Biomarkers of Alzheimer's Disease: F_2-isoprostanes

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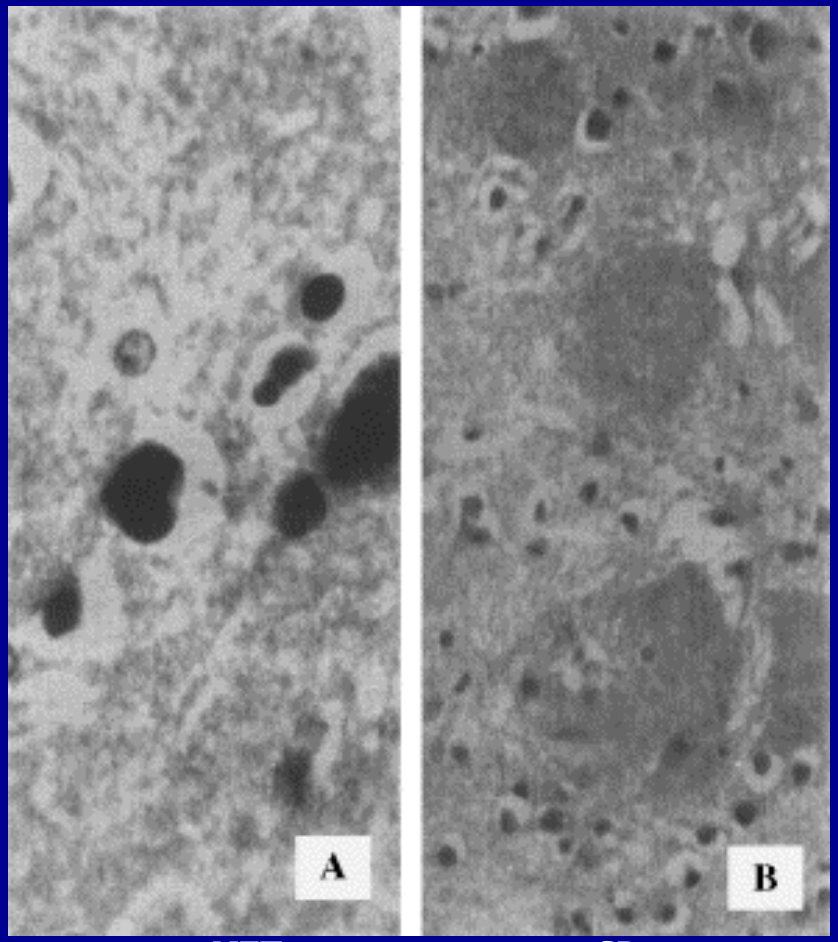
Biomarkers based on AD pathology

Pathological Features

- **Amyloid plaques**
- **Neurofibrilary tangles**
- **Lipid metabolism**
- **Oxidative stress**
- **Inflammation**

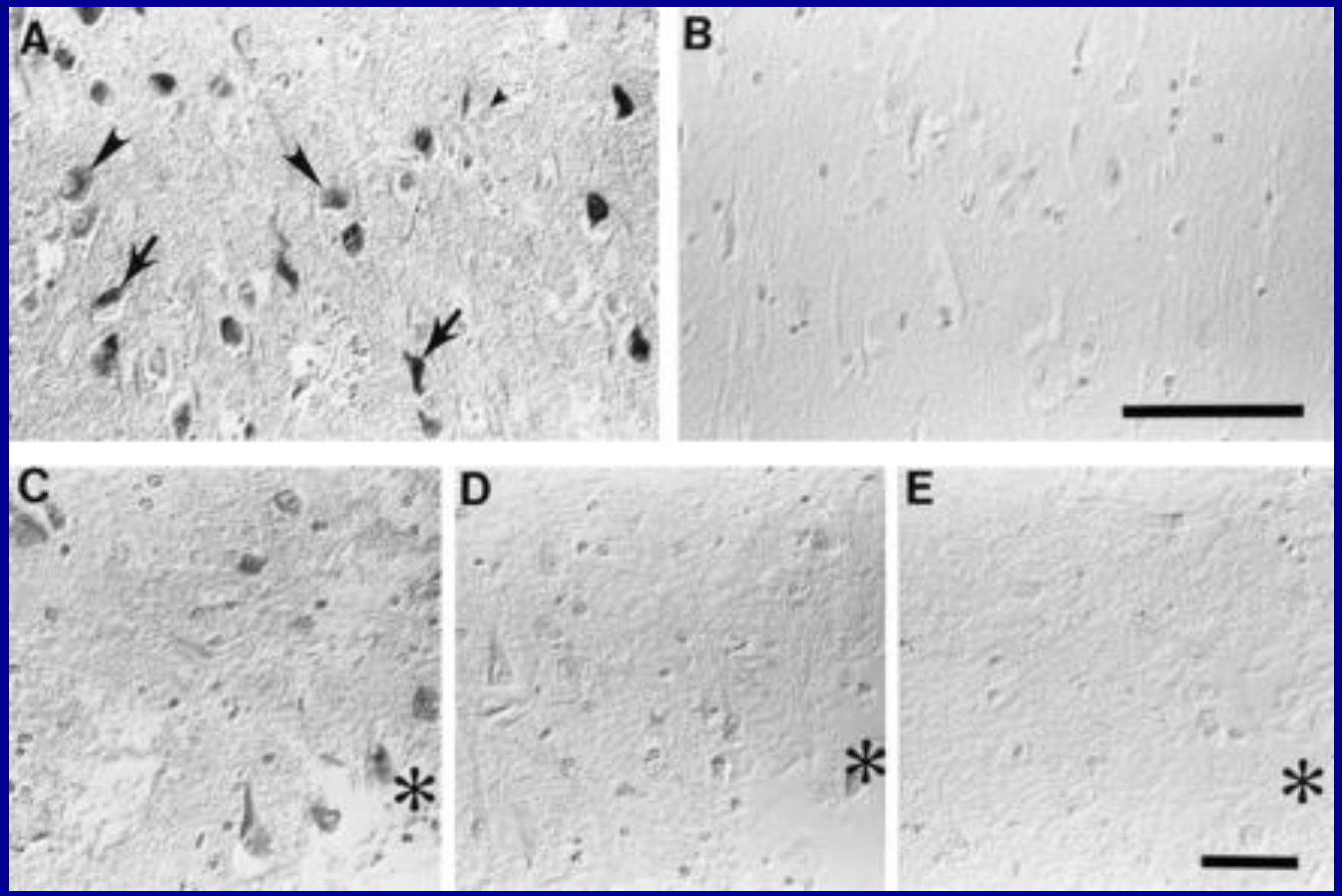
HNE immunopositive lesions in Alzheimer's Disease



NFT SP

Mol Aspects Med. 24;293-303, 2003

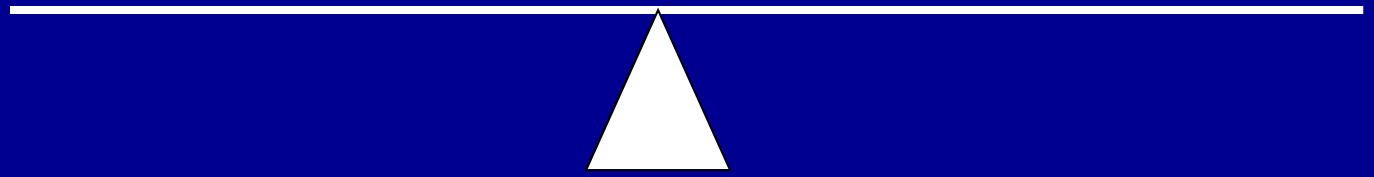
Protein carbonyl immunoreactions in Alzheimer's Disease



J. Hysto. Cyto. 46;731-736, 1998

Brain and Oxidative Stress

Pro-oxidants	*Anti-oxidants*
High use of Oxygen and Glucose	**Catalase ↓**
	SOD ↓
High PUFA	**GSH Px; GSH**
High transition metals	**Vitamin C ↑ / E**
	Uric Acid

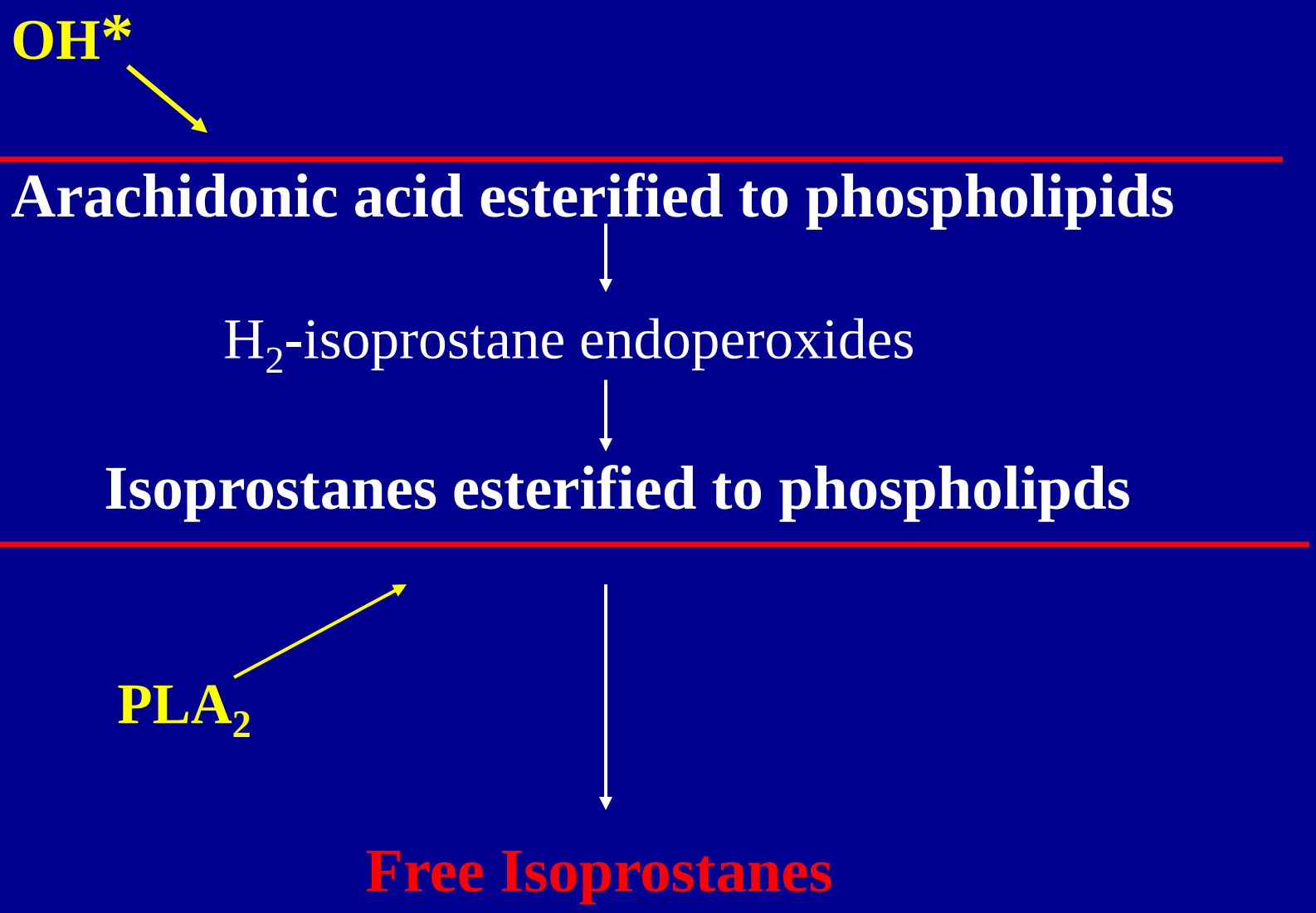


AD and Oxidative Stress

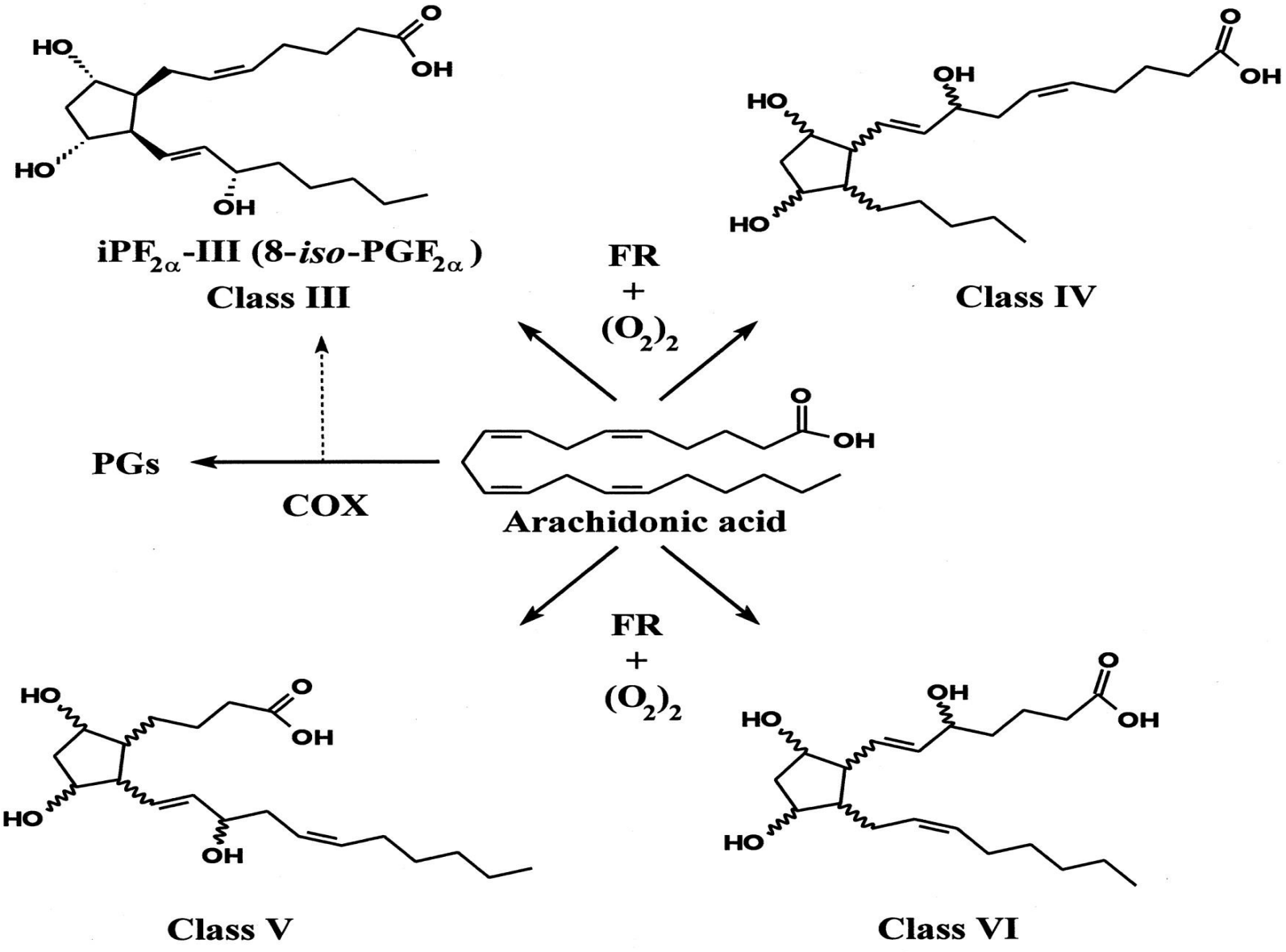
- Oxidative stress in the CNS predominantly manifests as Lipid Peroxidation because of its high content of PUFA.
- Assessment of Lipid Peroxidation in AD has been traditionally hampered by the use of assays that lack specificity and/or sensitivity.

The Isoprostane Family

- Prostaglandin isomers produced from oxidative modification of PUFA via a free radical-catalyzed mechanism.
- Accumulate in tissue, circulate in plasma and are excreted in urine.


OH*
Arachidonic acid esterified to phospholipids
H2-isoprostane endoperoxides
Isoprostanes esterified to phospholipds
PLA2
Free Isoprostanes

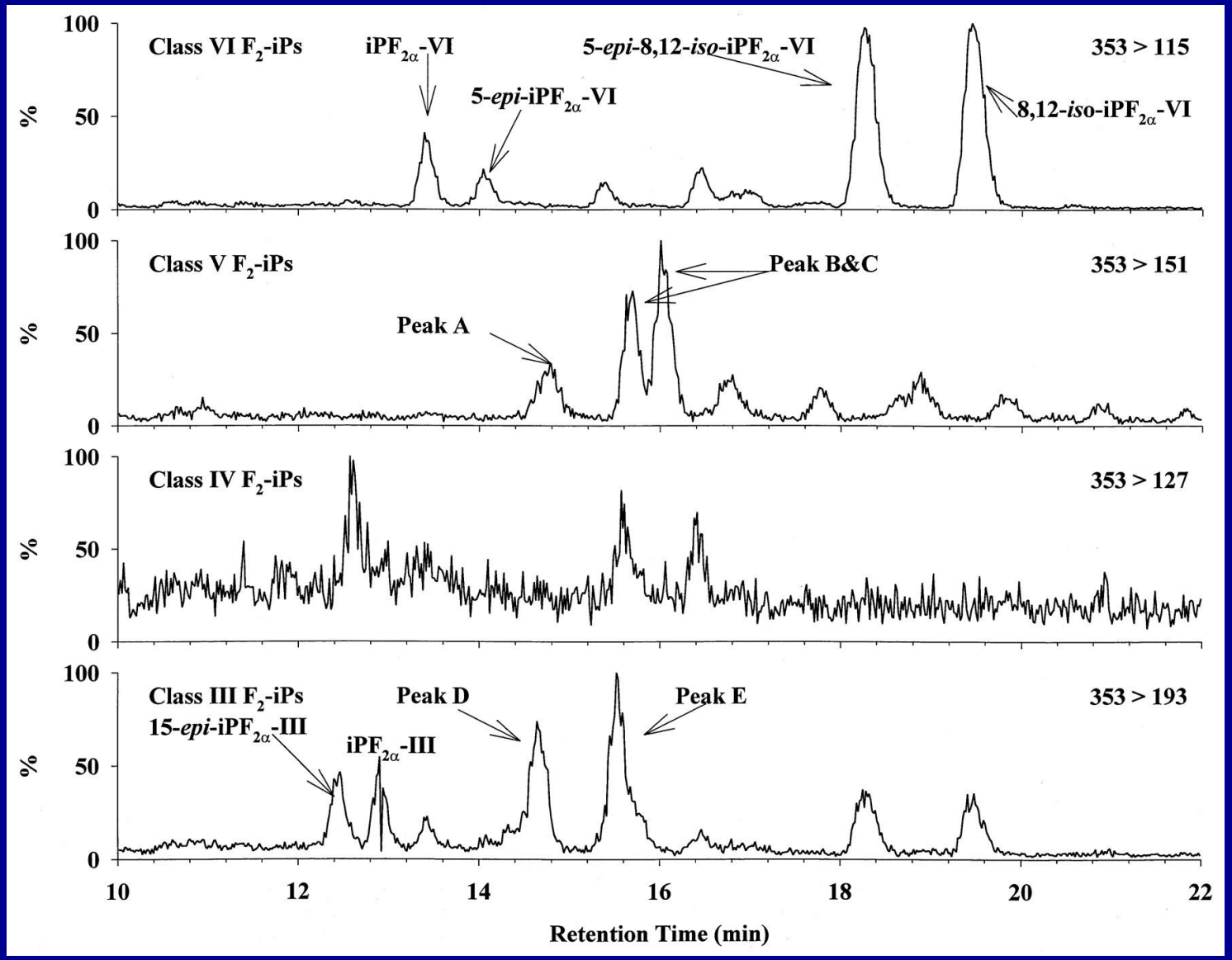
F_2-Isoprostane Family



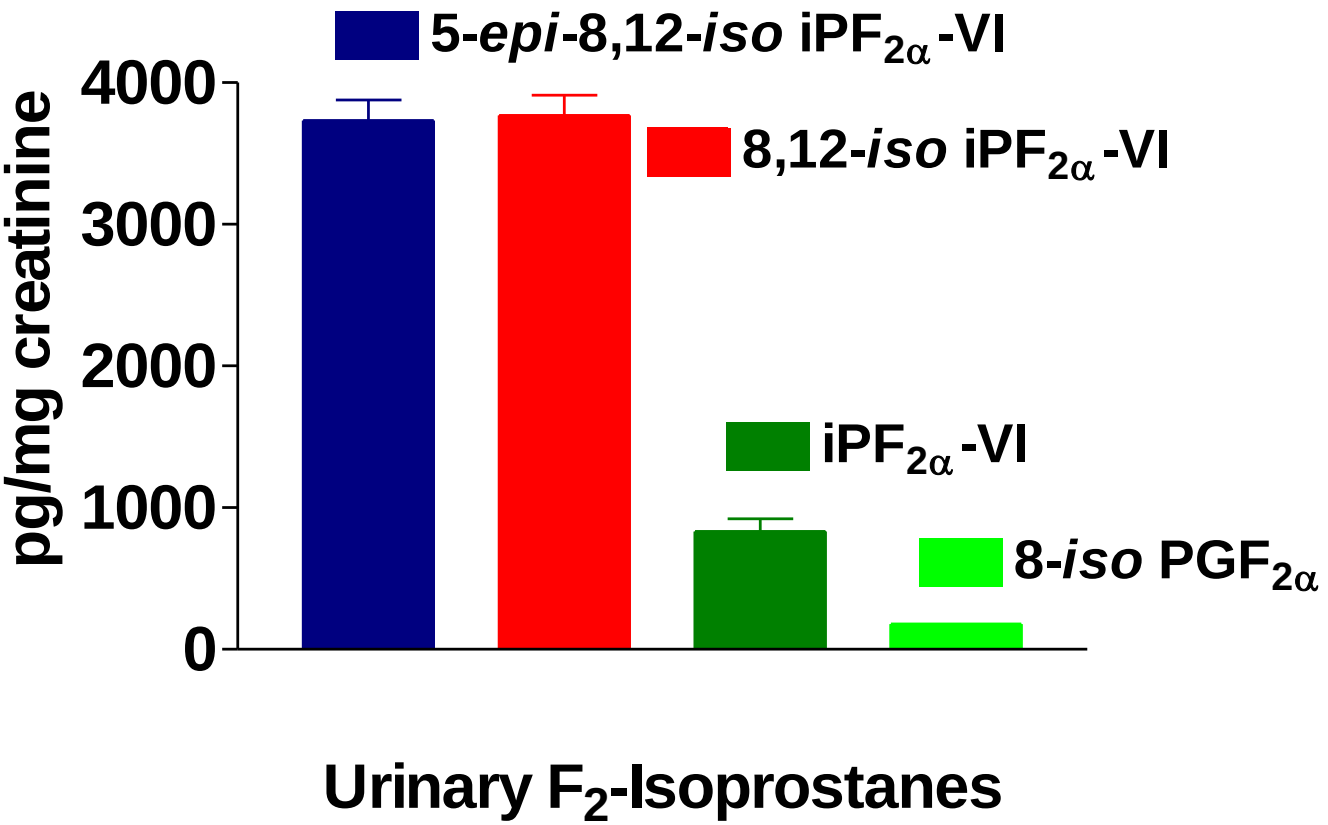
Methods to measure F_2-Isoprostanes

- **Original GC/MS Method**
  - **Serial peaks that co-migrates with $PGF_{2\alpha}$, which consist of at least 3 F_2-IsoPs (30% 8-iso$PGF_{2\alpha}$)**
- **Modified GC/MS Methods**
  - **Single peak that co-migrates with specific isomers**
- **ELISA**
  - **Relative affinity of antibody for different isomers not known**

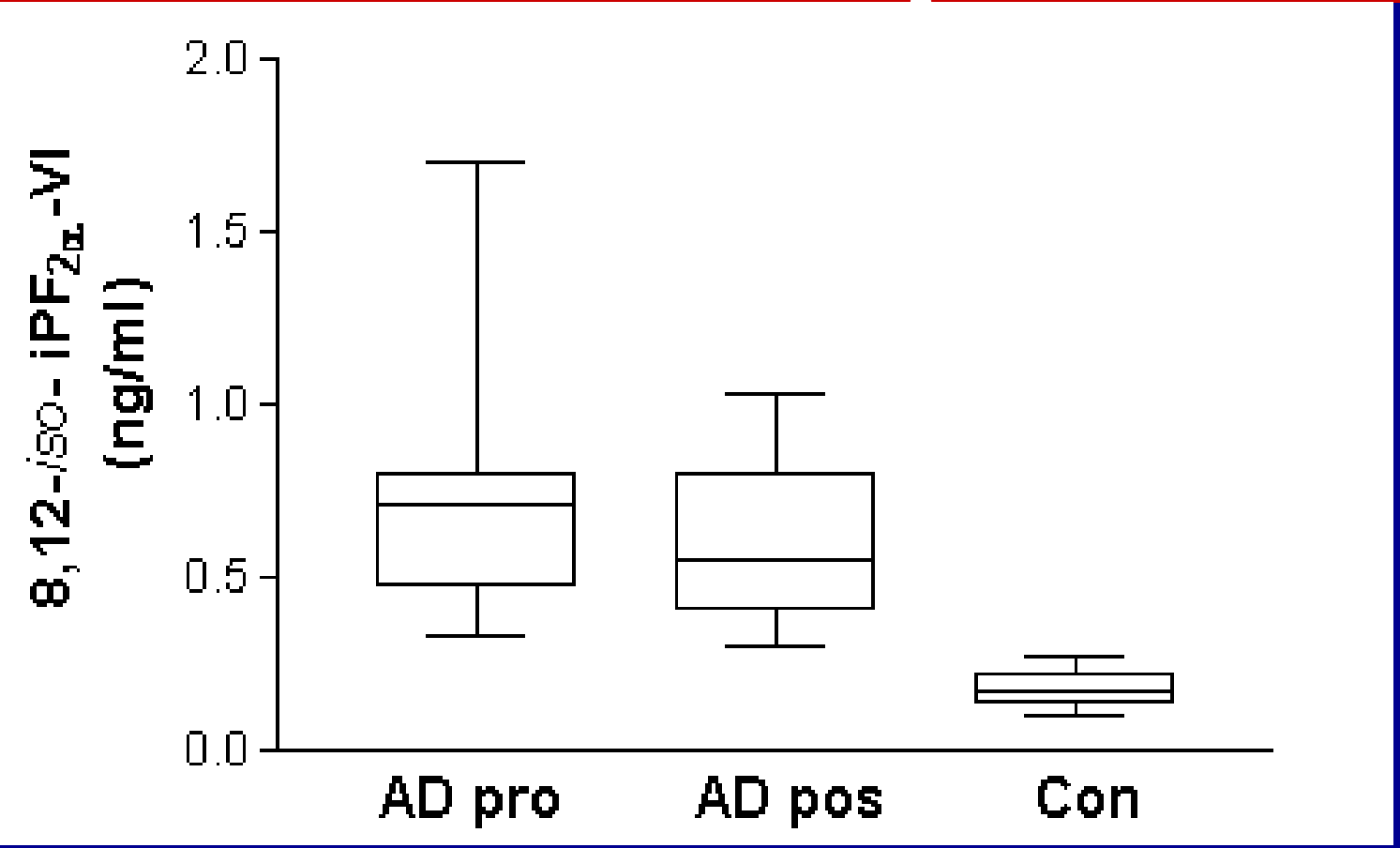
Preferential formation of F_2-iPs in vivo



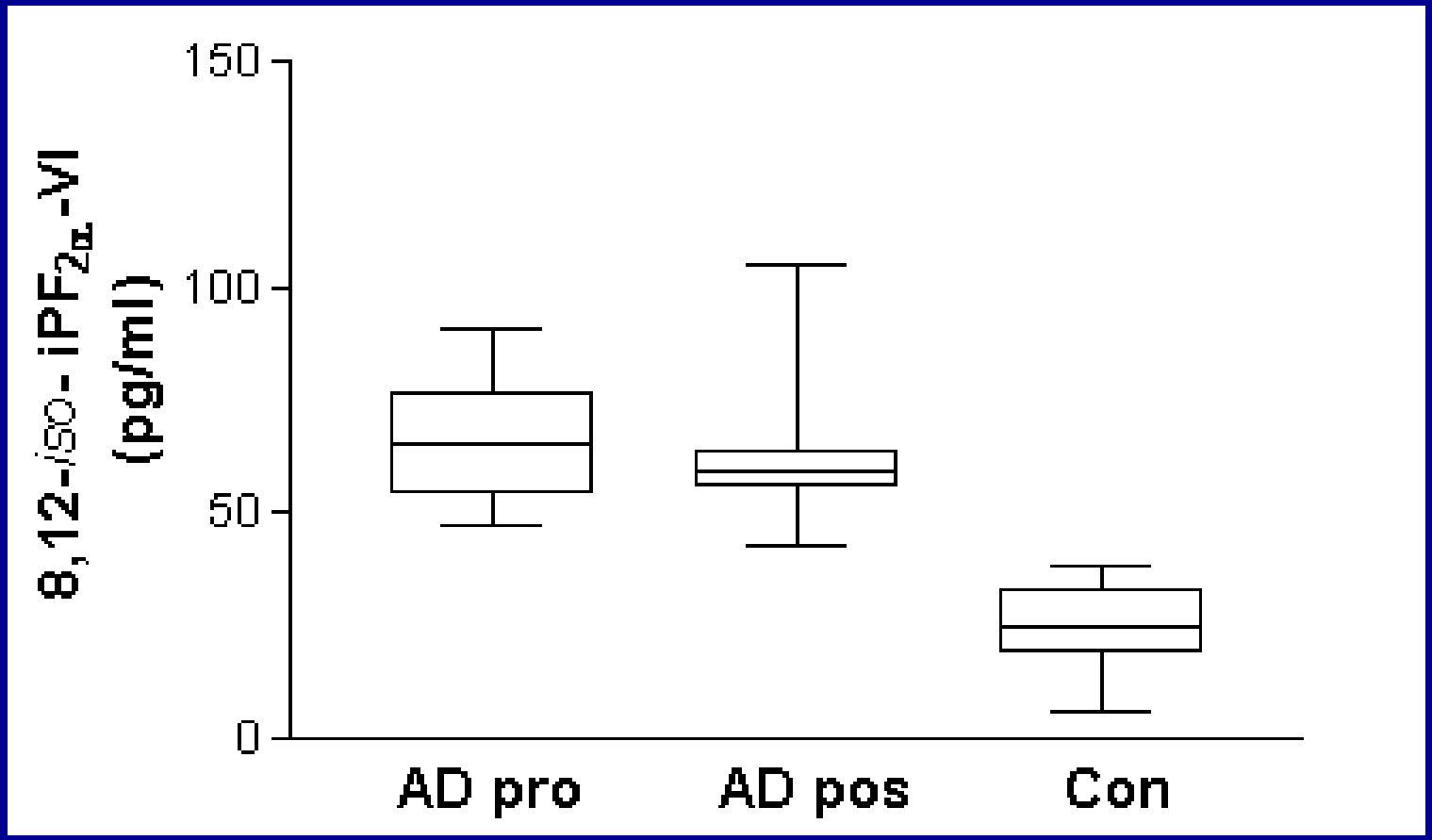
F_2-iPs in human urine



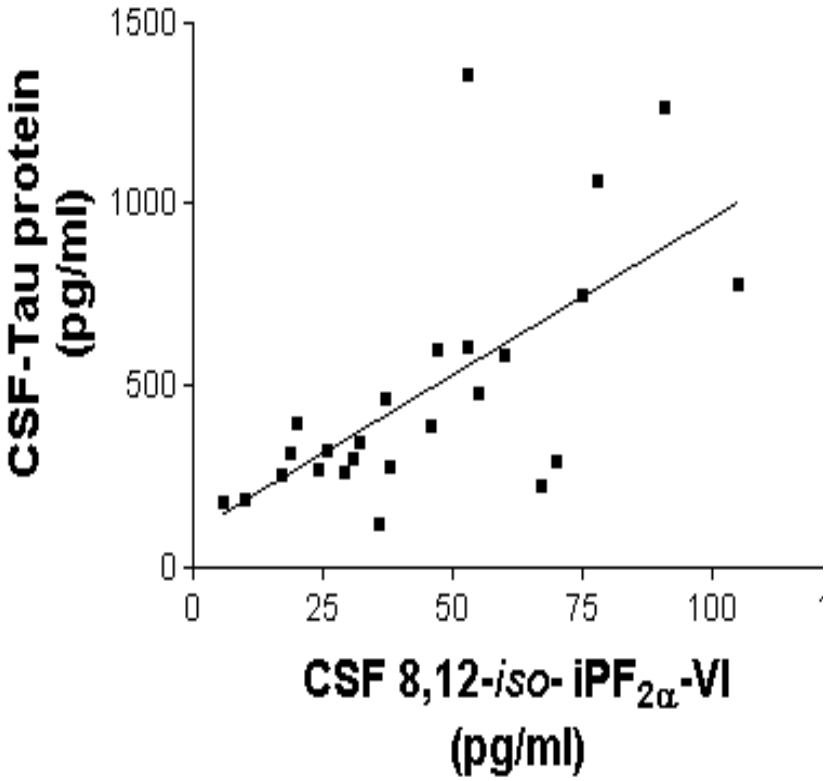
Plasma 8,12-*iso*-iPF$_{2\alpha}$-VI levels are elevated in AD patients

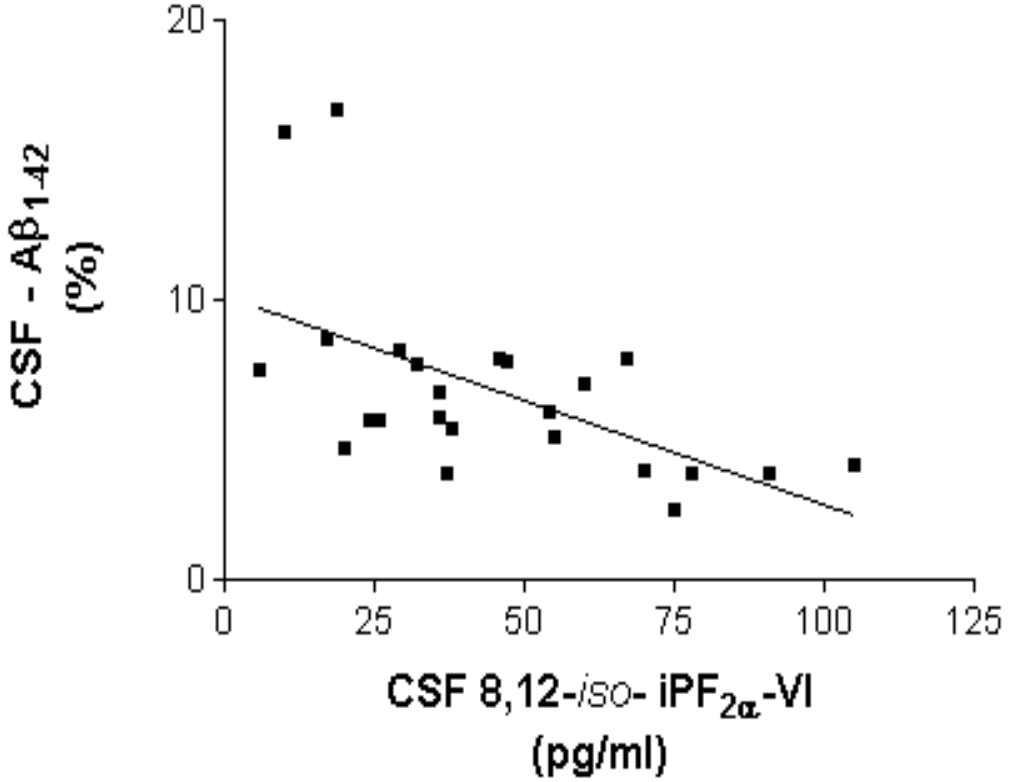


CSF 8,12-*iso*-iPF$_{2\alpha}$-VI levels are elevated in AD patients



CSF 8,12-*iso*-iPF$_{2\alpha}$-VI correlates with disease progression

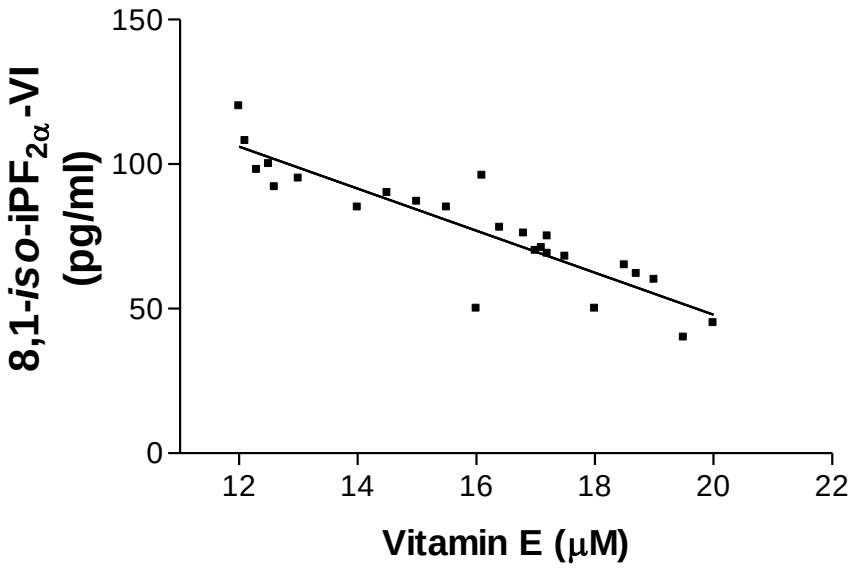


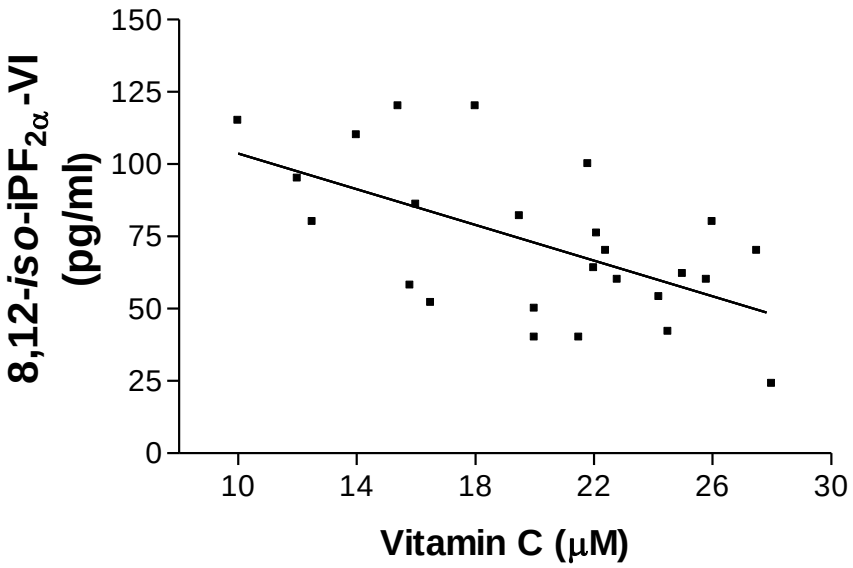


AD and the Antioxidant Status

	AD (25)	Control (25)
Vitamin C (μM)	16±5.8*	36±6.3
Uric Acid (μM)	210±41	238±59
Vitamin E (μM)	12±5*	30±5
Vitamin A (μM)	2±0.3	2.2±0.5
Lycopene (μM)	0.38±0.09*	0.72±0.19
α-Carotene (μM)	0.035±0.01*	0.071±0.01
β-Carotene (μM)	0.21±0.1	0.24±0.1
8,12-iso-iPF$_{2\alpha}$-VI (pg/ml)	110 ±15*	45 ±10

F_2-iPs and the Antioxidant Status





F_2-iPs in the CNS as markers of AD

Increased concentrations in AD patients compared to controls

- **Diseased regions of AD Brain**
  - **FASEB J 1998;12:1777-1783**
  - **Am J Pathol 2001;158:293-297**
- ***Post mortem* ventricular CSF**
  - **Ann Neurol 1998;44:410-413**
  - **Am J Pathol 1999;155:863-868**
- ***Intra vitam* lumbar CSF from mild AD**
  - **Neurology 1999;52:562-565**
  - **Ann Neurol 2000;48:809-812**
  - **Arch Pathol Lab Med 2001;125:510-512**

F_2-iPs in plasma and urine as markers of AD

- **Significant increase in AD compared to control:**
  - 2 studies (urine and plasma) using GC/MS, 1 study (urine) ELISA.
- **No difference between AD and control:**
  - 1 study (urine) using GC/MS, 1 study (plasma) ELISA

F_2-iPs and neurodegeneration

- Mechanism(s) underlying the oxidative imbalance and the increase in 8,12-*iso*-iPF_{2a}-VI in AD are unknown.
- It is unclear whether the increase in Lipid Peroxidation is a cause of a consequence of the neurodegenerative process *per se*, or they are two independent processes.

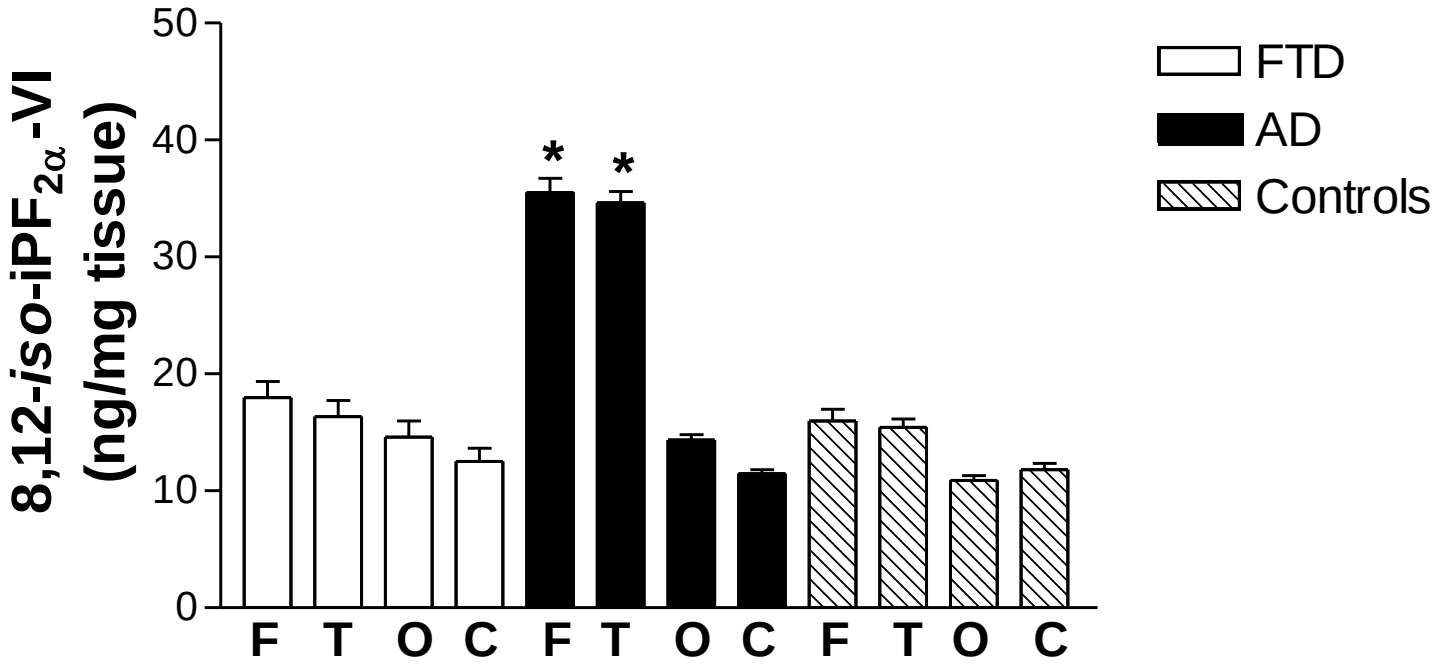
F_2-iPs levels and FTD

- Frontotemporal dementia (FTD) is a heterogenous group of neurodegenerative conditions that account for 3 to 10% of all dementia.
- FTD includes: Dementia lacking distinctive histopathology (DLDH), Progressive supranuclear palsy (PSP), FTD with parkinsonism linked to chromosome 17 (FTDP-17), Pick's disease.

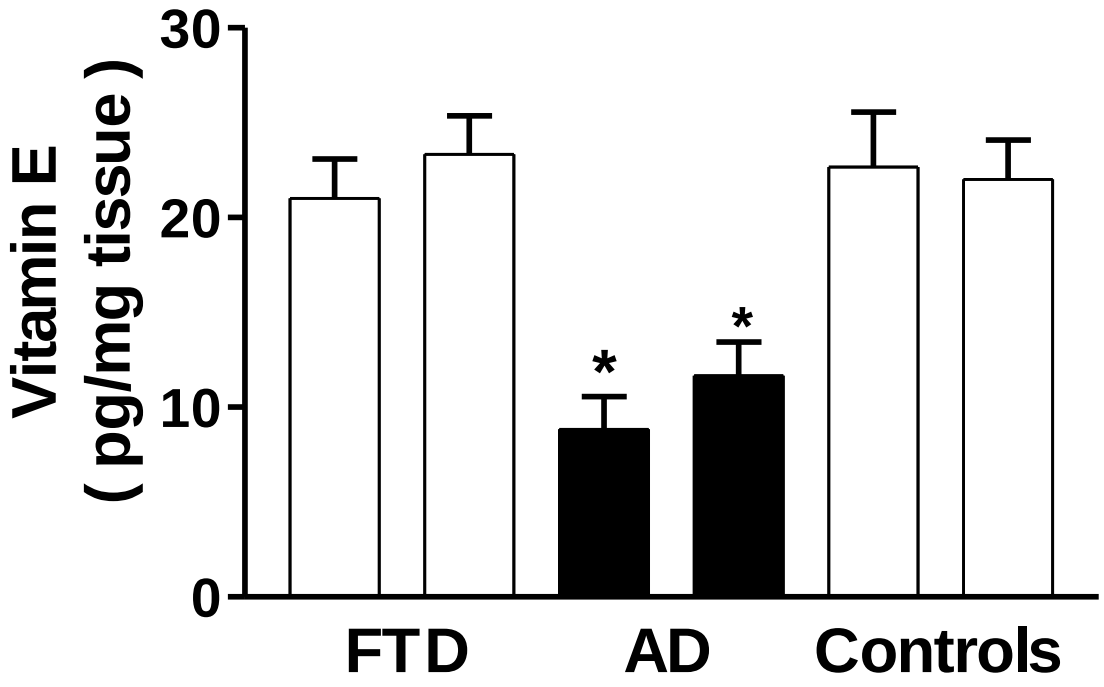
F_2-iPs levels and FTD

	n	M/F	Age	PMI
AD	23	11/12	75±2	9.3± 1
DLDH	8	2/6	74± 3	10± 1
Pick's	3	2/1	71± 2	8.5± 3
FTDP-17	2	M/F	55± 7	9± 3
PSP	6	2/4	75± 2	13± 2
Controls	14	8/6	76± 3	13± 2

8,12-*iso*-iPF$_{2\alpha}$-VI levels are elevated in AD but not in FTD



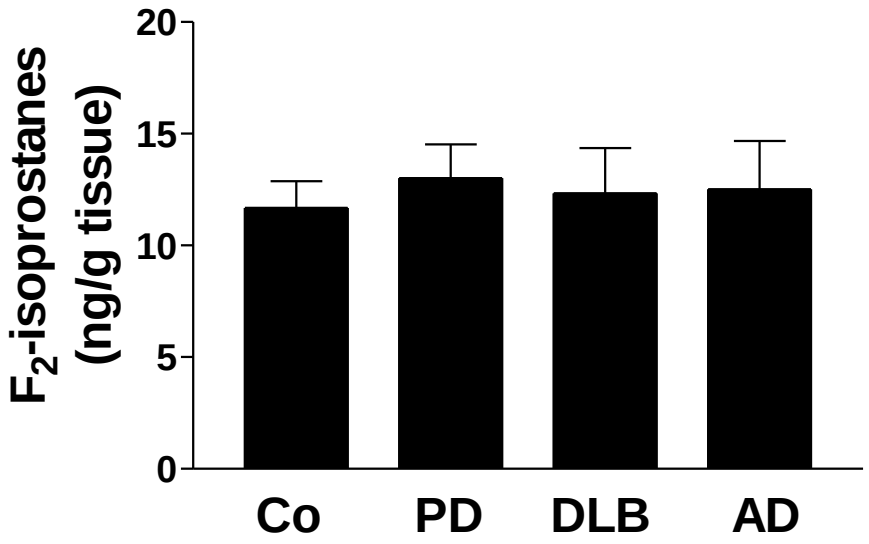
Vitamin E levels are decreased in AD but not in FTD



Brain 8,12-*iso*-iPF$_{2a}$-VI levels in FTD

	Frontal	Temporal	Occipi.	Cerebe.
AD	**35±2**	**34±2**	14±1	11± 0.5
DLDH	19±1.5	17±1.3	15± 1	13± 1
Pick's	18±5	21±7	16± 5	14± 4
FTDP-17	18±1	14±1.1	N/A	15± 1
PSP	1.5±2	12±2	12± 1.1	9.1± 2
Controls	15±2	16±1	11±1	12±1

F_2-iPs levels in PD substantia nigra

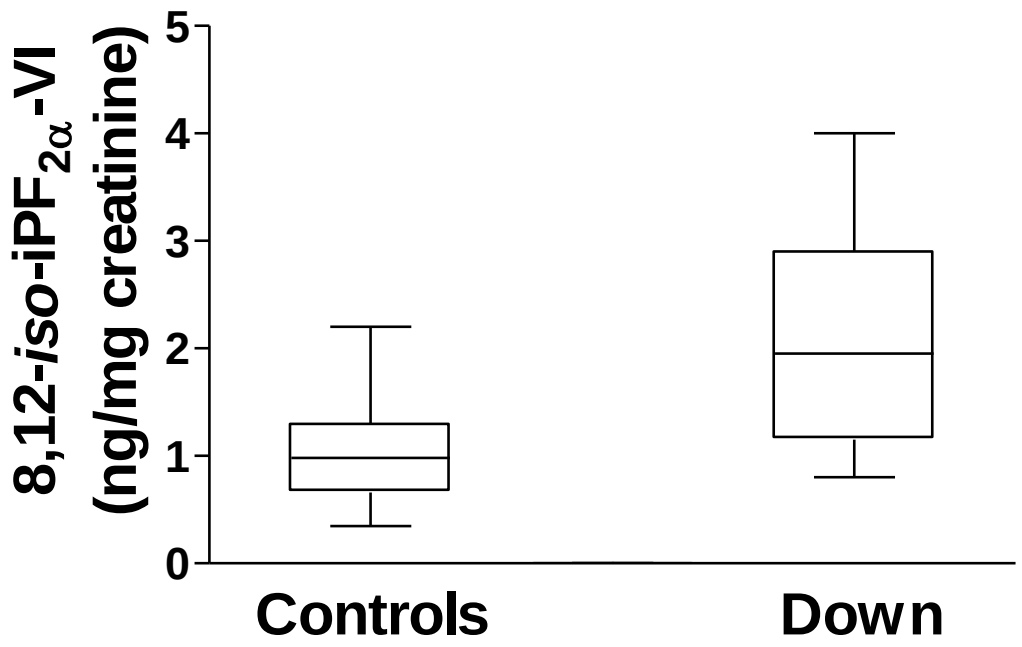


J.Neurochem. 85,645-650, 2003

8,12-*iso*-iPF$_{2\alpha}$-VI as an early marker of AD

- AD is characterized by an oxidative imbalance and an increase in 8,12-*iso*-iPF$_{2\alpha}$-VI .
- It is unclear whether the increase in Lipid Peroxidation is a cause of a consequence of the Aβ accumulation, or they are two independent processes.

8,12-*iso*-iPF$_{2a}$-VI is elevated in Down's syndrome

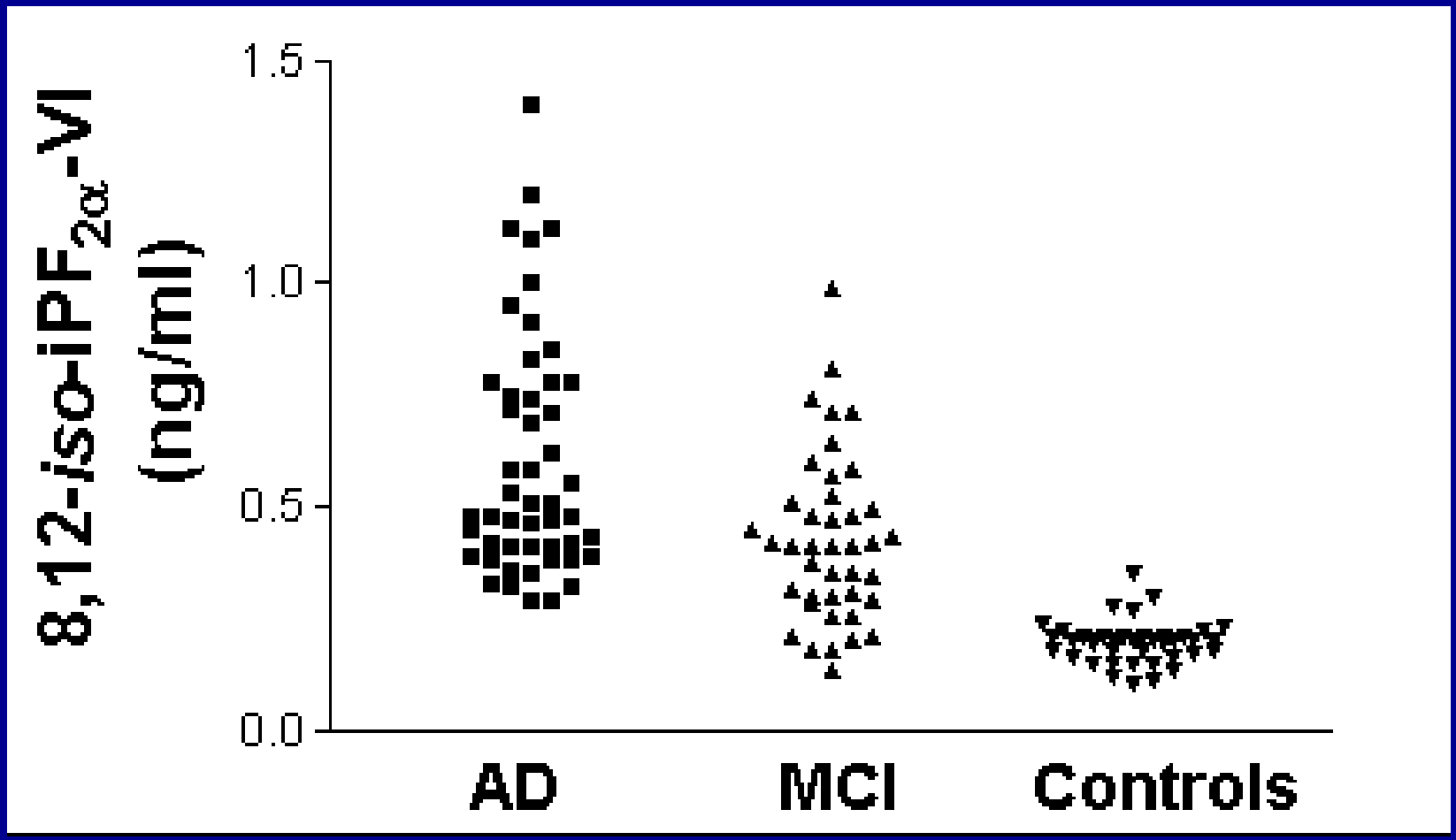


MCI and 8,12-*iso*-iPF$_{2a}$-VI levels

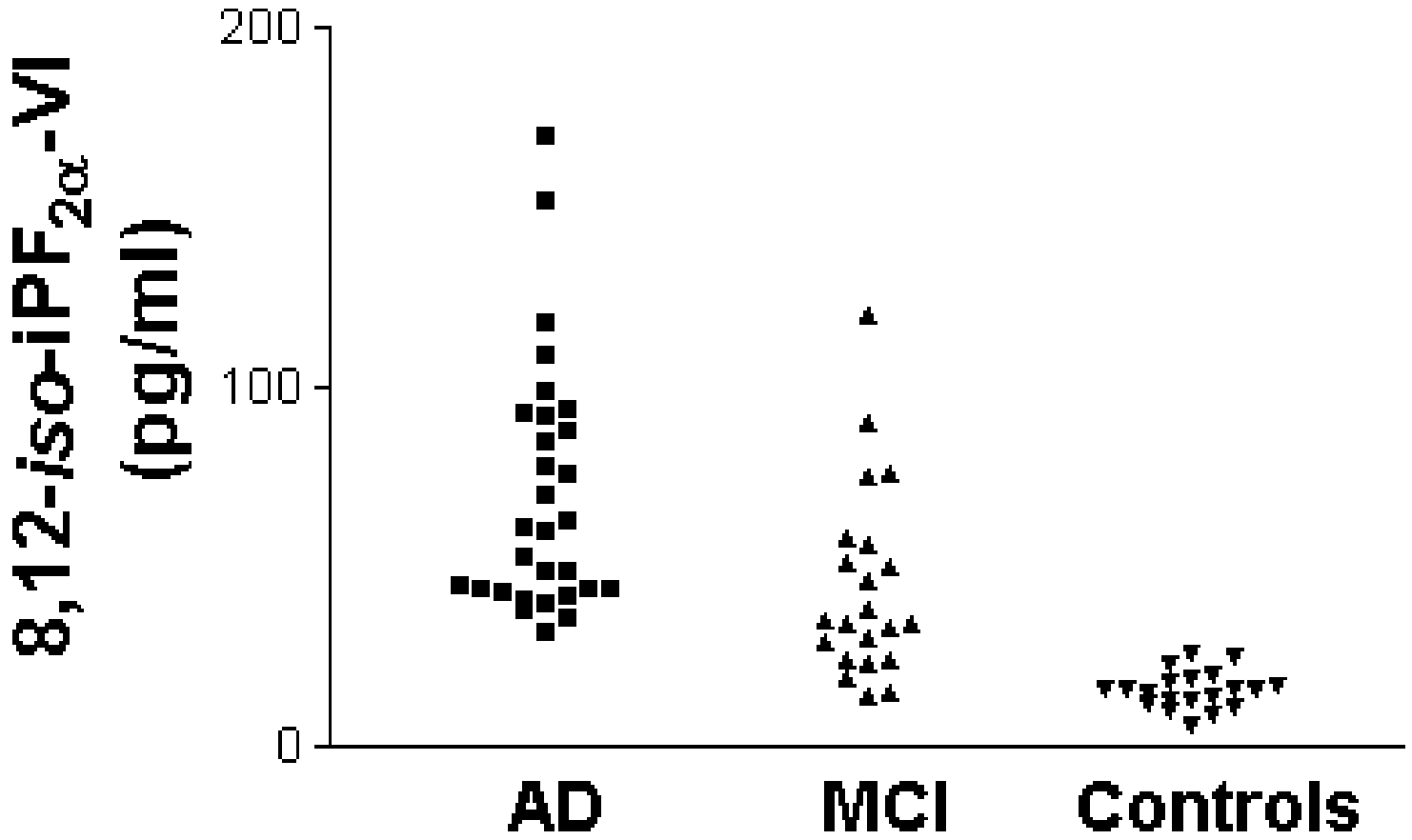
Since **MCI** subjects are felt to be a high risk to progress to a clinical diagnosis of AD,

do these individuals, like AD patients, manifest increased levels of this marker ?

Plasma 8,12-*iso*-iPF$_{2\alpha}$-VI levels are elevated in MCI



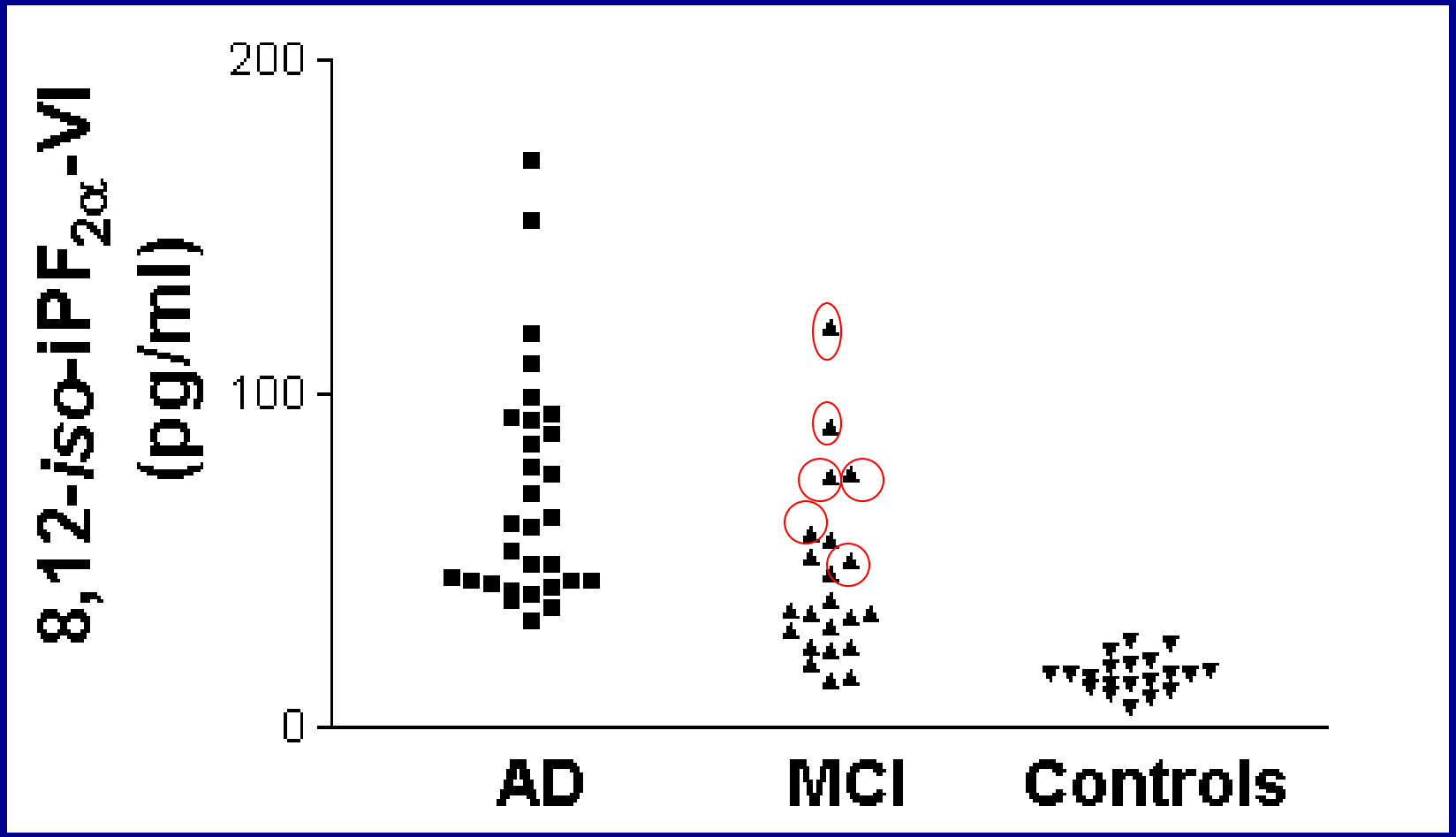
CSF 8,12-*iso*-iPF$_{2\alpha}$-VI levels are elevated in MCI



MCI: CSF biomarkers

	AD (n=30)	MCI (n=22)	Controls (20)
CSF tau (pg/ml)			
Mean (SE)	681 (63)*	381 (55)	313 (24)
Range	(293-1513)	(173-857)	(176-461)
CSF Aß$_{1-42}$(%)			
Mean (SE)	4.0 (0.29)**	4.7 (0.4)	6.7 (0.9)
Range	(2.1-9.2)	(1.7-7.9)	(3.4-16.7)

MCI with high 8,12-*iso*-iPF$_{2\alpha}$-VI levels converted to AD



Lipid Peroxidation is an early event in AD

- Patients who meet standardized clinical criteria for MCI have increased 8,12-*iso*-iPF$_{2\alpha}$-VI levels.
- No significant difference in CSF tau and the percentage of Aβ 1-40/1-42 was observed between MCI subjects and controls.
- The increase in 8,12-*iso*-iPF$_{2\alpha}$-VI is an early biomarkers for AD.

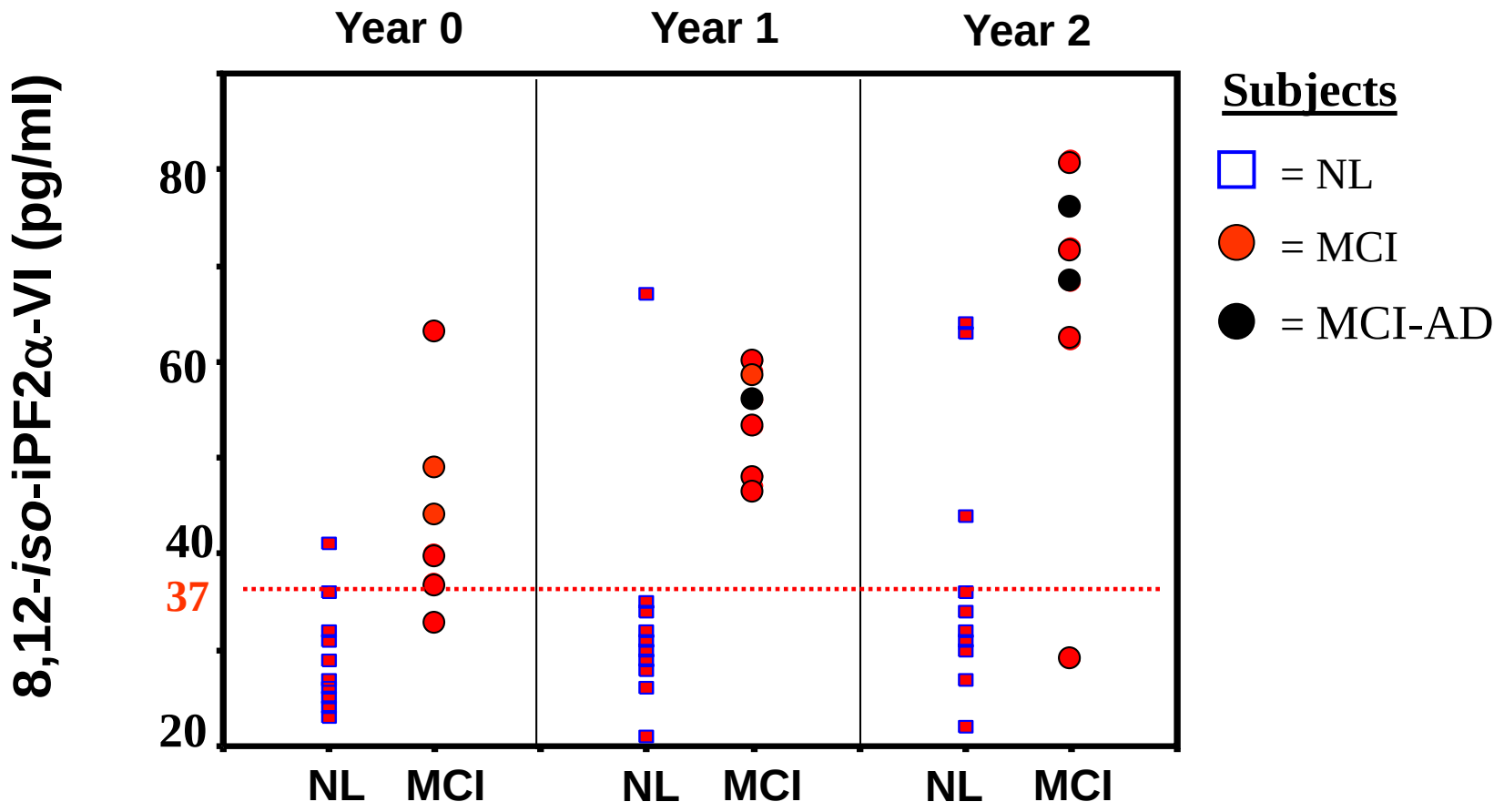
Annual CSF-MRI Study- 3Time points

Outcome Groups

	NL	MCI
Sample size	10	6
% Female	50	33
# Convert to AD	0	2
ApoE E4 +	1	2
Age	63	70
MMSE-baseline	30	28
Education	17	14

Annual Group Isoprostane Differences

NL n=10, MCI n=6



Sensitivity	83	100	83
Specificity	90	90	70
Overall	***88****	***94****	***75****

*p<.05

Classifications from Longitudinal Isoprostane Changes

NL(10) MCI(6)

Classification Accuracy with Sensitivity = 83%

Interval	Specificity	Overall
Year 0 ~ 1	90	88 *
Year 1 ~ 2	80	81 *

*p<.05

CNS F_2-iPs as AD biomarkers

- Advantages
  - Consistently increased even at the early stages of the disease
  - Closely reflect brain biochemistry and pathology
  - Specific for disease (FTD, PD)
- Disadvantages
  - Invasive procedure
  - Some overlap between controls and patients

Peripheral F_2-iPs as AD biomarkers

- Advantages
  - Much easier to obtain
- Disadvantages
  - Confounded by peripheral factors (selection criteria of the patients)

Application of F_2-iPs as AD biomarkers

- **Diagnosis (clinical, pre-clinical)**
- **Prediction of rate of progression**
- **Patients selection**
- **Rationale for dose-selection of therapeutics with and without anti-oxidant activity**

Thanks to the ADC directors and…..

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