Identification of Folate-sensitive signaling pathway in the Central Nervous System

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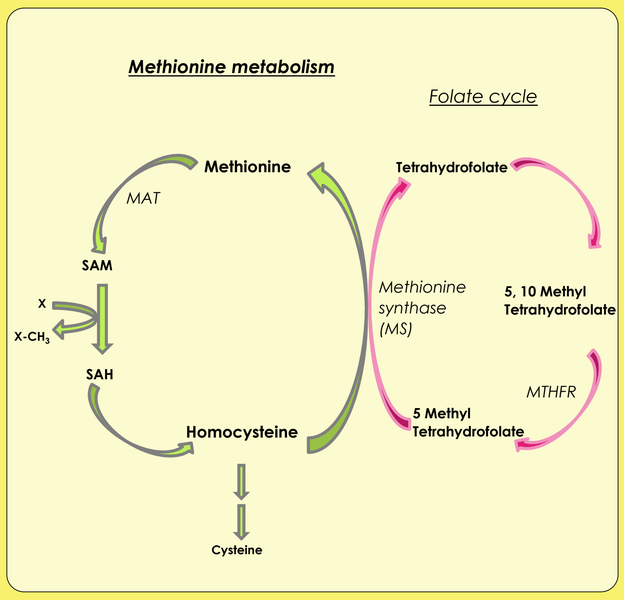
Background:

Folic acid or Vitamin B9 is well known for its important role in the nervous system development and correct closure of the neural tube. Folate is essential for numerous functions due to its role as one carbon group donor, Including: nucleotide synthesis, role in DNA methylation and other biological methylations[1].

Folate deficiency and elevated plasma homocysteine (which can be caused due to low levels of Folate) are associated with increased risk of age-related cognitive decline, cerebrovascular and neurodegenerative disease. Troen et al. previously found that methionine, a product of folate metabolism, can mitigate cognitive and neurochemical effects of folate-deficiency in young rats, independently of homocysteine (Troen et al, J Nutr 2008)[2] . However, as yet, these experiments have not been replicated in older rats. Furthermore, the molecular underpinnings of this response and their downstream effects on cellular function are unclear.

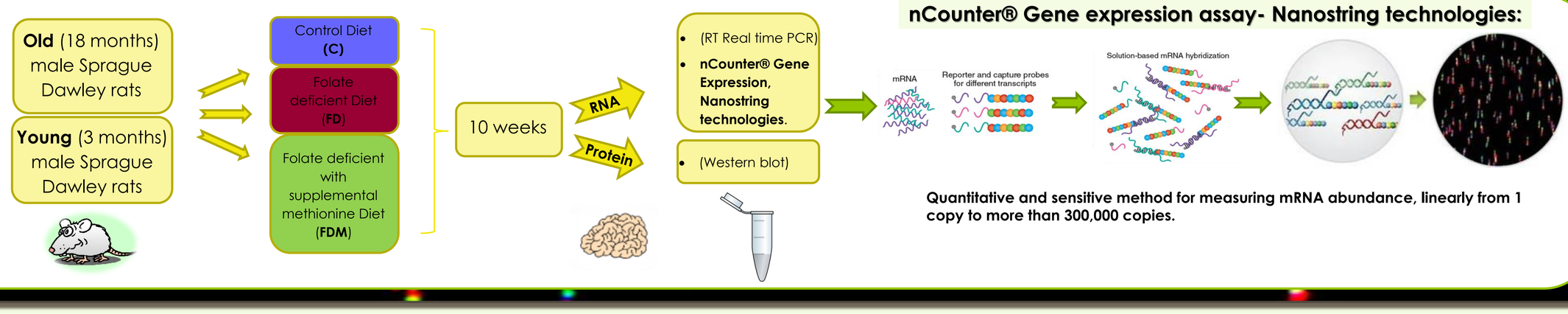
Our *working hypothesis* was that folate deficiency would increase the severity of brain aging, and this would be moderated by methionine.



Aim:

To identify folate- and methionine-responsive signaling pathways in brain that could mediate neurocognitive risk, and to determine their interaction with age.

Experimental design:



Results:

Plasma Homocysteine:

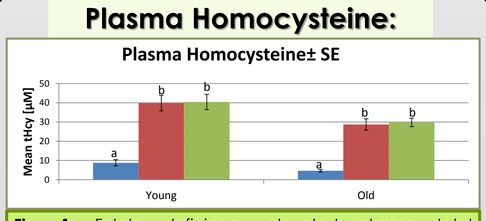


Fig. 1: Folate deficiency elevated plasma total homocysteine and was not corrected by methionine. No difference was seen by age.

Metabolic Response:

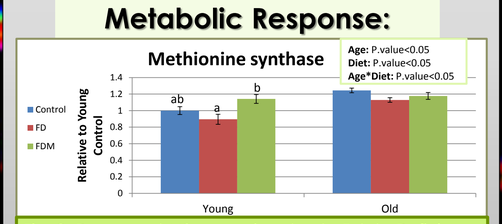


Fig. 2: Decreased MS expression due to Folate Deficiency was prevented by Methionine (independent of Hcy).

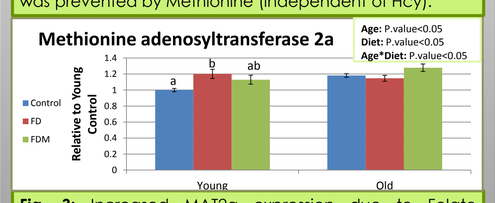
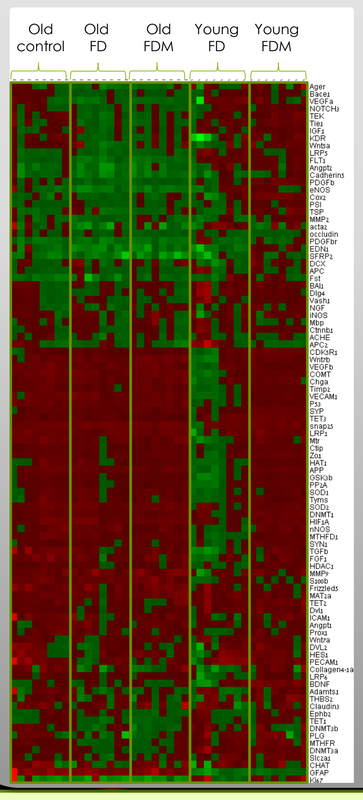


Fig. 3: Increased MAT2a expression due to Folate Deficiency was mitigated by Methionine (independent of Hcy).

Legend (Fig 1-3): Mean± SE in Old and Young Rats (n=8 per group). Different letters (a,b) differ significantly (P≤0.05). **C – Control FD – Folate Deficient FDM– Folate deficient + Methionine**

Overall effect:

Heat map for all tested genes:



Overall:

	Age	Diet	Interaction	N
	V	V	V	19
	V	V	-	10
	V	-	-	29
	-	V	-	6
	-	-	-	37
∑N:	58	35	19	101

Affect of Diet:

	C	FD	FDM	N
Folate responsive, correction by Methionine	a	b	a	4
Folate responsive, without Methionine sensitivity (possibly Homocysteine sensitive)	a	b	b	0
Methionine responsive	a	a	b	9
Trend of change by Folate and trend direction change by Methionine	ab	a	b	14
Methionine responsive depending on Folate availability	a	ab	b	6
Folate responsive, partial correction by Methionine	a	b	ab	1
			∑N:	34

*Groups with different letters (a,b) differ significantly (P≤0.05).

*Heat map is relative to Young control.
*Genes with expression less than 100 counts were excluded.

Vascular regulatory genes:

Angiogenesis:

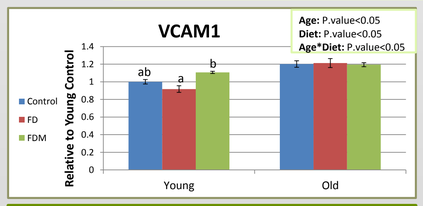


Fig. 4: Decreased VCAM1 expression due to Folate Deficiency was prevented by Methionine (independent of Hcy).

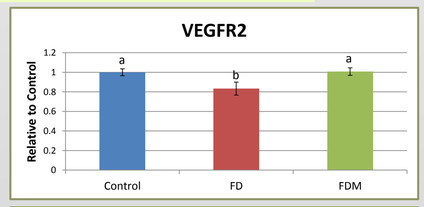
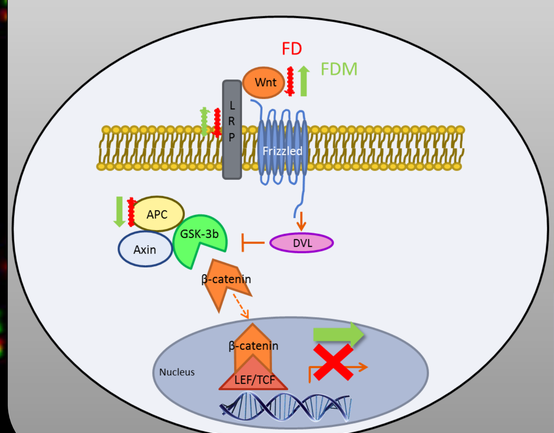


Fig. 5: Decreased VEGFR2 expression due to Folate Deficiency was prevented by Methionine (independent of Hcy).

Wnt signaling pathway:



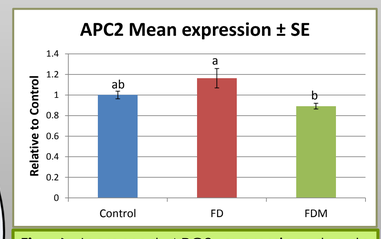


Fig. 6: Increased APC2 expression due to Folate Deficiency was prevented by Methionine (independent of Hcy).

Legend (Fig 4): Mean± SE in Old and Young Rats (n=8 per group).
Legend (Fig 5-6): Mean± SE in Old and Young Rats (n=16 per group).
Different letters (a,b) differ significantly (P≤0.05). **C – Control FD – Folate Deficient FDM– Folate deficient + Methionine**

Discussion:

- Key pathways in the brain, which are involved in cognitive decline, were affected by folate. In some cases these genes were responsive to methionine availability, independently of homocysteine. These affects were mild (10%-20% mean fold change, relative to young control) but significant, and may affect long-term brain health.
- We identified folate-responsive regulatory genes including members of the VEGF and Wnt signal transduction pathways, which are nececssary for maintaining brain vascular perfusion[3].
- Young rats are more affected by diet than old rats (in terms of significant changes). To the extent this is true for humans it suggests the importance of early intervention for the prevention of dementia.

References:

1. Sezgin et al. *Alzheimer's disease and epigenetic diet*. Neurochemistry International, October 2014; **78**: p. 105-116.
2. Troen AM et al. *Cognitive impairment in folate-deficient rats corresponds to depleted brain phosphatidylcholine and is prevented by dietary methionine without lowering plasma homocysteine*. The Journal of Nutrition, December 2008; **138**: p. 2502-2509.
3. Zerlin M et al. *Wnt/Frizzled signaling in angiogenesis*. Angiogenesis, 2008; **11**: p.63-69.