BM Systems

PREDICTIVE INTEGRATIVE BIOLOGY

Conditionnement durant la grossesse conduisant à des pathologies de l'adulte.

How can gestational malnutrition and chronic vitamin B12 shortage promote type 2 diabetes pathogenesis in adolescent offsprings?

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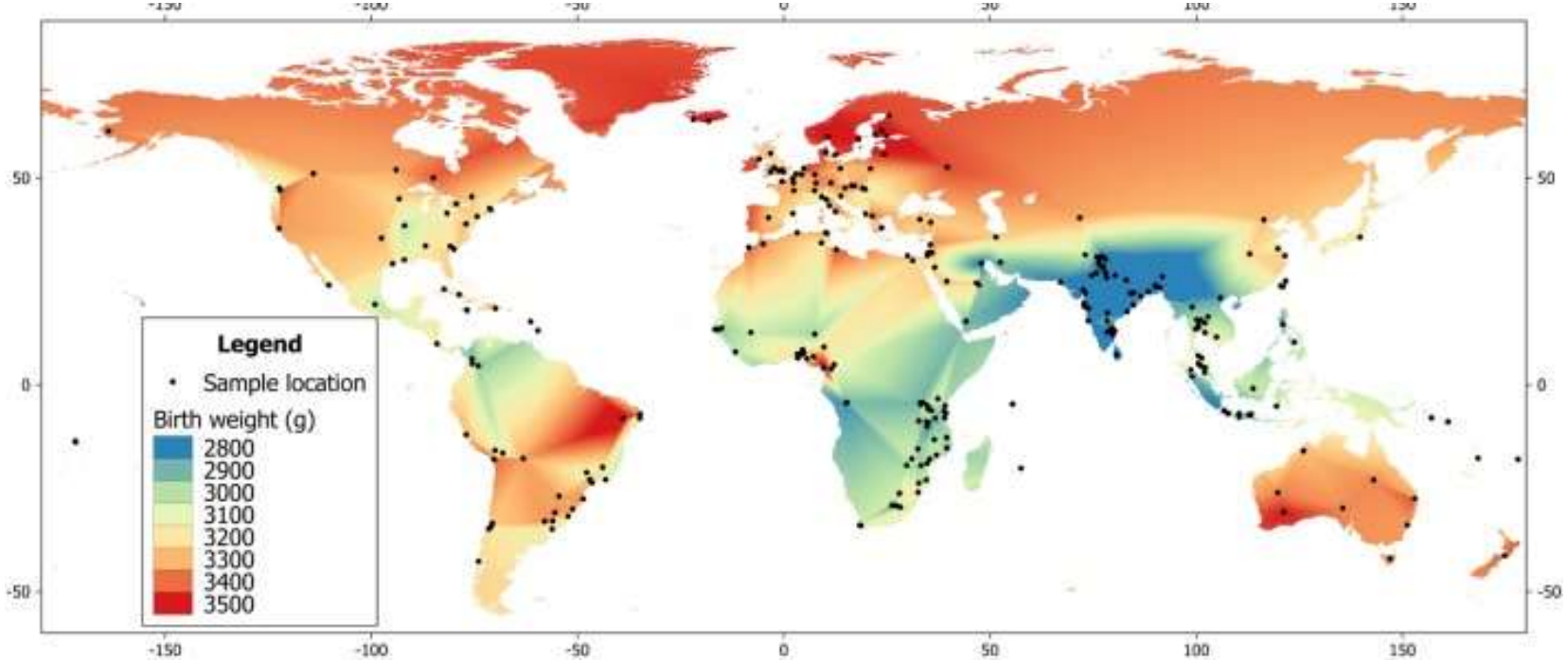
The problem

World-wide, 16 % of all births are LBW. Almost 95 % of LBW births occur in developing countries, India accounting for nearly 40 % of the global burden (B. Vishnu Bhat &B. Adhisivam (2013): Indian J Pediatr . **80**(1):60–62).



Global heat map of mean birth weight, based on data from the World Health Organization

LBW individuals present a 3.8-times increased risk of glucose intolerance & T2D, and a 6.5-times increased risk of hypertension in adulthood (C. S. Yajnik (2002): Obes Rev.**3**(3):217-24).

India has become known as the "diabetes capital" of the world, substantial further increases being anticipated (J.C. Wells et al. (2016): Front Public Health. **4**: 145).

The basic facts

From the Pune Maternal Nutrition Study (Katre P et al. (2016): Eur J Clin Nutr.**70**(6):687-93; Yajnik CS et al. (2003):Int J Obes Relat Metab Disord. **27**(2):173-80).

Maternal blood biochemistry in early pregnancy (2^{d} month).

- Low B12, low LDL cholesterol;
- High folate (5m-THF), high homocysteine;
- Increased triglycerides, increased carbohydrates, increased total cholesterol.

Placental characteristics at parturition

- Reduced placental weight, high cord-blood leptin, high insulin, low adiponectin.

Infant characteristics at Birth

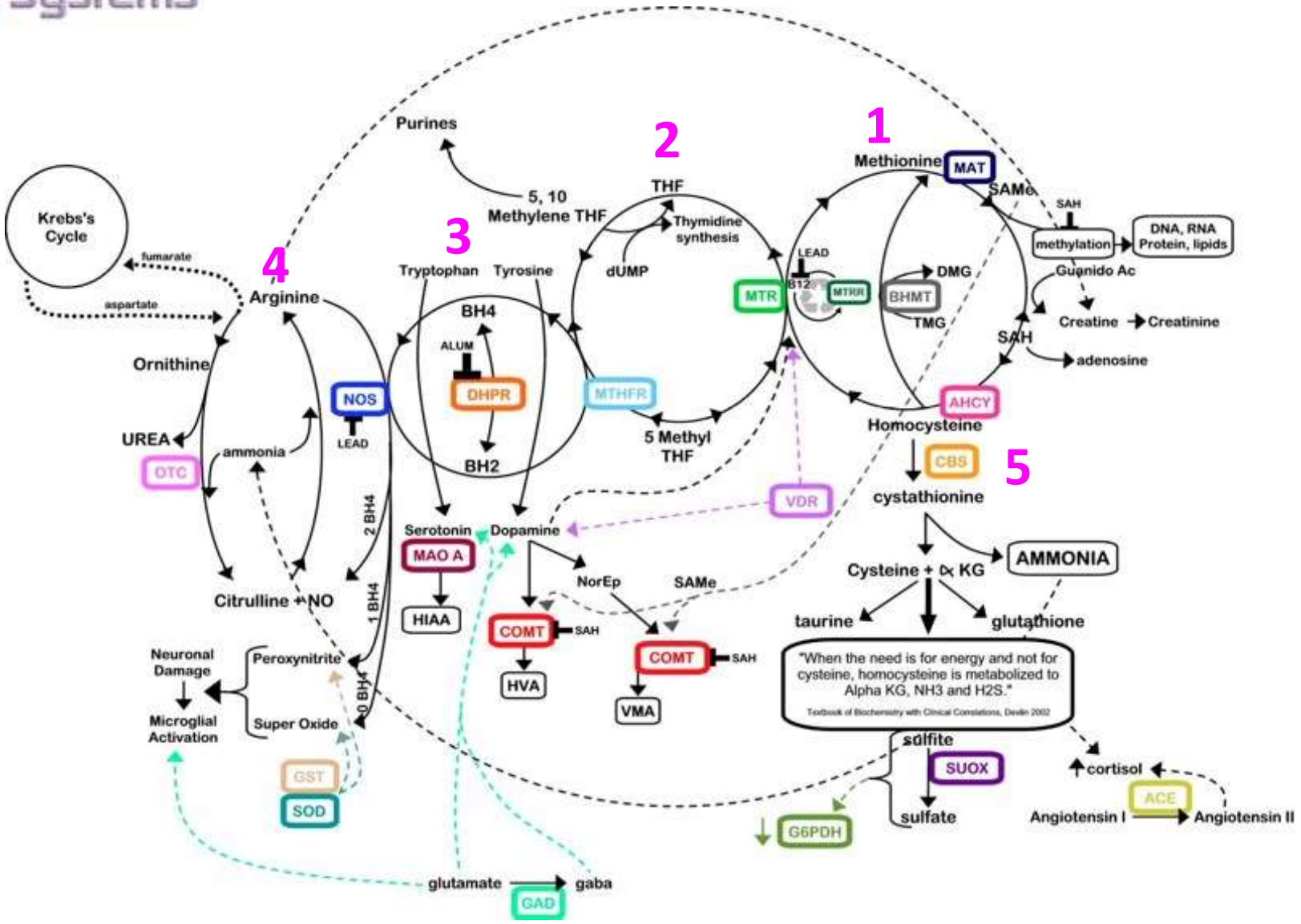
- Low birth weight ($<$ 2.5kg), short ($<$ 47.5 cm), decreased non-fat soft tissues (abdominal viscera and skeletal muscle) but increased subcutaneous fat (thin-fat phenotype).

Infant characteristics at 6 years of age

- Increased subcutaneous fat and body-mass index (BMI);
- Hyperinsulinemia and hyperleptinemia.

How does maternal nutrient restriction coupled with defective one-carbon metabolism alter the foetal development program, leading to enhanced predisposition to T2D in adolescence?

The B12-dependent metabolic network



Cycle 1: Methylation cycle; **Cycle 2**: Folate cycle; **Cycle 3**: BH4 cycle; **Cycle 4**: Urea-NO cycle; **Pathway 5**: Trans-sulphuration.

Consequences of defective maternal one-carbon metabolism

(Early pregnancy)

B12 chronic deficit → MTR activity ↓↓ → SAM and THF supply ↓↓ + homocysteine (Hcy) and mTHF ↑↑

mTHF ↑↑ + SAM ↓↓ → BH4 cycle disrupted (BH2 ↑↑)

BH4 ↓↓ → NO production ↓↓ → placental vascularisation ↓↓

Chronically elevated Hcy →

↓

Altered lipid metab.

+

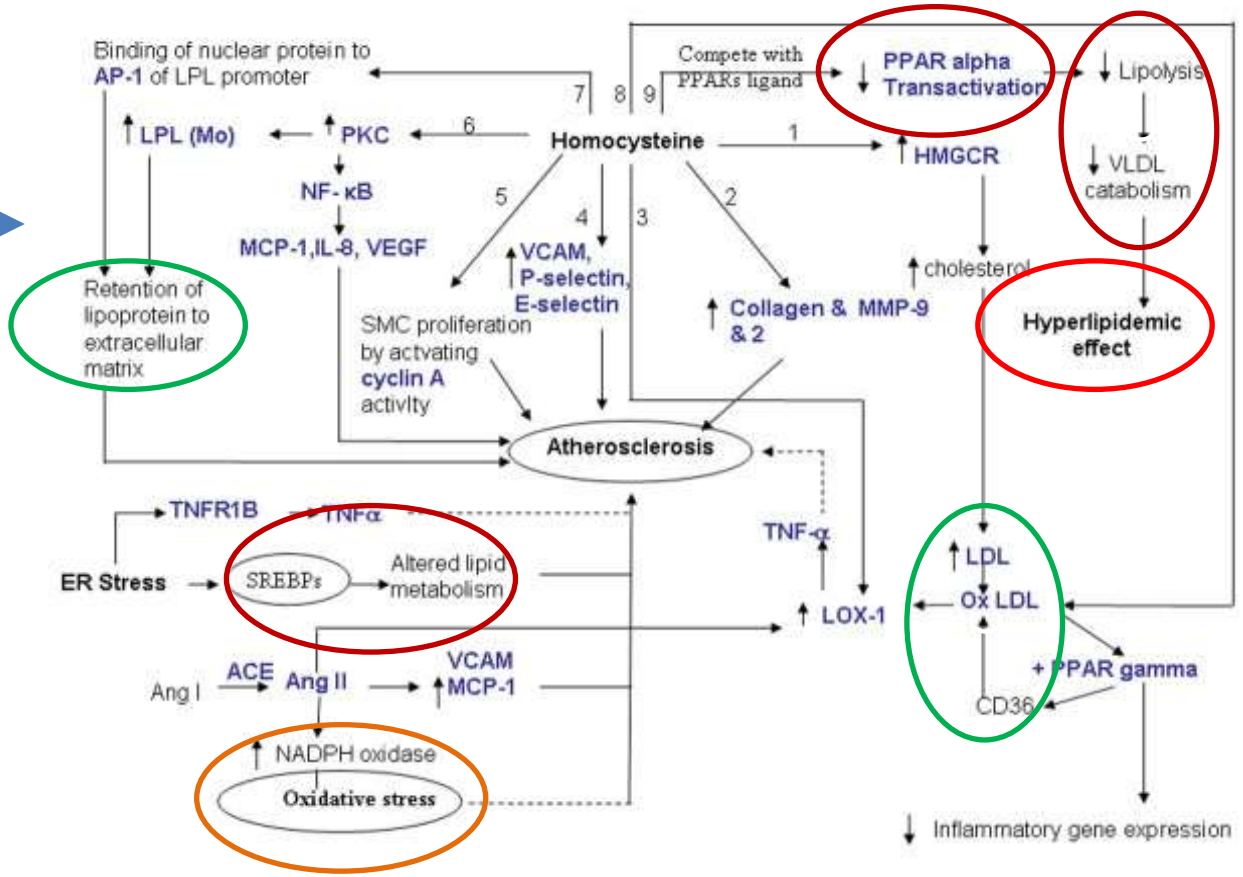
Elevated circulating TGs

+

Elevated circulating cholest.

+

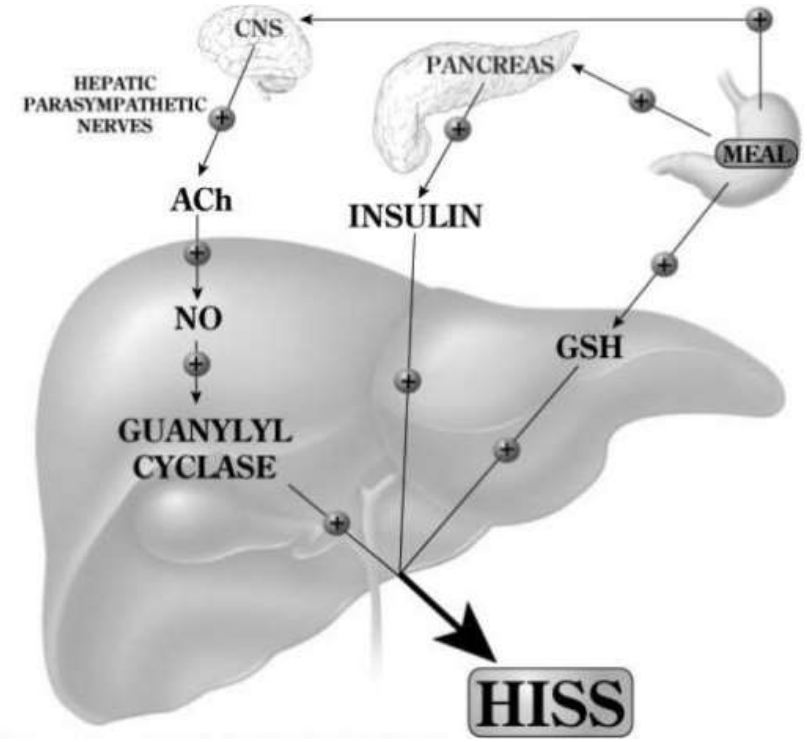
Elevated H2S production.
(as a result of cystathionine and homolanthionine elevation)



Consequences of defective maternal one-carbon metabolism

(Early pregnancy)

Feeding results in an increase of hepatic glutathione (GSH) and a parasympathetic signal to the liver that acts, via acetylcholine, on muscarinic receptors to activate NO release which, in turn, activates guanylyl cyclase.
Both signals are required for insulin to cause the release of hepatic insulin sensitizing substance (HISS).



HISS acts selectively on skeletal muscle. Blockade of any portion of the above pathways leads to blockade of HISS release and a state of HISS-dependent insulin resistance.

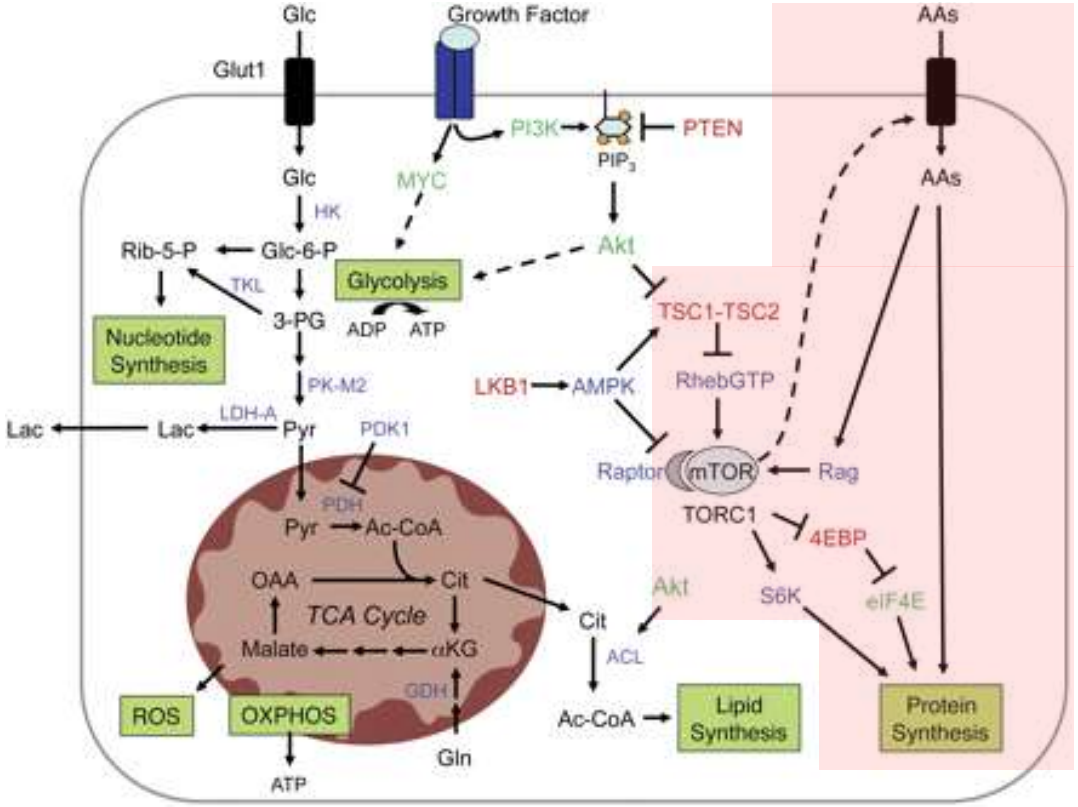
Low B12 supply concurrently with low BH4 supply and high Hcy circulating levels stands to severely impair maternal HISS mechanisms at the parasympathetic level as well as at the NO and GSH levels, leading to postprandial

- hyperglycemia,
- hyperinsulinemia,
- hyperlipidemia, and
- increased oxidative stress.

Consequences of maternal low protein high carbohydrate diet

(Early pregnancy)

Under low protein diet , high carbohydrate signals antagonize the TORC1 complex through the LKB1–AMPK pathway, favouring activation of the AMPK-mediated autophagic mechanisms leading to essential amino acids preservation .



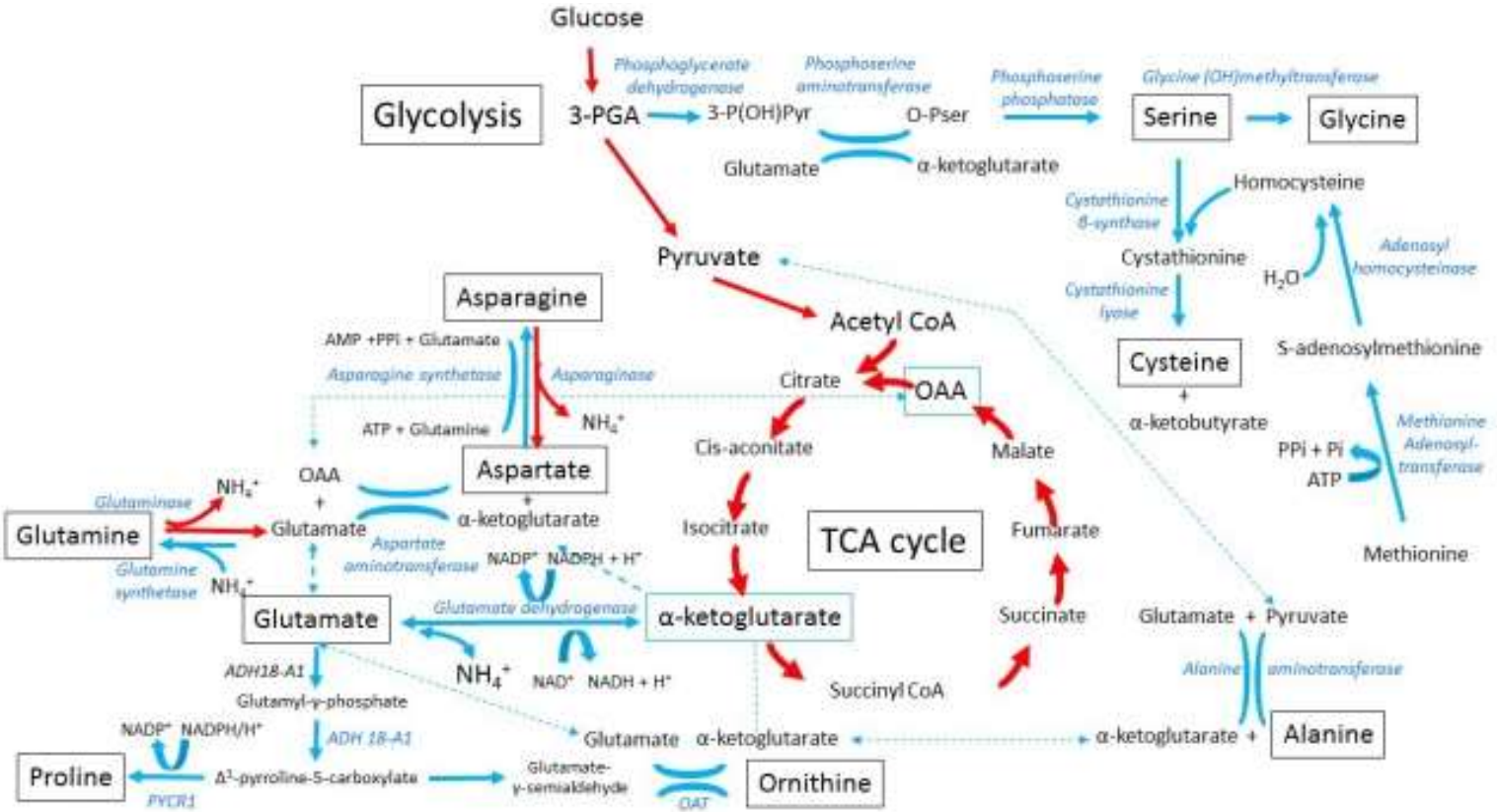
Most of the excess acetyl-CoA will now be channelled to cytoplasmic fatty acids synthesis, particularly so in preparation for lactation.

This will exacerbate the effects of elevated Hcy which impedes β-oxidation, thereby promoting hypertrigycerylemia, while also leading to NADH overproduction.

Consequences of maternal low protein high carbohydrate diet

(Early pregnancy)

In parallel, a significant proportion of carbohydrates will be channelled to non-essential amino acids (N.E αA.A) production, attenuating but not inhibiting AMPK-mediated autophagy.



3 main consequences:

- Elevated N.E αA.A : E. αA.A ratio;
- Oversupply in pyruvate + NADH overproduction;
- Elevated circulating carbohydrates

Consequences of maternal metabolic dysregulations

(Early to late pregnancy)

- Maternal hypercholesterolemia and hypertriglyceridemia during pregnancy is correlated with foetoplacental endothelial dysfunction;
- Maternal low B12 and high circulating Hcy leads to reduction in placental weight;
- Hcy impairs bradykinin-induced intermediate- and small-conductance calcium-activated potassium (IKCα and SKCα) channels activity (vasodilation);
- The above effects will result in placental endoplasmic reticulum (ER) stress with subsequent increased placental leptin production and constitutive mild placental hypoxia;
- ER stress represses cell surface expression of IKCα and SKCα channels, now inducing endothelial dysfunction.

The cumulative effects are likely to result in substantial placental dysfunctions.

At placental level, the foetus will be expose to bouts of maternal postprandial hyperglycemia and hyperinsulinemia (the equivalent of mild gestational diabetes) together with chronically elevated placental leptin, while being supplied with a “diet” of

- high lipids and cholesterol,
- high carbohydrates,
- high Hcy and elevated pyruvate together with
- poor B12 and tetrahydrofolate supply and
- low essential amino acids, all this associated with
- mild hypoxic conditions.

Serological markers of maternal metabolic dysregulations

- Elevated acylcarnitines (indicating Hcy-mediated impaired β-oxidation);

- Elevated lactate at rest (reflecting Ac-CoA over-production);

- Elevated ratio of non-essential over essential amino acids (indicative of increased carbohydrates channelling to non-essential amino acids production while suggesting concurrent activation of autophagy);

- Elevated urinary phosphatidylethanolamine-conjugated form of microtubule-associated protein 1 light chain 3 (MAP1LC3A) **and possibly also 8-hydroxy-2'-deoxyguanosine** (8-OHdG), both of which are indicative of activation of AMPK-mediated hepatic autophagy (mTOR-independent pathway),

- Increased circulating levels of cystathionine and homolanthionine, indirect indicators of increased H2S production associated with overconsumption (i.e. not the object itself but its shadows), as well as

- Decrease in circulating postprandial somatostatin (suggestive of defective HISS response).

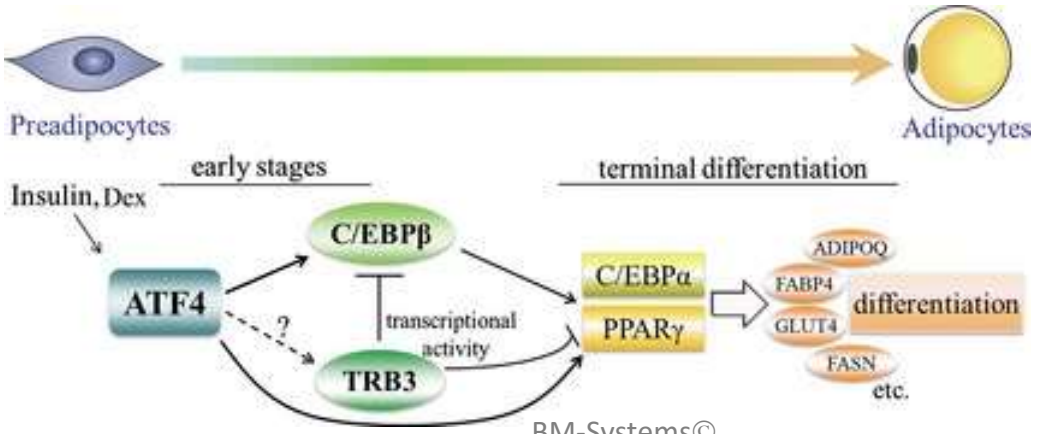
Consequences of maternal metabolic dysregulations on the foetal side

Placental vasculature supplies the foetus with
- elevated insulin and leptin,
- high lipids and cholesterol,
- high carbohydrates,
- high Hcy, elevated pyruvate together with
- low B12 and tetrahydrofolate
- low essential amino acids,

all this associated with mild hypoxic conditions.

- ❖ Metyl donor deficiency;
- ❖ SIRT inhibition;
- ❖ Epigenetic inhibition of the PPARγ co-activator PGC-1α;
- ❖ Early sustained Akt & mTORC1 activation;
- ❖ Alteration of metabolic control via ATF4 and ATF6β

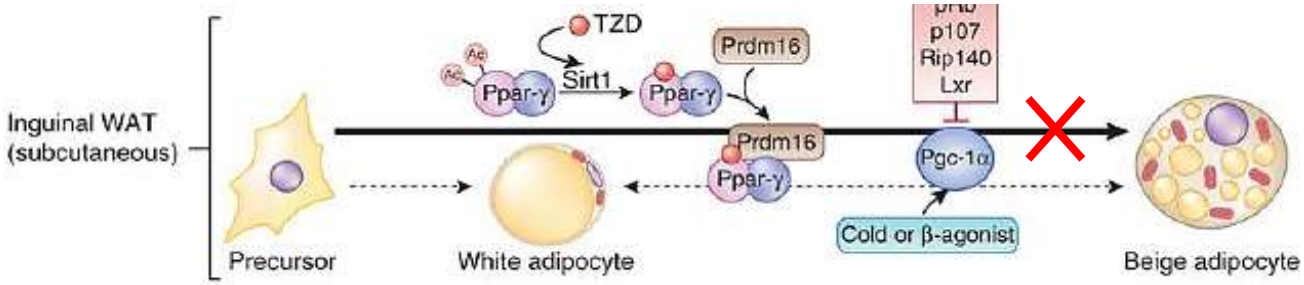
Foetal transcriptional, translational, and metabolic reprogramming are all interconnected through ATF4 and C/EBPβ, and in particular adipocyte differentiation and patterning where C/EBPβ drives epigenetic mechanisms that play an essential role in adipogenesis while leptine promotes white adipocytes terminal differentiation.



In-utero adipocytes patterning

Adipocytes origins and differentiation

In inguinal fat, β-adrenergic stimulation triggers predominantly de novo differentiation of precursor cells (large arrow) and mature white fat cells can transdifferentiate into beige cells (small dashed arrow).



PPARγ agonists promote beiging by increasing the stability of Prdm16 and through the Sirt1-dependent deacetylation of PPARγ, which recruits Prdm16 to PPARγ target genes (**Sirt1 is inhibited by elevated Hcy**).

PGC-1α is epigenetically inhibited by methyl donor deficiency and high Hcy, blocking beige adipocyte development.

Two distinct subtypes of preadipocytes have been characterized in human fat (Myf5+ and Myf5-), the Myf5+ lineage being also a skeletal muscle precursor.

Akt-mediated PTEN deactivation (mTORC1 activation) → IR1β signalling dominant → Myf5+ adipocytes lineage expansion → selective expansion of fats exclusively derived from Myf5+ precursors.

- The constitutive presence of high leptine (placental origin) largely favours subcutaneous depot patterning at the expense of visceral patterning (subcutaneous hypertrophy with remodelling).

PTEN-deficient-like skewing of adipocytes patterning has very significant by-stander effects

- Differentiation & hypertrophy without preceding division (Akt-mediated inhibition), followed by
- Myf5+ progenitor pool exhaustion, impacting skeletal muscle development potential.

thereby precipitating *in utero* insulin resistance.

Markers of in-utero adipocytes patterning
(abortuses/stillbirths)

cell surface markers of adipose cell types:

- Amino acid transporter SLC7A10 (*Asc-1*)***:*** *white adipocyte-specific cell surface protein****;***

- Amino acid transporter SLC36A2 *(PAT2)* ***and purinergic receptor P2RX5 :*** *cell surface markers in classical brown and beige adipocytes.*

Gene expression markers *distinguishing brown, beige and white adipocytes :*

- ***leptin, Hoxc8 and Hoxc9*** *for white fat****;***
- ***Tbx1 and Tmem26*** *for beige fat****; and***
- ***UCP-1, CIDEA and Prdm16*** *for brown fat (Prdm16 is also expressed in non-fat tissues).*

The situation on either sides of the placenta

Pregnancy

Maternal side

Placental nutrients supply:

TG↑↑, Chol↑↑ CarbHyd↑↑ Hcy↑

NE αAA ≈* , EαAA +/-↓**

B12↓, THF↓, HISS↓

The situation during infancy

Infancy **Post-Partum**

CarbHyd ↑, TG ↑, Proteins ↓
NE αAA ≈ , EαAA +/-
B12 ↓, THF ↓, HISS ↓

Female infants will experience insulin resistance but will largely escape the testosterone-induced exacerbation into overt clinical DM

The overall scenario

Mother's diet:
Low Protein
+
Low vitamines
(B12; C; etc.)

Mother's bood:
Low essential amino acids
+
High homocycteine

Fœtus growth conditions:
Poor: High homocycteine
+
High lipids
+
High carbohydrates
+
Limiting essential amino acids

Infant:
Develops in normal environment.

Adolescent:
Testosterone becomes constitutively present

Fœtus adapts to poor conditions:
Small size
+
Changes in
fat tissue patterning
(High subcutaneous
+
Very low visceral)

Implementing a solution

To be prevented, intergenerational adiposity, diabetes and other related conditions requires drastic changes in dietary behaviour very early in pregnancy.

However, dietary behaviours, including meals frequency and timing, are heavily influenced by geography, religions, traditions, seasons, cultural specificities, economic burden and psychosocial beliefs.

These qualitative factors in turn affect physiological characteristics as well as potential means of intervention.

A single globally applicable agronomic solution cannot exist.
Any such solution will have to be adapted to the locally relevant key qualitative variables.

A Federal research program under the direction of Prof. S. Galande, head of the National Centre of Excellence in Epigenetics at the IISER in Pune, aiming to test the model briefly presented here and involving a campaign of populations-wide samples collection concurrently with the locally relevant key qualitative variables, under the supervision of Dr. P. Katre, is currently underway.

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Questions