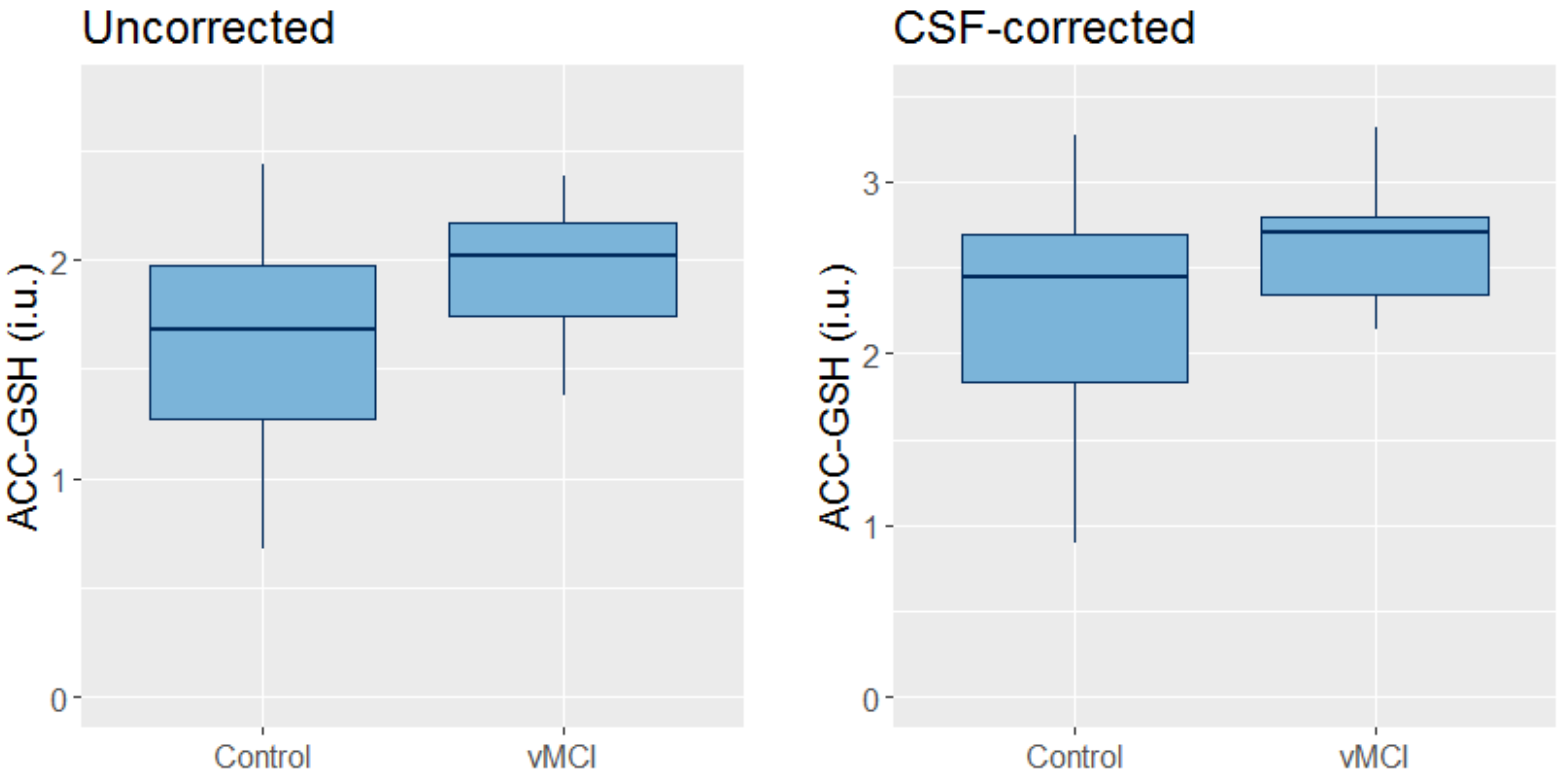
in vivo Brain GSH - Magnetic Resonance Spectroscopy (MRS)



- Cerebrospinal Fluid (CSF)-corrected levels obtained using T1-weighted images and SPM12

- 2 regions – ACC and OC
- Bonferroni correct for primary outcomes
  - threshold $\alpha<0.025$

Brain ACC-GSH is higher in vMCI



- ACC-GSH was significantly higher in vMCI, difference remained after CSF correction ($\mathbf{F_{(1, 31.2)}=7.3, p=.01}$)
  - OC-GSH was not different between vMCI and controls

Post hoc controlling for cofounders

- Between-group difference in years of education, mean arterial pressure (MAP), comorbid hypertension
- Potential age and sex differences in GSH (literature)

Outcome Variable	Independent Variable	B [SE]	t, p	Adj R^2
ACC-GSH (uncorrected)	vMCI status*	0.30 [0.13]	2.3, P=.03	0.29
ACC-GSH (CSF-corrected)	vMCI status*	0.36 [0.18]	2.1, p=.048	0.22
ACC-GSH (uncorrected)	vMCI status+	0.36 [0.14]	2.7, p=.01	0.32
ACC-GSH (CSF-corrected)	vMCI status+	0.48 [0.18]	2.7, p=.01	0.26

controlling for: age, sex, years of education,* ***MAP*

+controlling for: age, sex, years of education, ***hypertension status***

Exploratory: ACC-GSH was correlated to executive function performance

Outcome Variable	Independent Variable*	$B_{ACC\text{-}GSH}$ [SE]	t, p	Model significance	Adj R^2
Executive Function composite z score	ACC-GSH (CSF-corrected) +vMCI status	-0.50 [0.22]	-2.2, p=.04	$F_{(2, 23)}$ = 17.7, p<.001	0.57
Executive Function composite z score	ACC-GSH (CSF-corrected) +vMCI +YED	-0.50 [0.23]	-2.2, p=.04	$F_{(3, 22)}$ = 11.3, p<.001	0.55

**Diagnosis was controlled for in all models*

- Executive function - domain most often affected in vMCI
- All vMCI & subset of controls (n=6) completed executive function scales

Exploratory: ACC-GSH was correlated to executive function performance

Model: Outcome ~ Predictor	Res. DF	RSS	DF	SS	F, p
Model 1 (base): Executive Function ~ vMCI status	24	5.07			F=5.01, p=.035
Model 2 (complex): Executive Function ~ vMCI + CSF- corrected ACC-GSH	23	4.16	1	0.91	

Exploratory: ACC-GSH was correlated to executive function performance

Model: Outcome ~ Predictor	Res. DF	RSS	DF	SS	F, p
Model 1 (base): Executive Function ~ vMCI status	24	5.07			F=5.01, p=.035
Model 2 (complex): Executive Function ~ vMCI + CSF- corrected ACC-GSH	23	4.16	1	0.91	

- Controlling for diagnosis, other cognitive domains were not significantly associated with ACC-GSH
  - verbal memory, visual memory, working memory, and processing speed
- Analysis repeated with OC-GSH levels

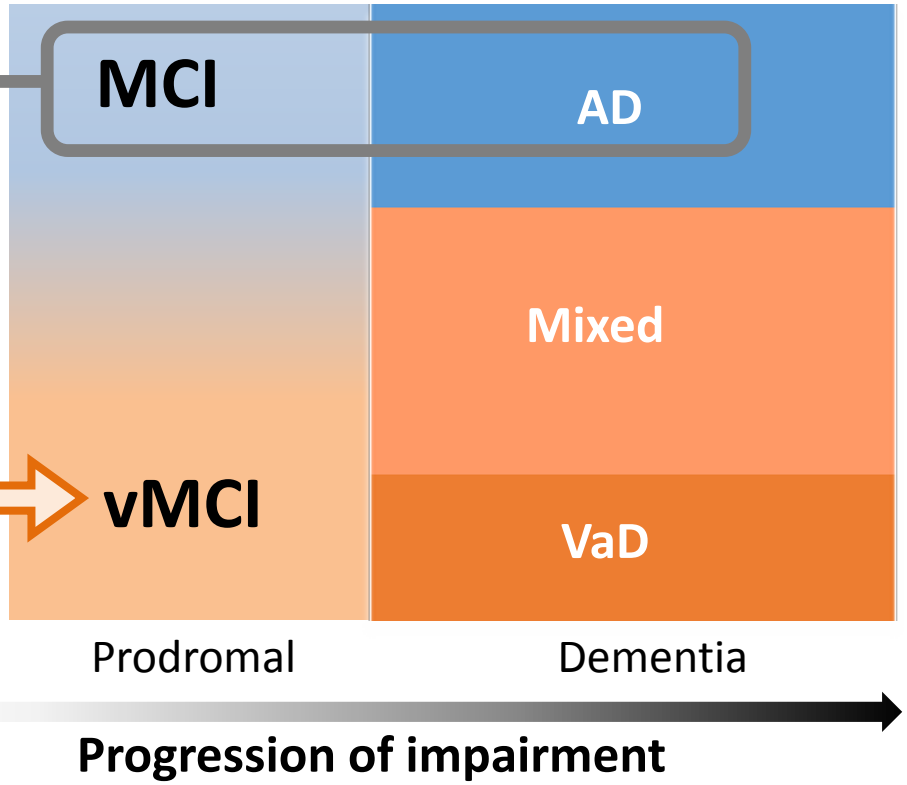
Study 2: Summary

Study 1:

- Blood GSH : ↓ in AD & MCI
- Brain GSH : ↓ in AD & MCI (MEGA-PRESS only)

Study 2:

- ↑ ACC-GSH in vMCI
- ↑ ACC-GSH ∝ ↓ executive function (regardless of dx)

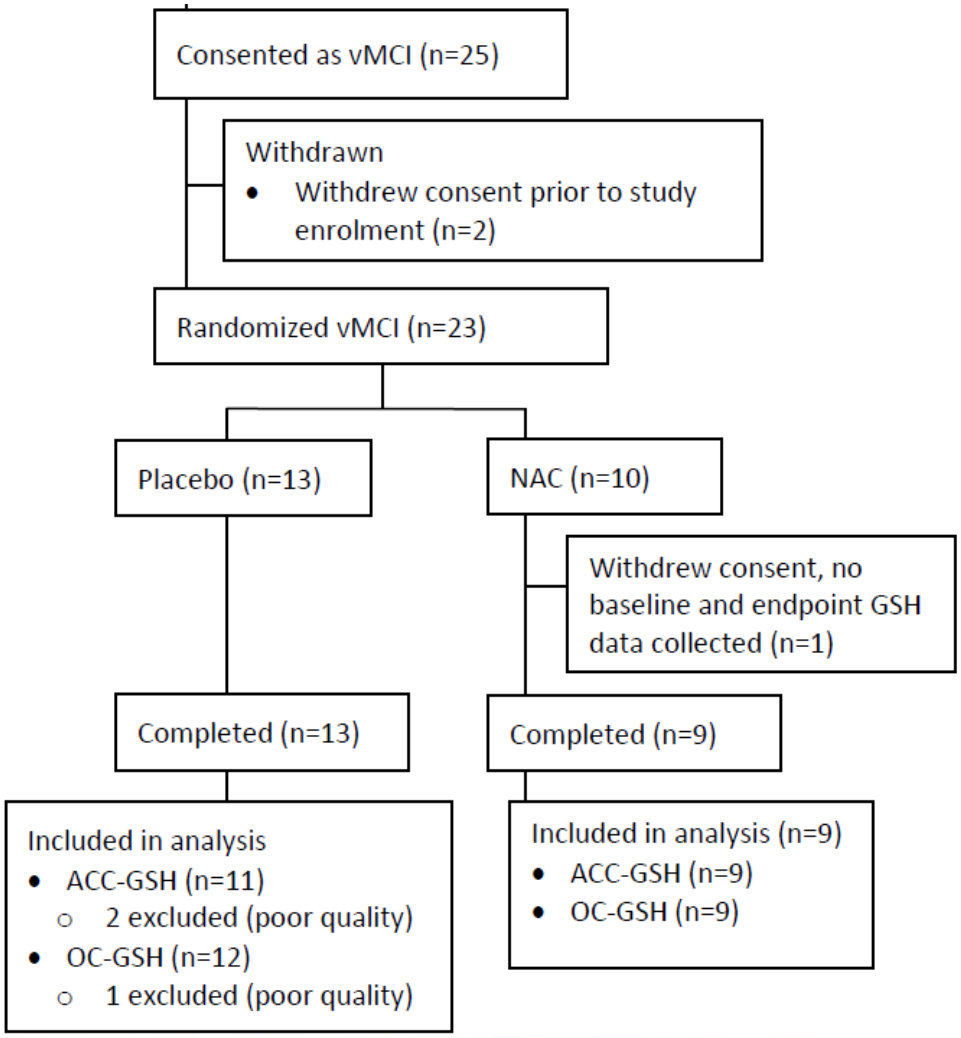


Study 2: Limitations

- Limited number of controls with in-depth neurocognitive testing
- Small sample size
- No longitudinal measures of GSH in controls
- Reduced:oxidized GSH ratio vs. reduced GSH-only
- vMCI participants may have AD contributions to cognitive impairment

Study 3 – vMCI only, GSH over time with NAC

- 6 month randomized control trial
- Oral NAC (2400mg/day)
- Conducted in conjunction with supervised exercise



Study 3: Clinical/Demographic Characteristics

	Placebo (n=13)	NAC (n=9)
Age, mean (SD)	67.6 ± 7.3	67.0 ± 7.8
Male, %	77%	67%
Caucasian, %	46%	67%
Drinks per week, mean (SD)	3.00 (5.28)	0.89 (0.78)
History of smoking, %	31%	33%
Years of education, years (SD)	15.0 ± 4.3	15.8 ± 1.7
Mean Arterial Pressure, mmHg (SD)	93.9 (9.2)	99.7 (8.2)
Hypertension, %	62%	89%
Diabetes, %	31%	22%
Dyslipidemia, %	62%	78%
BMI>=30, %	15%	0%
Current smoker, %	8%	0%
Number of vascular risk factors (SD)	1.77 (1.54)	1.89 (0.93)

Study 3: Concomitant Medications

Drug category	Placebo N (%)	NAC N (%)
Aspirin	9 (69%)	9 (100%)
Other antiplatelet medications	8 (62%)	3 (33%)
Statins	12 (92%)	8 (89%)
Thiazide diuretics	0 (0%)	1 (11%)
Calcium channel blockers	3 (23%)	2 (22%)
Systemic beta-blockers	9 (69%)	5 (56%)
ACE inhibitors	5 (39%)	2 (22%)
Thyroid hormone	1 (8%)	0 (0%)
Vitamins, minerals and supplements**	5 (39%)	4 (44%)

n.s. between placebo and NAC group for all major drug categories

Study 3: Cognition over time

	Placebo (n=13)		NAC (n=9)		t (df)	p
	Baseline	Endpoint	Baseline	Endpoint	Time main effects	
Global cognition						
MoCA	22.9 (2.4)	24.7 (2.87)	23.1 (1.3)	26.0 (2.45)	4.23 (21)	**p<.001**
Composite z-scores						
Executive function	-0.46 (0.45)	-0.17 (0.50)	-0.28 (0.56)	-0.11 (0.32)	2.37 (21)	**p=.028**
Verbal memory	-0.97 (0.97)	-0.69 (1.0)	-0.73 (0.88)	-1.11 (1.01)	0.04 (21)	p=.97
Visual memory	-0.08 (0.97)	0.30 (1.32)	-0.30 (0.65)	0.63 (1.07)	3.64 (20)	**p=.002**
Working memory	-0.26 (0.55)	-0.05 (0.68)	-0.14 (0.75)	-0.15 (0.62)	1.20 (21)	p=.25
Processing speed	-0.13 (0.61)	0.14 (0.64)	0.02 (0.77)	0.10 (0.74)	2.3 (21)	**p=.032**

- Significant increase over time in some cognitive domains
- No treatment effects nor significant treatment and time interactions.

Whole brain volumetrics

- Decrease over time in white matter hyperintensity volume
- No treatment effects nor treatment and time interactions

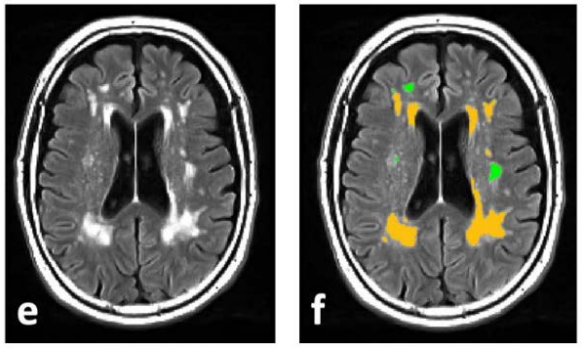
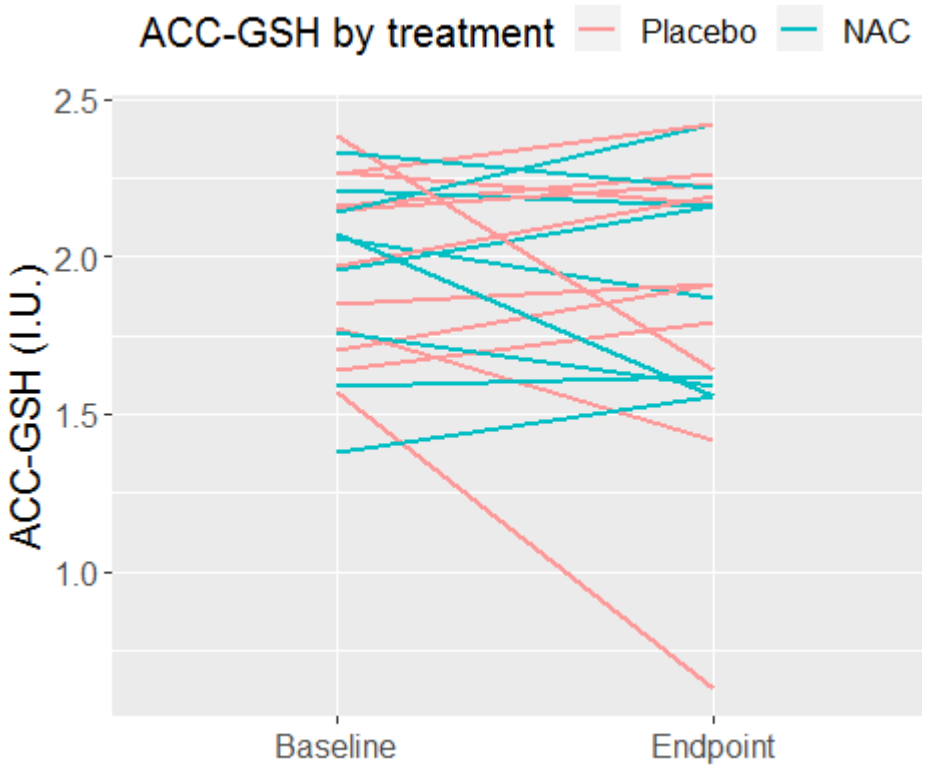
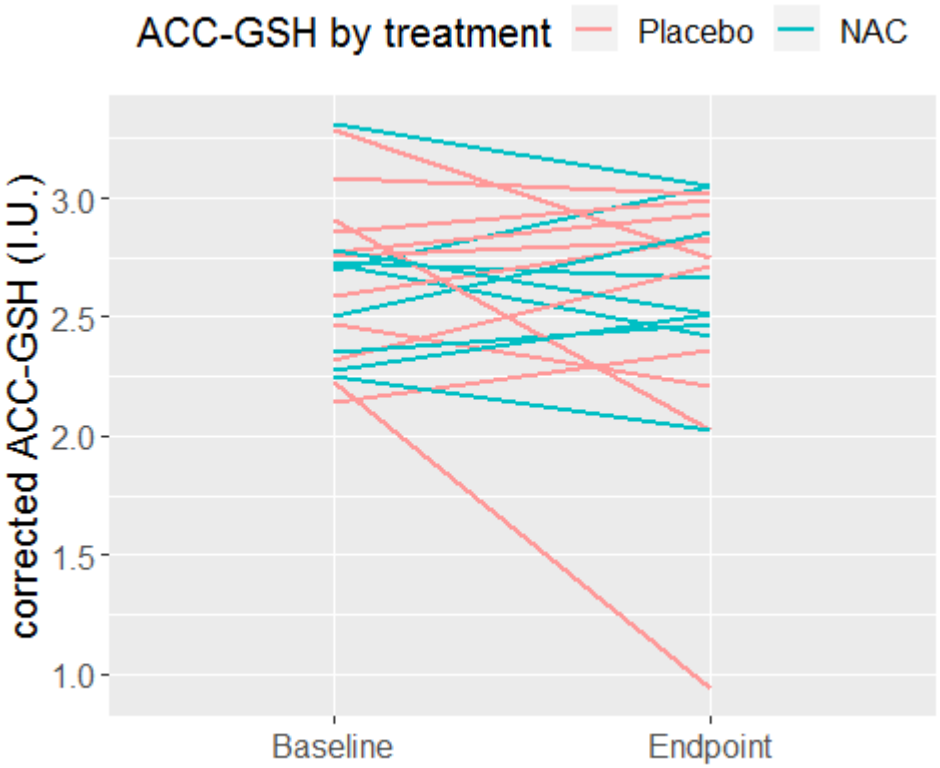


Image from Ramirez et al., 2013

cm^3 (SD)	Placebo (n=13)		NAC (n=9)		t (df)	p
	Baseline	Endpoint	Baseline	Endpoint	Time main effects	
Normal-Appearing White Matter	425 (84)	420 (84)	427 (77)	424 (80)	**-4.01 (21)**	**P<.001**
Normal-Appearing Grey Matter	549 (60)	542 (65)	553 (77)	553 (75)	-1.6 (21)	p<.13
Sulcal CSF	226 (58)	226 (54)	241 (74)	239 (74)	-.20 (21)	p<.84
Ventral CSF	161 (244)	37 (27)	137 (211)	34 (16)	**-2.5 (21)**	**p=.02**
Periventricular WMH	56 (89)	91 (16)	43 (77)	8.2 (7.4)	**-2.4 (21)**	**p=.03**
Deep WMH	10.8 (24.9)	.67 (.74)	10.1 (22.5)	1.3 (1.2)	-1.9 (21)	p=.07

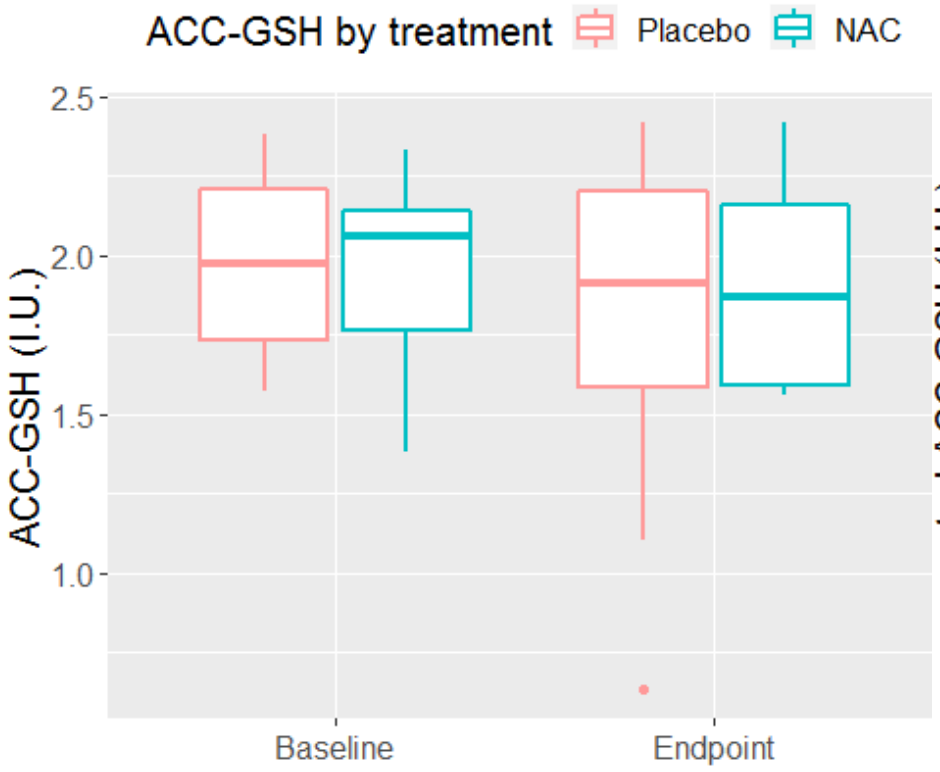
Primary outcome: does GSH change over time with NAC treatment?

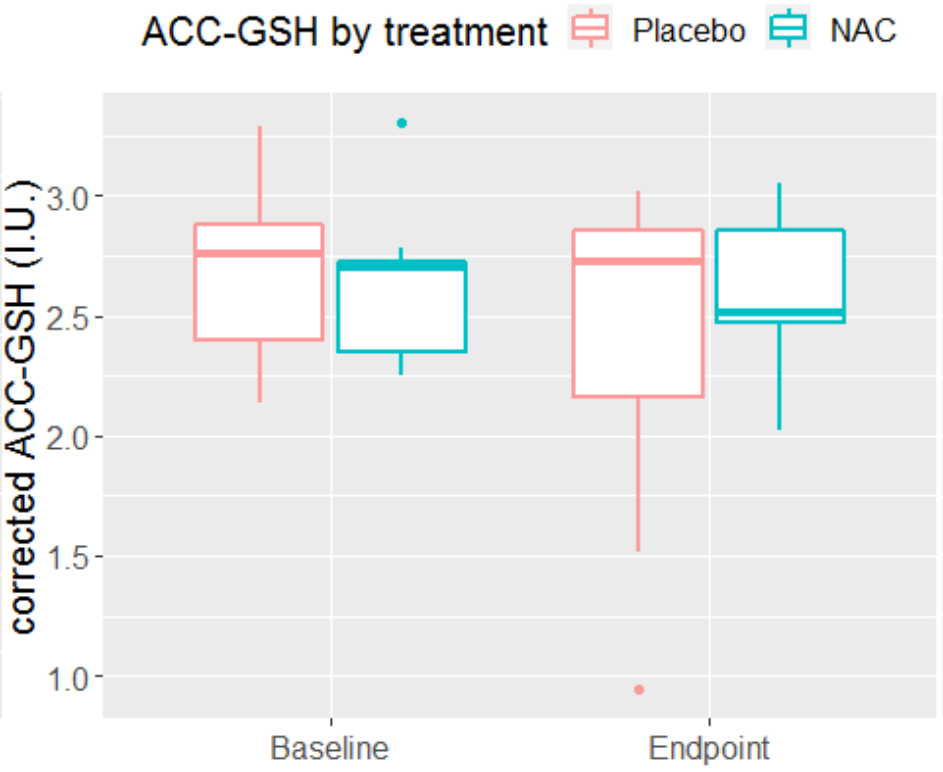




- No time, treatment, nor treatment by time interaction effects detected in ACC-GSH (n=21)
- Similar results in OC-GSH (n=22)

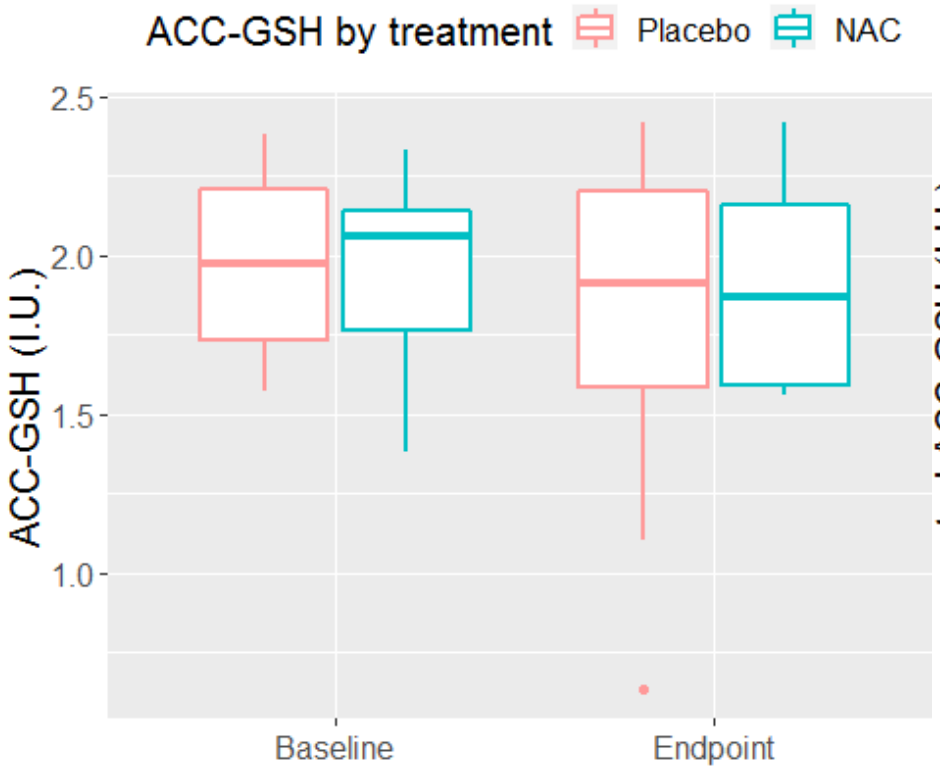
Primary outcome: does GSH change over time with NAC treatment?

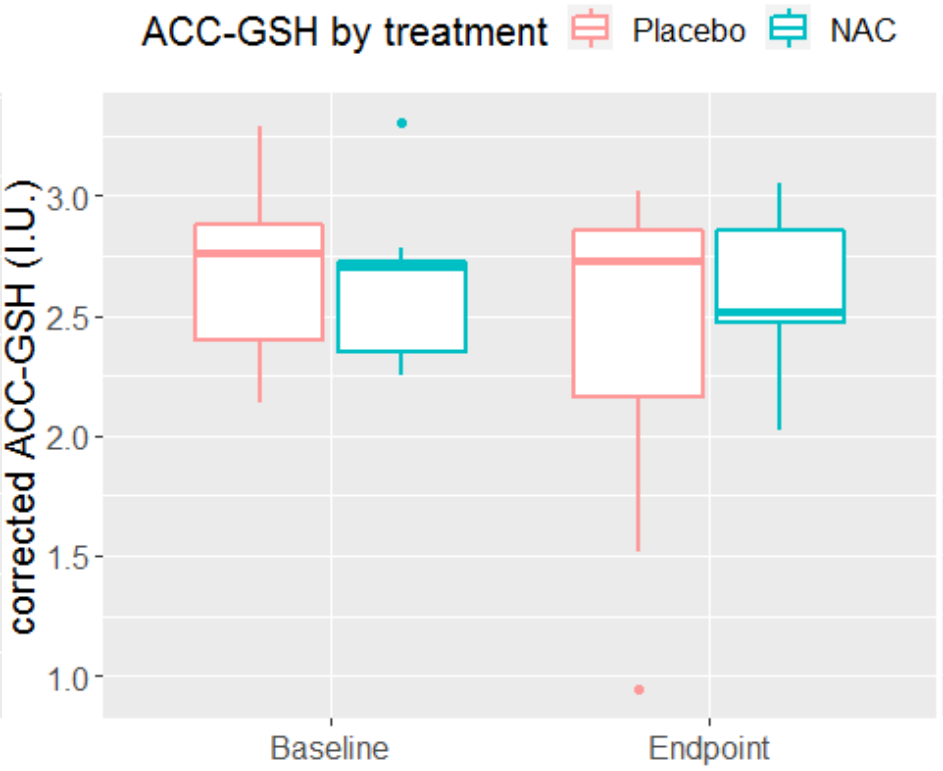




- No time, treatment, nor treatment by time interaction effects detected in ACC-GSH (n=21)
- Similar results in OC-GSH (n=22)

Primary outcome: does GSH change over time with NAC treatment?





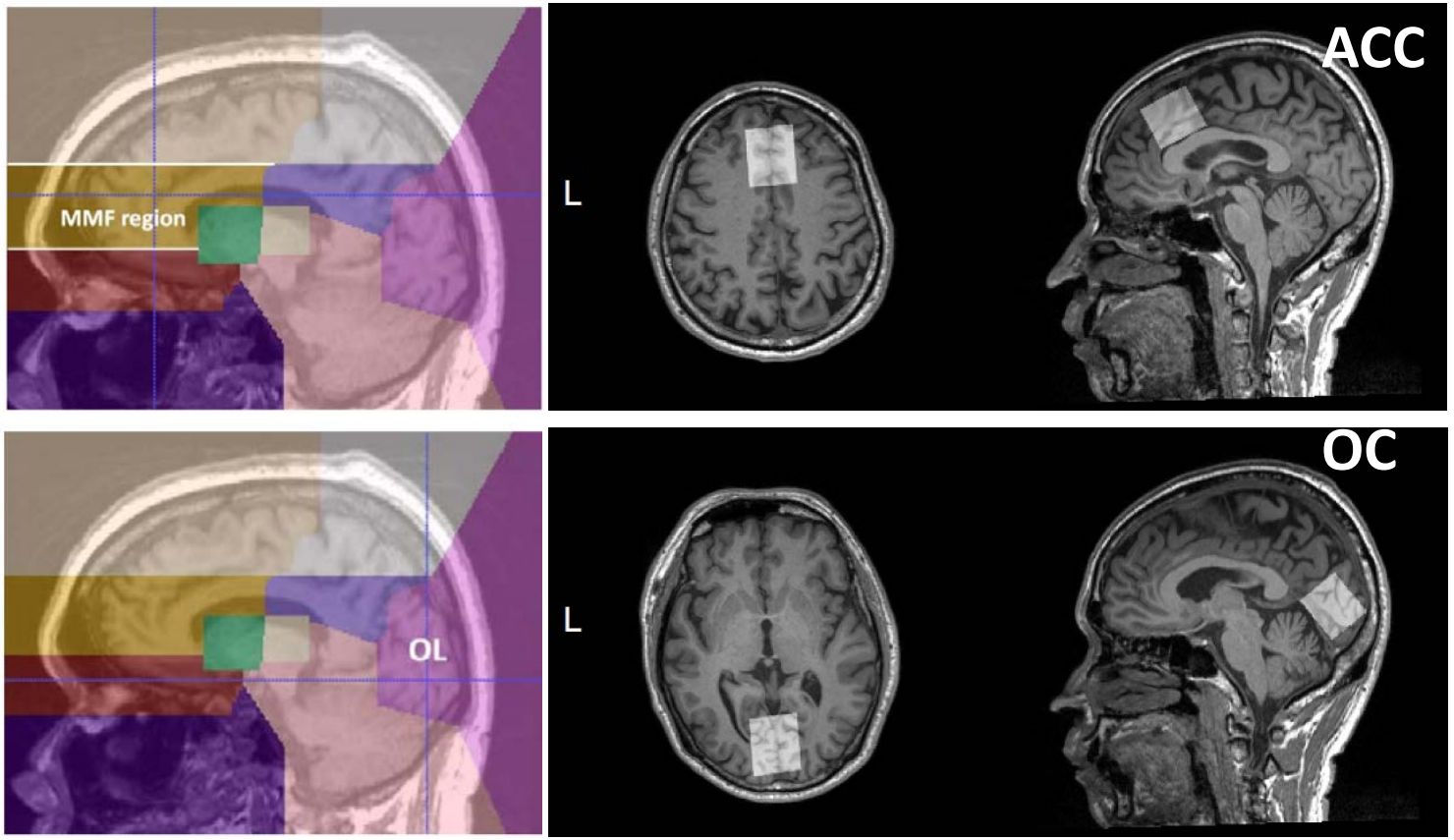
- Post hocs adjusting for confounders:
  - Amyloid status, comorbid hypertension, statin use

Exploratory: does GSH track with cognition?

Outcome	Fixed Effects	B [SE]	t (df)	p
Global Cognition	Time	2.53 [4.3]	0.59 (23)	p=.56
	CSF-corrected ACC-GSH	0.11 [3]	0.04 (27)	p=.97
	Time*CSF-corrected ACC-GSH	-0.20 [1.62]	-0.12 (23)	p=.91
Executive Function	Time	-0.52 [0.82]	-0.64 (24)	p=.53
	CSF-corrected ACC-GSH	-0.56 [0.57]	-0.99 (28)	p=.33
	Time*CSF-corrected ACC-GSH	0.26 [0.31]	0.86 (24)	p=.40
Processing Speed	Time	-0.09 [0.75]	-0.11 (20)	p=.91
	CSF-corrected ACC-GSH	-0.10 [0.55]	-0.19 (22)	p=.85
	Time*CSF-corrected ACC-GSH	0.1 [0.28]	0.35 (20)	p=.73
Visual memory	Time	1.78 [1.81]	0.99 (20)	p=.34
	CSF-corrected ACC-GSH	1.27 [1.38]	0.92 (21)	p=.37
	Time*CSF-corrected ACC-GSH	-0.53 [0.84]	-0.63 (21)	p=.54

- Change in ACC-GSH not associated with change in cognition over time
- Similar findings for OC-GSH

Exploratory: does GSH track with brain volumetrics?

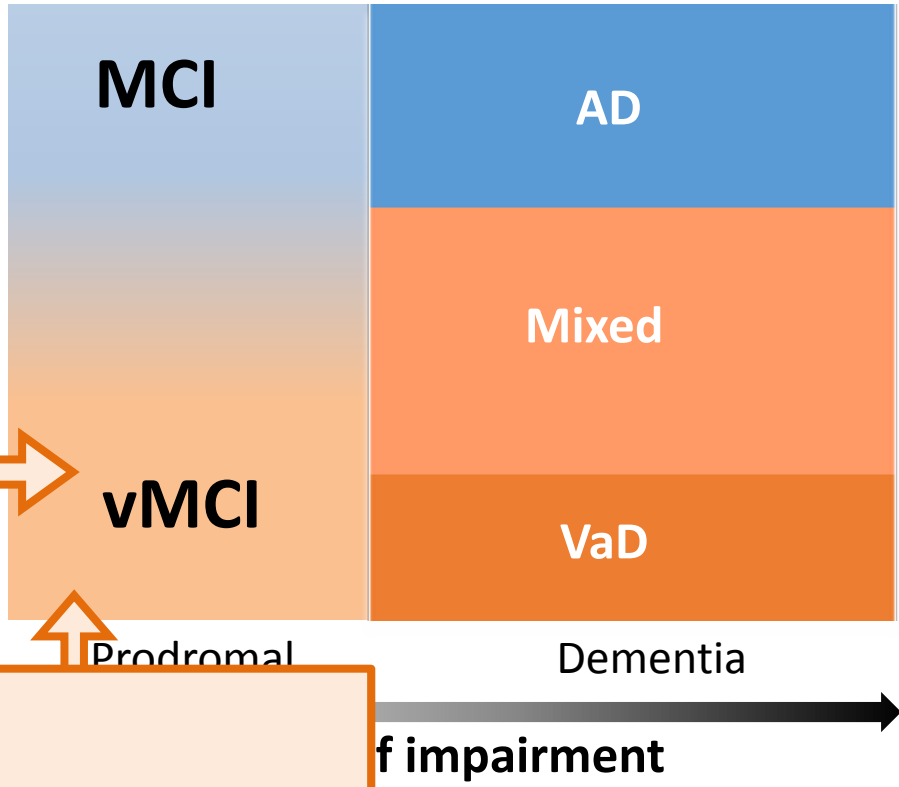


SABRE images (left) adapted from Dade, L.A., Gao, F.Q., Kovacevic, N., Roy, P., Rockel, C., O'Toole, C.M., Lobaugh, N.J., Feinstein, A., Levine, B., Black, S.E.(2004). Semiautomatic brain region extraction: a method of parcellating brain regions from structural magnetic resonance images. *NeuroImage, 22*, 1492-1502.

Exploratory: does GSH track with brain volumetrics?

Outcome	Fixed Effects	B [SE]	t (df)	p
MMF-NAWM (cm^3)	Time	4.11 [4.59]	0.89 (18)	p=.38
	CSF-corrected ACC-GSH	4.2 [3.4]	1.23 (20)	p=.23
	Time*CSF-corrected ACC-GSH	-1.68 [1.73]	-0.97 (18)	p=.34
MMF-NAGM (cm^3)	Time	2.84 [5.04]	0.56 (21)	p=.58
	CSF-corrected ACC-GSH	2.99 [3.59]	0.83 (25)	p=.41
	Time*CSF-corrected ACC-GSH	-1.29 [1.9]	-0.68 (21)	p=.51
MMF-sCSF (cm^3)	Time	-1.68 [2.52]	-0.66 (17)	p=.51
	CSF-corrected ACC-GSH	-0.08 [1.89]	-0.04 (18)	p=.97
	Time*CSF-corrected ACC-GSH	0.5 [0.95]	0.53 (17)	p=.6
MMF-vCSF(cm^3)	Time	2.18 [1.63]	1.34 (17)	p=.2
	CSF-corrected ACC-GSH	2.15 [1.22]	1.76 (17)	p=.1
	Time*CSF-corrected ACC-GSH	-0.84 [0.61]	-1.37 (17)	p=.19
MMF-pWMH (cm^3)	Time	-0.75 [0.51]	-1.48 (20)	p=.16
	CSF-corrected ACC-GSH	-0.57 [0.37]	-1.53 (22)	p=.14
	Time*CSF-corrected ACC-GSH	0.27 [0.19]	1.42 (19)	p=.17
MMF-dWMH (mm^3)	Time	-19.11 [20.8]	-0.92 (17)	p=.37
	CSF-corrected ACC-GSH	-13.94 [15.41]	-0.9 (18)	p=.38
	Time*CSF-corrected ACC-GSH	5.47 [7.83]	0.7 (17)	p=.49

Study 3: Summary



Study 2:

- ↑ ACC-GSH in vMCI
- ↑ ACC-GSH ∝ ↓ executive function (regardless of dx)

Study 3:

- No longitudinal change in GSH over 6 months
  - With/without NAC intervention
- Change in cognition and brain volumetrics not correlated with GSH over time

Study 3 - discussion and limitations

- GSH levels do not reflect *in vivo* NAC activities
- GSH is not consumed/oxidized effectively
- Small oral dose (2400mg) vs. literature
  - Small effect size?
  - Single 150mg/kg infusion (~9000mg for a 60kg patient)
  - 6000mg oral NAC/day for 28 days
  - chronic over-supplementation is a concern

Implications

- Recap: GSH - primary antioxidant in the brain
- AD and MCI patients (meta analysis)
  - ↓ Blood GSH
  - ↓ Brain GSH (MEGA-PRESS subgroup)
- vMCI patients
  - ↑ brain GSH (anterior cingulate)
  - ACC-GSH correlated to executive function
- Inconclusive results in longitudinal study with NAC intervention

Conclusion

- Findings support the use of brain GSH as a possible biomarker in NCD
- Brain GSH response to OS differed based on disease etiology, region- and disease stage.
- Prodromal NCD has little promising interventions
  - Inconclusive evidence for efficacy of NAC
  - Alternative candidates

Future directions

- Studies with larger sample sizes
- Increasing the bioavailability of NAC?
- Other interventions that target other antioxidant pathways (e.g. GLP-1)
- Meta analysis – multiple brain regions
  - For vMCI patients, these should be considered for evaluation
- Peripheral measures of GSH and GSSG, enzymes associated with GSH metabolism

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Dr. Ana Andreazza & lab
Dr. Paul Oh & TRI
Dr. Simon Graham
Dr. Sandra Black

MOVE-IT team

Coordinator: Kate Survilla

Team members: Ethan and Yejin

Neuropsychopharmacology Research Group

Committee

Drs. Andreazza, Black, MacIntosh

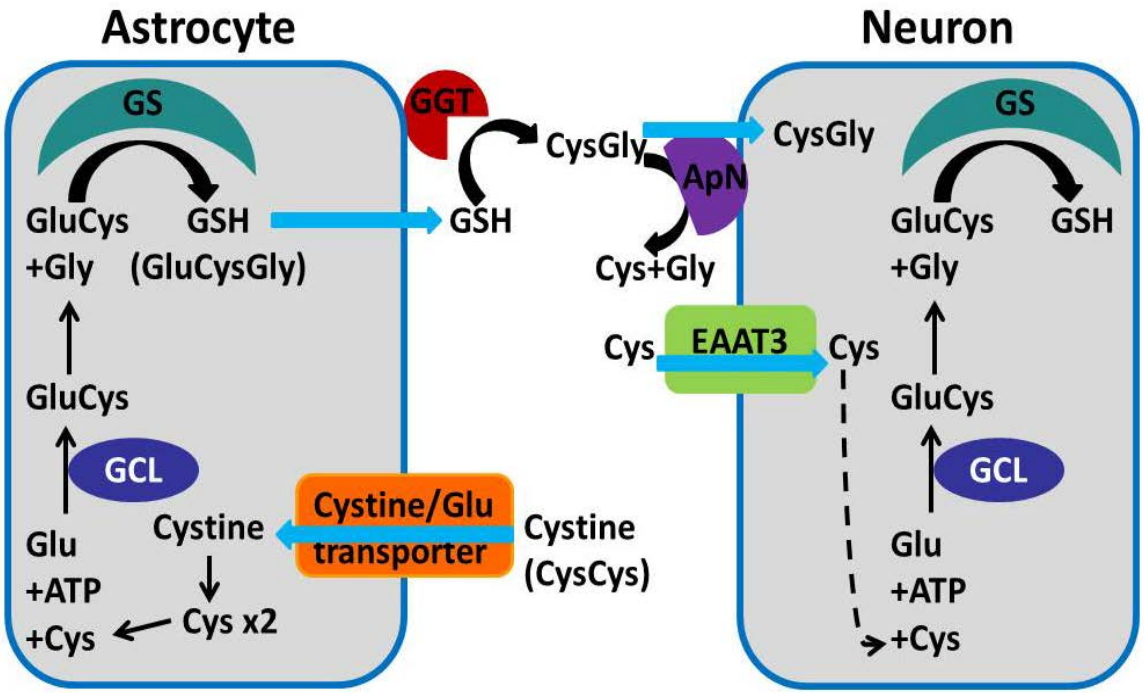
Thank you!!!

ADDITIONAL SLIDES FOR REFERENCE

NAC Pharmacokinetics

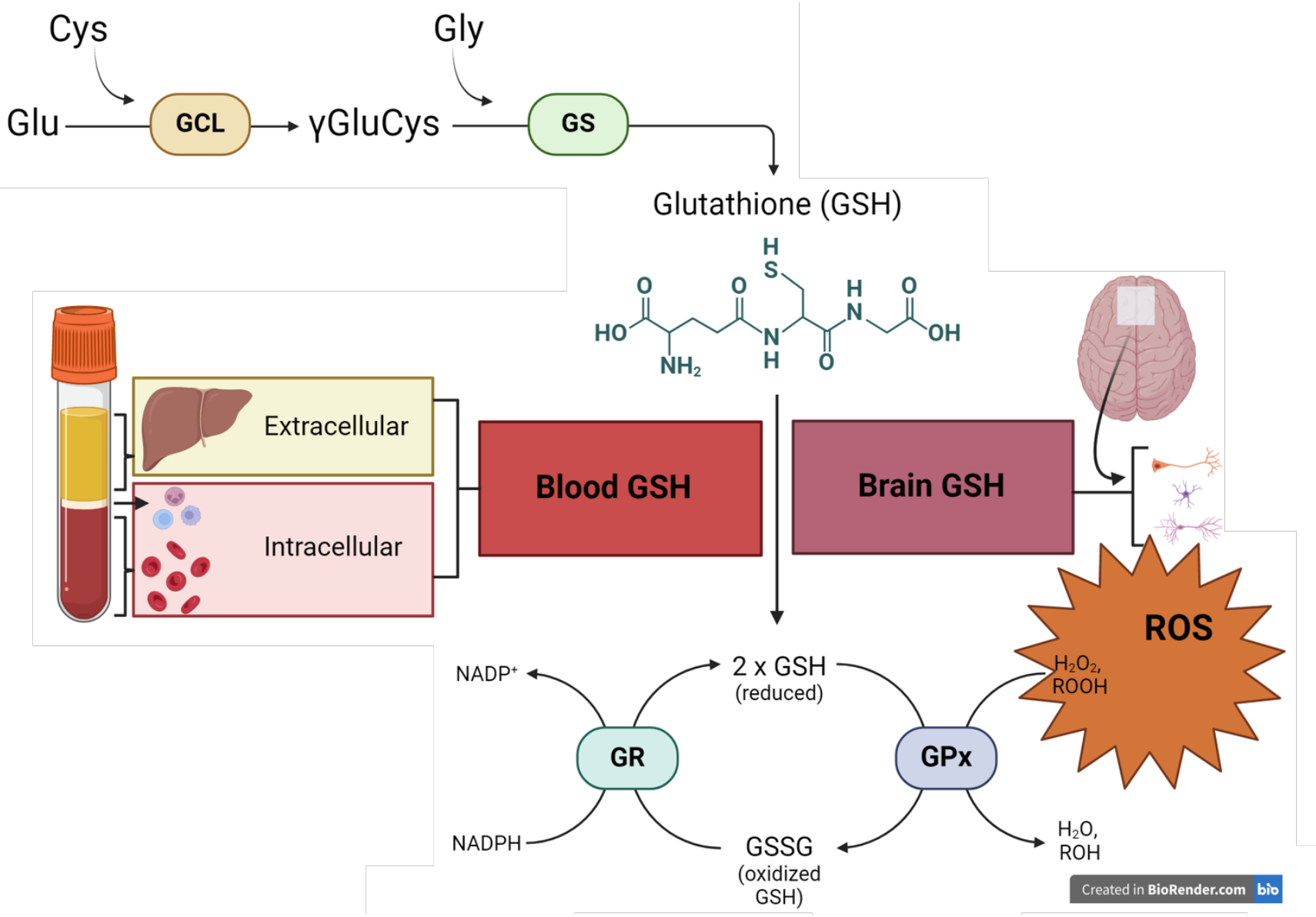
- orally bioavailable (10%)
- volume of distribution 0.33-0.47 L/kg
  - 50-83% plasma protein-bound
- Hepatically de-acetylated to cysteine
- 30% renally cleared
- t1/2 = 5.6-6.25 hours

Brain GSH

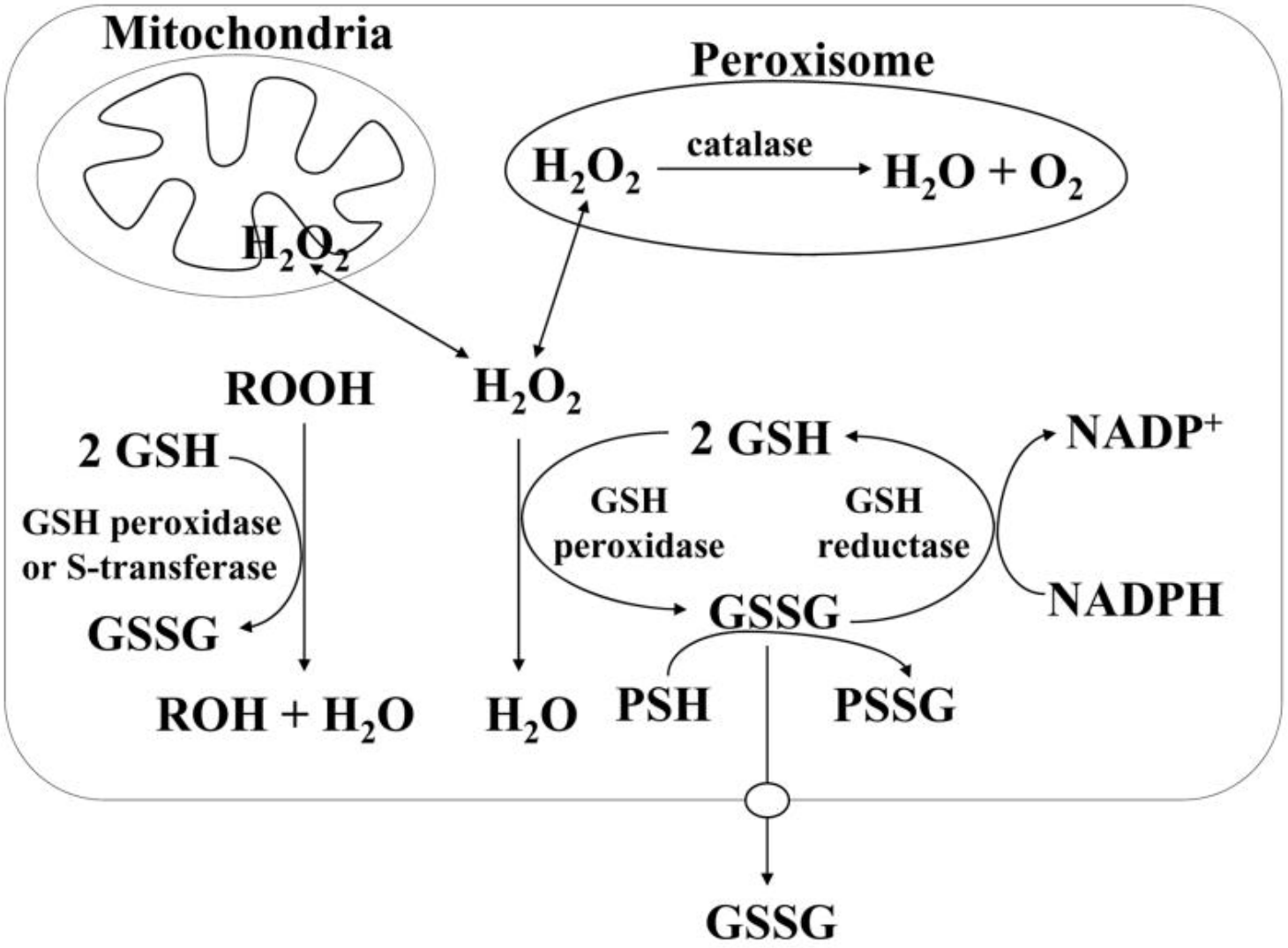


- Star-shaped glia, provide nutrients and support to neurons, help in neuronal repair
- Highest cytosolic GSH (primary astrocytes 8–10 mM)

- Maintain antioxidant levels in neurons
- GSH-mediated antioxidant capacity of both astrocytes and neurons is dependent on cysteine availability in astrocytes


Cys
Gly
Glu
GCL
γGluCys
GS
Glutathione (GSH)
Extracellular
Intracellular
Blood GSH
Brain GSH
ROS
H_2O_2, ROOH
NADP+
2 x GSH (reduced)
GR
GPx
NADPH
GSSG (oxidized GSH)
H_2O, ROH
Created in BioRender.com

Result	Patient, Sample Size	NAC dose	Study type, Length	Study
Maintenance of cognitive performance	AD, n=24	Nutraceutical containing NAC	Open Label, 12 mo.	Remington et al., 2016
Improved DRS and CLOX-1	MCI, n=34	Nutraceutical containing NAC	RCT with Open Label extension, 6 mo. + 6mo.	Remington et al., 2015
Improved DRS	AD, n=106	Nutraceutical containing NAC	RCT, 3 mo.	Remington et al., 2015
Improvement in Letter Fluency Task, Wechsler Memory Scale Immediate Number Recall	Probable AD, n=43	50 mg/kg/day	RCT, 6 mo.	Adair et al. 2001



Lu, S. C. (2009). "Regulation of glutathione synthesis." Molecular aspects of medicine **30**(1-2): 42-59.

Brain GSH Subgroup – MRS sequences

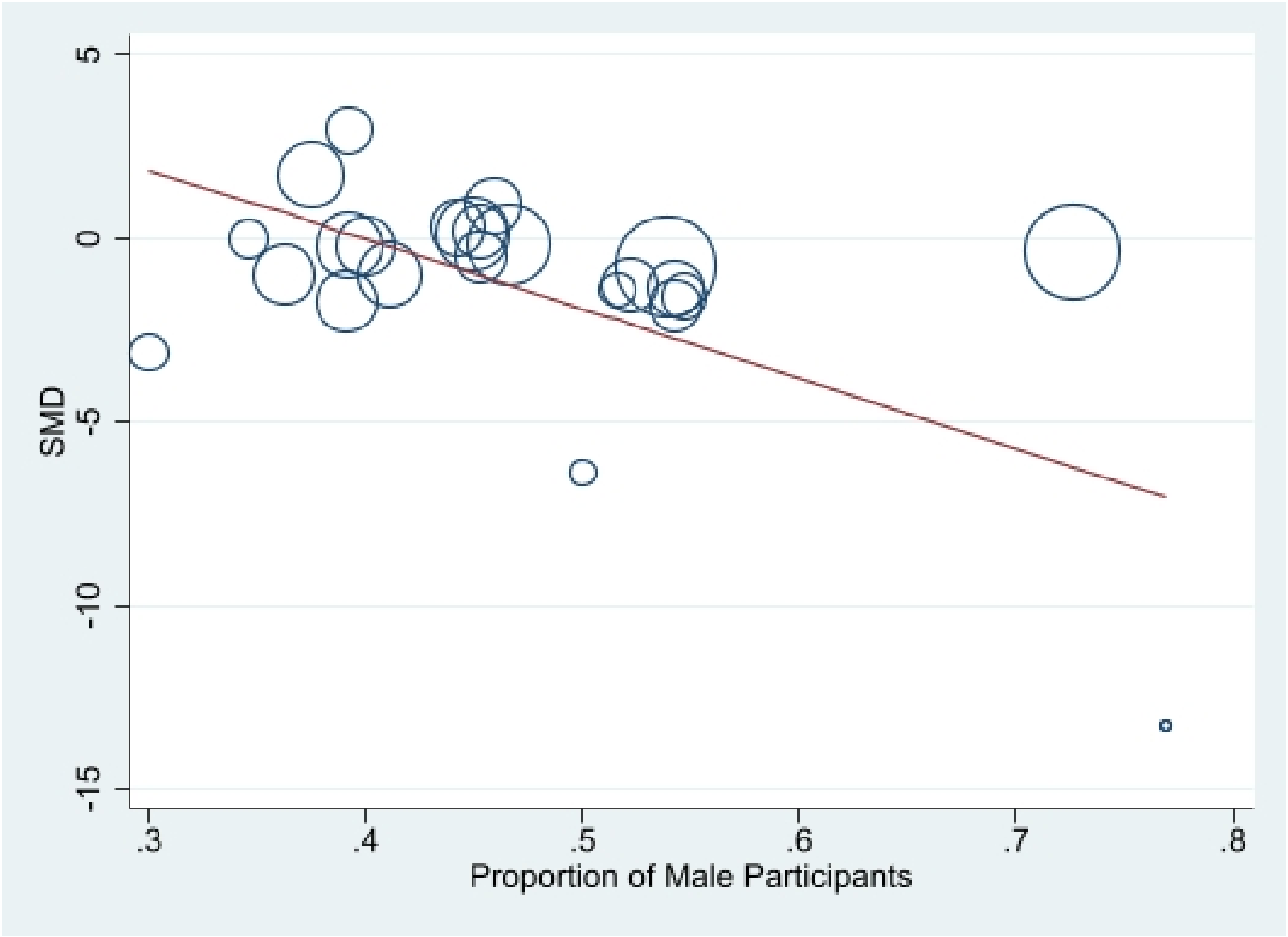
- MEGA-PRESS, PRESS, and STEAM
- MEGA-PRESS detecting full range of GSH phantoms with accurate linear relationship ($R^2 = 0.99$) through 0–24 mM
  - PRESS could not reliably determine physiological concentrations (<4 mM) of GSH
- Brain and liver MRS studies using PRESS and STEAM report different sensitivities to J coupling effects
  - Difference in fat quantification

Extra: Study 1 Methods

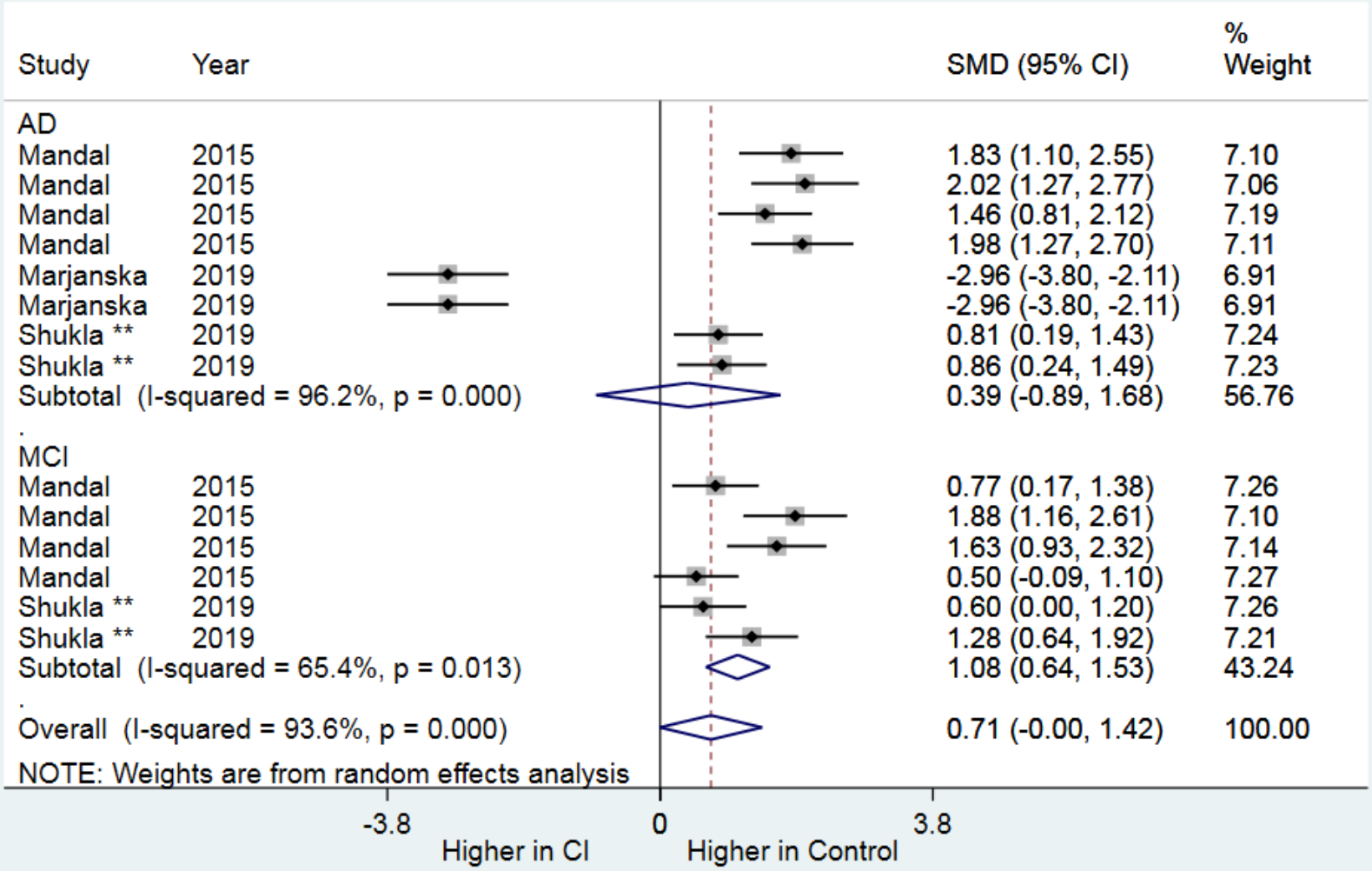
- *In vivo* brain or blood GSH in MCI or AD with a healthy control (HC)
  - Medline, PsychInfo, and Embase (1947-June 2020)
- Stata IC16, Random effects models, standardized mean differences (SMD)
- A-priori subgroups:
  - Brain GSH: Meshcher-Garwood Point Resolved Spectroscopy (MEGA-PRESS) vs. non
  - Blood GSH: intra- vs. extra-cellular
- Assessed heterogeneity

Sample Search Strategy (PRISMA guidelines)	
Population	"Alzheimer Disease" OR "Dementia" OR "Dementia, Vascular" OR "Dementia, multi-infarct" OR "cognitive dysfunction" **Note MCI is indexed under these
Method of measurement	"Magnetic resonance spectroscopy" OR "Proton Magnetic Resonance Spectroscopy"
Comparison	AD or MCI vs. Controls
Outcomes	"Glutathione" OR "Oxidative Stress" OR "Antioxidants"
Type of question	Screening/diagnosis/prognosis
Type of Study	Randomized control trials, controlled trials, prospective/cohort/longitudinal follow-up studies, cross sectional studies, case control studies Exclude: case reports, research in progress, conference abstracts, dissertations, books, scientific meeting reports

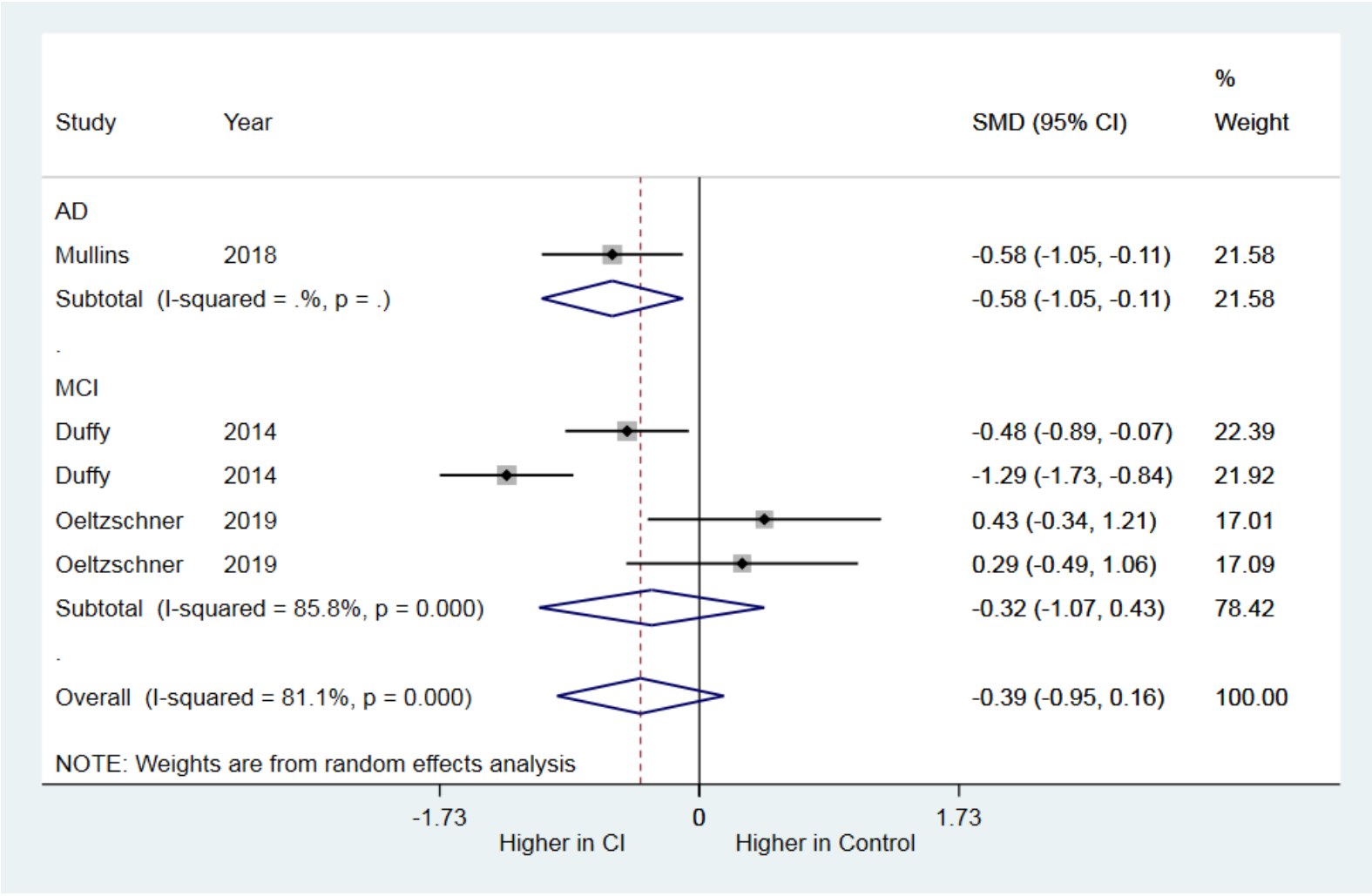
EXTRA: Study 1
Meta-regression of blood GSH in AD



EXTRA: Study 1
Brain GSH subgroup – water reference



EXTRA: Study 1
Brain GSH subgroup – creatine reference



EXTRA: Study 2
OC-GSH Primary and Post Hoc

Occipital Cortex	Control (n=21)	vMCI (n=20)	Significance
Uncorrected brain GSH (i.u. ± SD)	1.65 (0.27)	1.79 (0.25)	$F_{(1, 38.3)}$=0.83, P=.37
CSF-corrected brain GSH (i.u. ± SD)	2.00 (0.29)	2.14 (0.27)	$F_{(1, 40)}$=2.5, p=.12

Outcome Variable	Independent Variable	B [SE]	t, p	Adj R^2
OC-GSH (uncorrected)	vMCI status*	0.07 [0.09]	0.8, p=.4	0.26
OC-GSH (CSF-corrected)	vMCI status*	0.07 [0.11]	0.6, p=.5	0.16
OC-GSH (uncorrected)	vMCI status+	0.12 [0.09]	1.3, p=.2	0.20
OC-GSH (CSF-corrected)	vMCI status+	0.17 [0.11]	1.5, p=.13	0.08

**controlling for: age, sex, MAP*

+controlling for: age, sex, hypertension status

EXTRA: Study 2
Blood Markers

	Control (n=21)	vMCI (n=22)	Significance
4-HNE (fmol/µg)	10.2 (1.4)	10.4 (1.2)	$F_{(1,35)}$=0.19, p=.67
8-ISO (pg/mL)	10.4 (9.8)	8.5 (7.2)	$F_{(1,26.7)}$=0.57, p=.50
LPO (µM)	21.7 (22.7)	9.7 (7.8)	$F_{(1,16.6)}$=3.8, p=.07
(4-HNE + 8-ISO)/LPO ratio	1.86 (1.6)	4.83 (6.0)	$F_{(1,22.4)}$=4.5 p=.04
Log [4-HNE]	2.31 (0.13)	2.34 (0.12)	$F_{(1,36)}$=0.51 p=.48
Log [8-ISO]	2.02 (0.78)	1.89 (0.67)	$F_{(1,36)}$=0.31 p=.58
Log [LPO]	2.72 (0.82)	1.84 (1.09)	$F_{(1,33)}$=7.2 p=.012
Log [(4-HNE + 8-ISO)/LPO]	0.21 (0.98)	1.02 (1.08)	$F_{(1,33)}$=5.4 p=.026

- Lipid hydroperoxide (LPO) was lower in vMCIs
- Ratio of early (LPO) to late stage (8-ISO and 4-HNE) OS markers significantly higher in vMCIs

EXTRA: Study 2
MMF volumetrics vs. ACC-GSH

Outcome	Predictors	Predictor B [SE]	t, p	Model Significance	Adj R^2
MMF NAWM (cm^3)	Uncorrected ACC-GSH	4.76 [1.76]	2.7, p=.01	$F_{(3, 37)}$ = 10.6, p<.001	0.42
	vMCI status	-16.4 [6.6]	-2.5, p=.02		
	ACC-GSH * vMCI status interaction	8.75 [3.47]	2.5, p=.02		
MMF NAWM (cm^3)	CSF-corrected ACC-GSH	2.52 [1.71]	1.47, p=.15	$F_{(3, 37)}$ = 2.7, p=.06	0.11
	vMCI status	-9.88 [9.50]	-1.04, p=.31		
	ACC-GSH * vMCI status interaction	4.07 [3.66]	1.11, p=.27		

- vMCI status was associated with lower MMF-NAWM volume
- Higher MMF-NAWM volume was associated with each additional unit of **uncorrected** ACC-GSH in those with vMCI vs. controls
  - Controls, every unit of ACC-GSH increase ∝ 4.76cm^3 more NAWM volume
  - vMCIs, every unit of ACC-GSH ∝ with 13.51cm^3 more NAWM volume