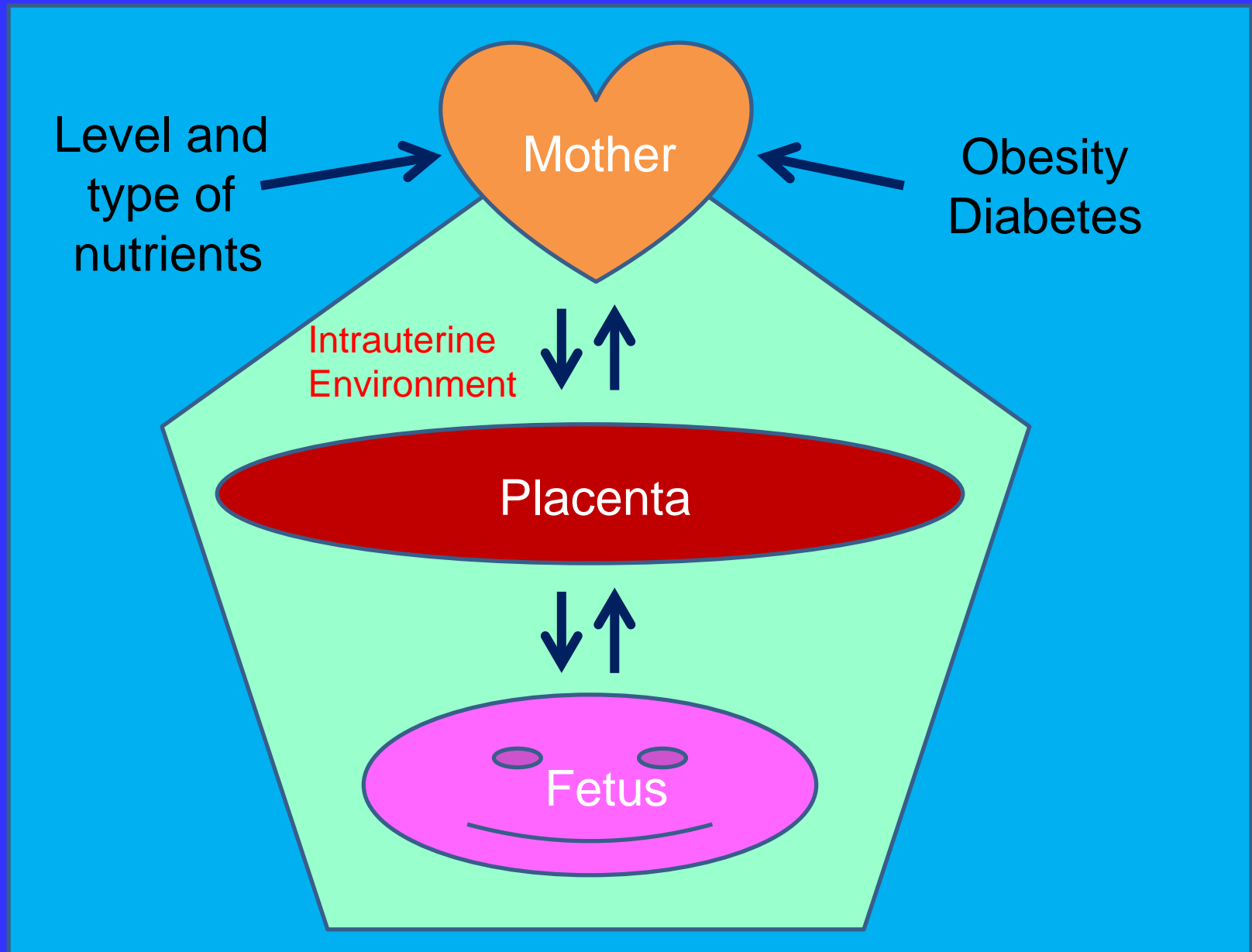


Role of the Placenta in Delivering Nutrients and in Developmental Programming

Leslie Myatt PhD FRCOG
Dept of Obstetrics and Gynecology
Oregon Health & Science University



Maternal-Placental-Fetal Unit



Developmental (Fetal) Programming

(Barker Hypothesis)

(Fetal Origins of Adult Disease)

(Developmental Origins of Health and Disease [DOHaD])

- Life in utero determines risk of development of disease in adult life

Cardiovascular

Diabetes (Insulin resistance/Metabolic syndrome)

Obesity

Stroke

Osteoporosis

Obstructive Airway Disease

Cancer

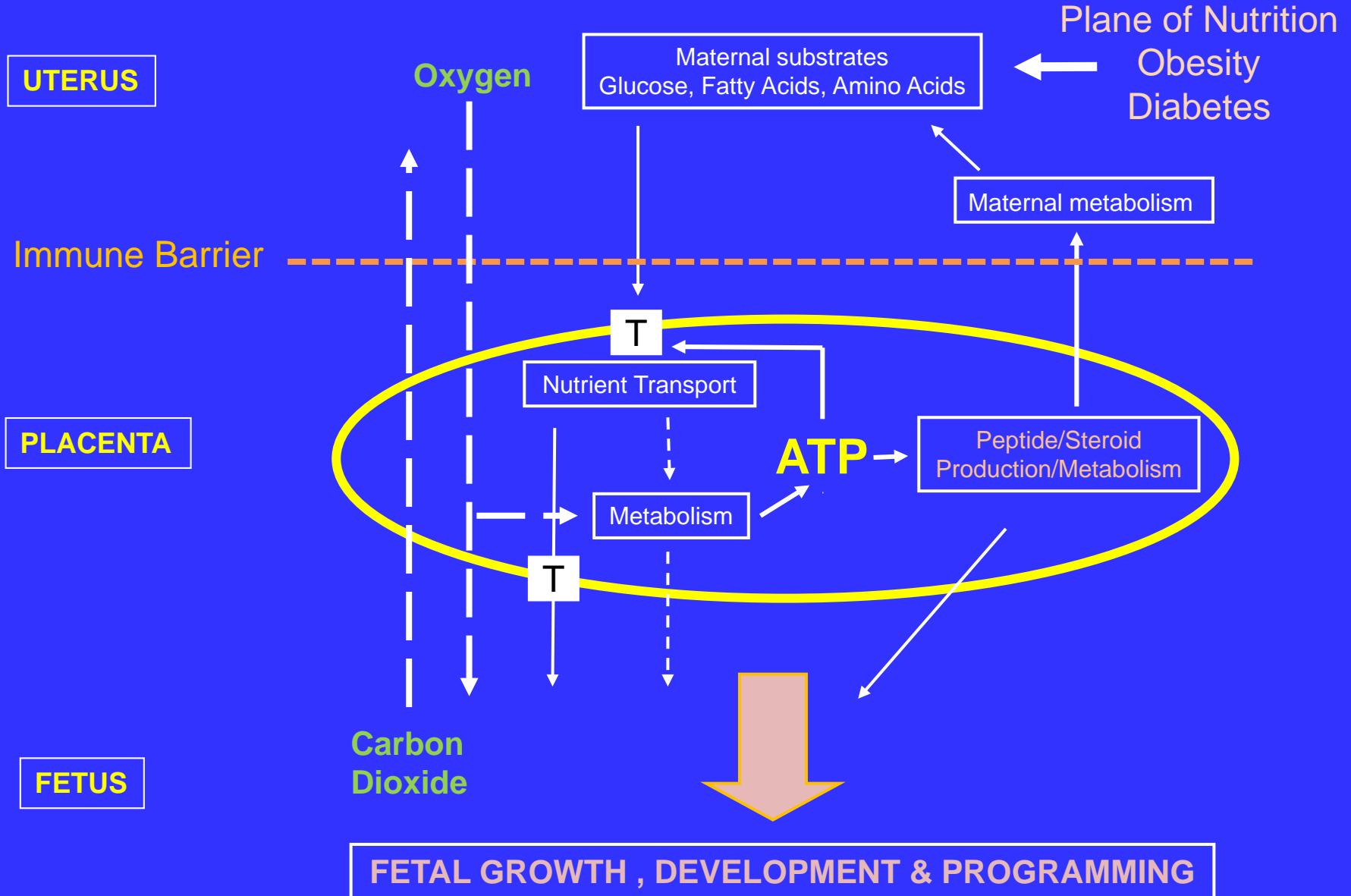
Disordered HPA axis

Behavioral abnormalities

- Sexual dimorphism in effect
- Epigenetic mechanisms

Histone modification, DNA methylation

Roles of the Placenta



Placenta – Not Just a Conduit

- In human placenta consumes 50% of oxygen and 30% of glucose supplied to uterus
- Metabolic activity of placenta 4-6-fold higher per unit weight than fetus
- One third of placental oxygen consumption used for de novo generation of peptides, one third to maintain cation gradient across membrane for transport
- Therefore the placenta is not simply a conduit. It is a selfish organ - regulates nutrient composition and supply from mother to fetus

Mechanisms of Transport

- Flow mediated diffusion
 - oxygen, ions, (fatty acids), mol wt <1,000
- Active transport
 - amino acids
- Facilitated transport
 - glucose, fatty acids
- Endocytosis-exocytosis
 - insulin, IgG

How does the Placenta Mediate the Effect of Nutrition on Health and Disease

- Regulation of type and amount of nutrients transferred
 - Regulates maternal metabolism (Supply)
 - Regulates fetal growth and development (Demand)
 - Expression of type and quantity of transporters
 - Buffering/storage of nutrients
 - Alteration in placental metabolism that consumes/produces nutrients and alters/limits transfer to the fetus
- Adaptive responses to altered supply
 - Increased/decreased transport of nutrients, altered production, metabolism or storage
 - Altered regulatory signals to mother and fetus
 - Epigenetic responses

Sexual Dimorphism in Fetal Outcomes

- Different evolutionary strategies for males and females.
- Male fetuses appear to keep growing, are larger but have more adverse outcomes:
 - preterm birth, PPRROM, placenta previa, preeclampsia, lagging lung development, macrosomia, late stillbirths, poorer maternal B cell function and increased risk of GDM.
 - “Boys live dangerously in the womb” (*Eriksson et al 2010*)
- Females adapt growth rate to optimize survival in a poor environment
- Differences in fetal programming of metabolic syndrome based on sex of fetus.

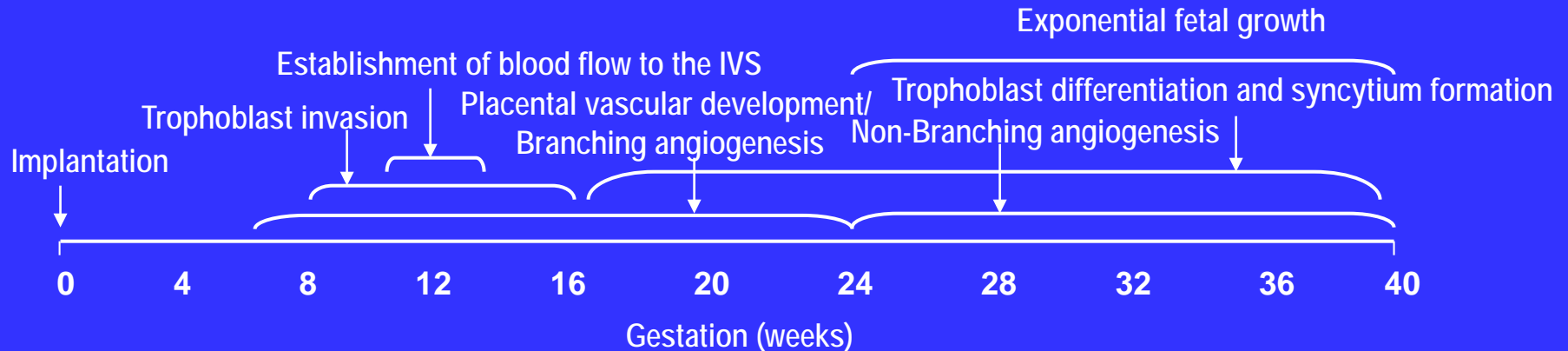
Evidence for Sexual Dimorphism in Placental Function

- Differences in gene expression, 1st trimester and term, linked to escape from X chromosome inactivation
- Inflammatory, hypoxia, apoptosis and autophagy responses
- Expression of antioxidant defense enzymes
- Fatty acid transporters
- Fatty acid oxidation
- Response to maternal adiposity and inflammatory status
- microRNA expression in normal pregnancy
- Steroid synthesis
- All linked to difference in outcomes male vs female

Placental Growth and Development Throughout Gestation

	<u>6 weeks</u>	<u>Term</u>
Placental Weight (g)	6.0	470
Fetal Weight (g)	1.1	3500
Fetal/Placental Weight Ratio	0.18	7.23
Villous volume occupied by vessels (%)	2.7	28.4
Trophoblast Surface area (m ²)	0.08	12.5
Mean Trophoblast Thickness (μm)	18.9	4.1
Maternofetal Diffusion Distance (μm)	55.9	4.8

Critical periods during placental development



Placental development can be affected by type, severity, timing and duration of a challenge – clearly seen in animal models [structure/function].

An insult e.g. nutritional, applied at a specific time will have a specific effect on placental development /function.

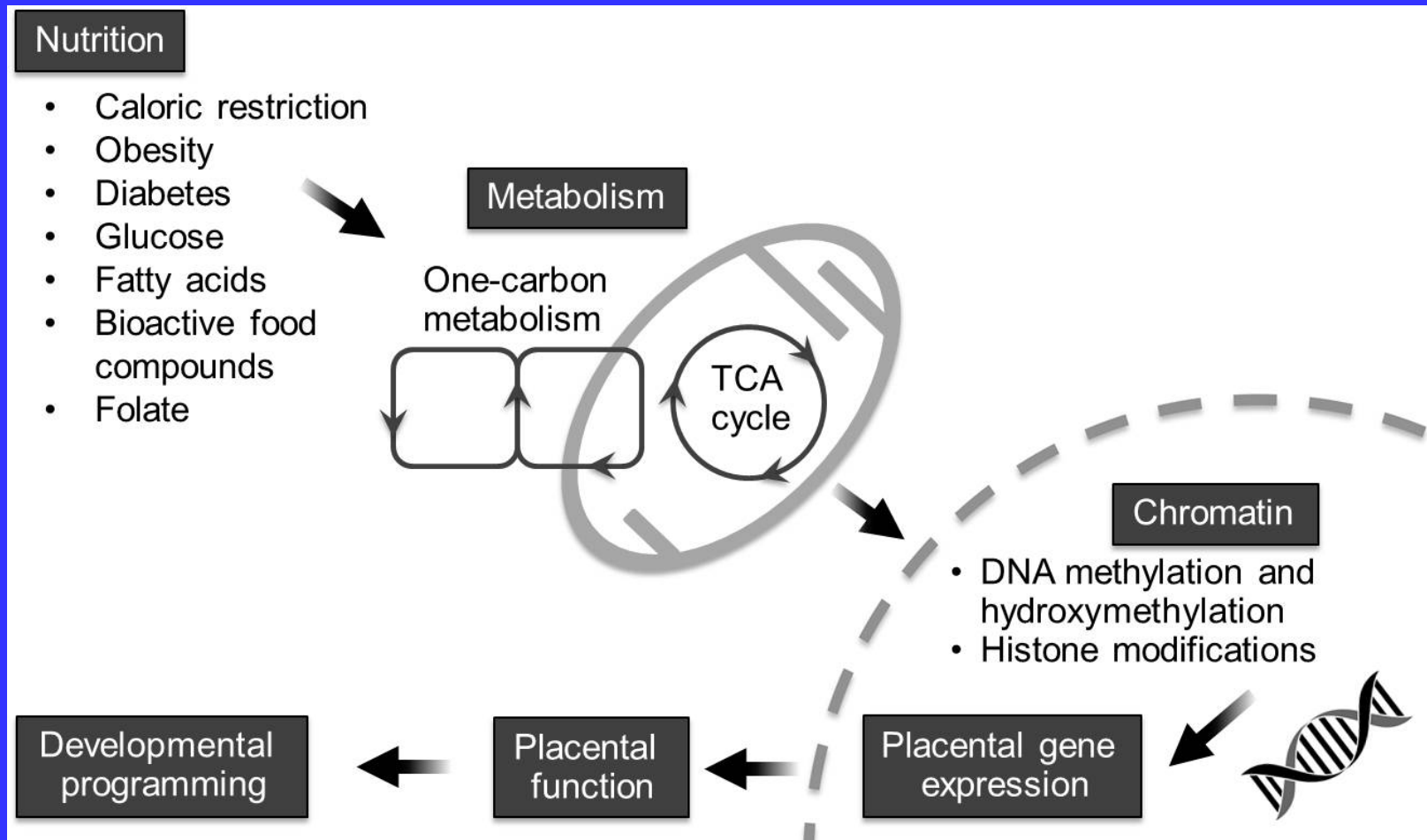
The same insult at a different times may have different effect

E.g. IDDM plus LGA gives increased Glut 1 in BM and increased system A aa transporter, whereas GDM plus LGA no change in Glut 1 in BM but increased system A

Influence of Nutrition or the Metabolic Environment on Epigenetic Modifications

- Epigenome responds to changes in nutrients including methyl donors, folate supplementation, fat, glucose and caloric restriction
- Differences in DNA methylation reported in individuals exposed to the Dutch Hunger Winter
- Variations in DNA methylation associated with many aspects of diabetes mellitus and metabolic/inflammatory milieu of obesity

Links between Nutrition and Epigenetic Changes



Nutrients that affect Epigenetic Modification

Folate

Vitamin B12

Methionine

Choline

Betaine

Biotin

Niacin

Pantothenic acid

Resveratrol

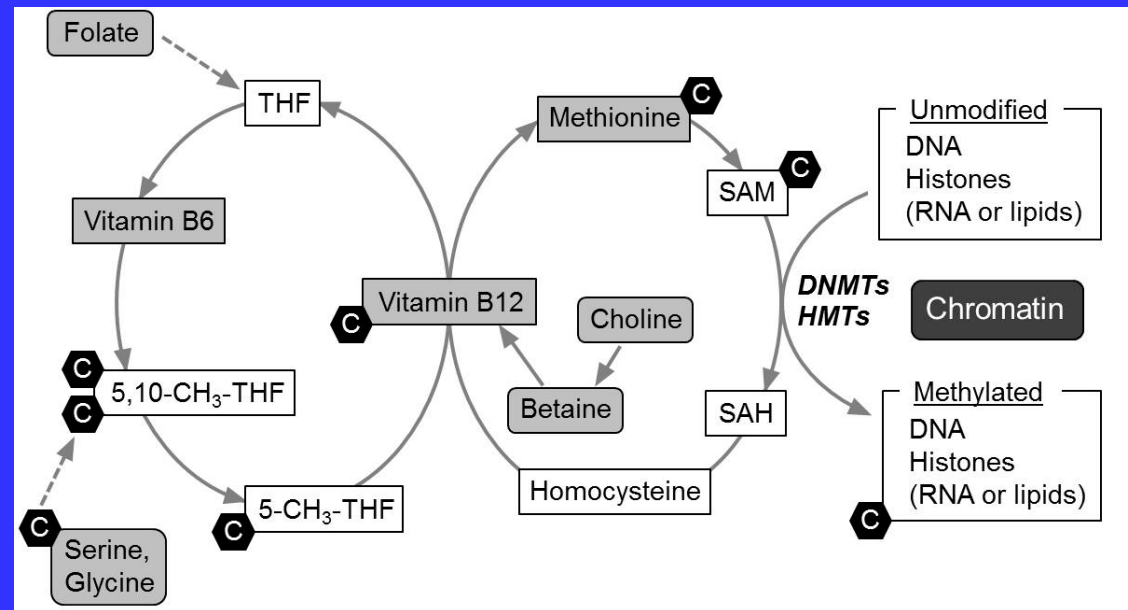
Butyrate

Curcumin

Genestein

Polyphenols

Tea catechin



Effect of Obesity on Placental DNA Methylation

Methylated regions identified by
NimbleGen 2.1 M arrays (NimbleScan)

Number of methylated regions (peak score>3)	5mC	
	Normal	Obese
All tiled regions	12, 319	14,233
TSS1500 (1500 bp upstream to 500 bp downstream of TSS)	3,187	3,844
TSS100 (100 bp upstream - 100 bp downstream of TSS)	1,459	1,676
CpG islands	3,294	3,375
CpG island shores (2 kb flanking CpG islands)	3,127	3,774
CpG island shelves (2kb flanking shores)	1,502	1,764
Gene body	8,560	9,607
microRNA (-15 kb to +1 kb)	429	492

Oxidative Stress In Pregnancy

- Antioxidants protect cells from oxidative stress which causes cellular damage of DNA, lipids and protein
- Normal pregnancy is a state of increased oxidative stress which is increased further in pathologic pregnancies
- The placenta is a source of oxidative stress due to its high metabolic activity with mitochondria being a major source
- The inflammatory conditions of obesity and gestational diabetes heighten oxidative stress and deplete antioxidant defenses often in a sexually dimorphic manner (Evans and Myatt 2017)

Sources of Antioxidants

Nutritional and Supplemental:

- Vitamin C, Vitamin E, Resveratrol, N acetylcysteine (NAC),
Omega 3 fatty acids, vegetables, selenium

Intracellular Reducing Elements:

- Glutathione (NAC, glutamine and glycine)

Extracellular antioxidants:

- Transferrin
- Ceruloplasmin, Uric acid, Bilirubin

Enzymes:

- Superoxide Dismutase (SOD)
- Glutathione Peroxidase (GPx – selenocysteine)
- Thioredoxin-Thioredoxin reductase
- Catalase

Selenium

- In humans, selenium is a trace element nutrient that functions as a cofactor for reduction of antioxidant enzymes such as glutathione peroxidases and certain forms of thioredoxin reductase
- The glutathione peroxidase family of enzymes (GSH-Px) catalyze reactions that remove reactive oxygen species such as hydrogen peroxide and organic hydroperoxides:
$$2 \text{ GSH} + \text{H}_2\text{O}_2 \xrightarrow{\text{GSH-Px}} \text{GSSG} + 2 \text{H}_2\text{O}$$
- Selenium is a component of the unusual amino acids selenocysteine and selenomethionine.
- Selenium deficiency in soil is associated with increased incidence of preeclampsia and dietary supplementation is being studied

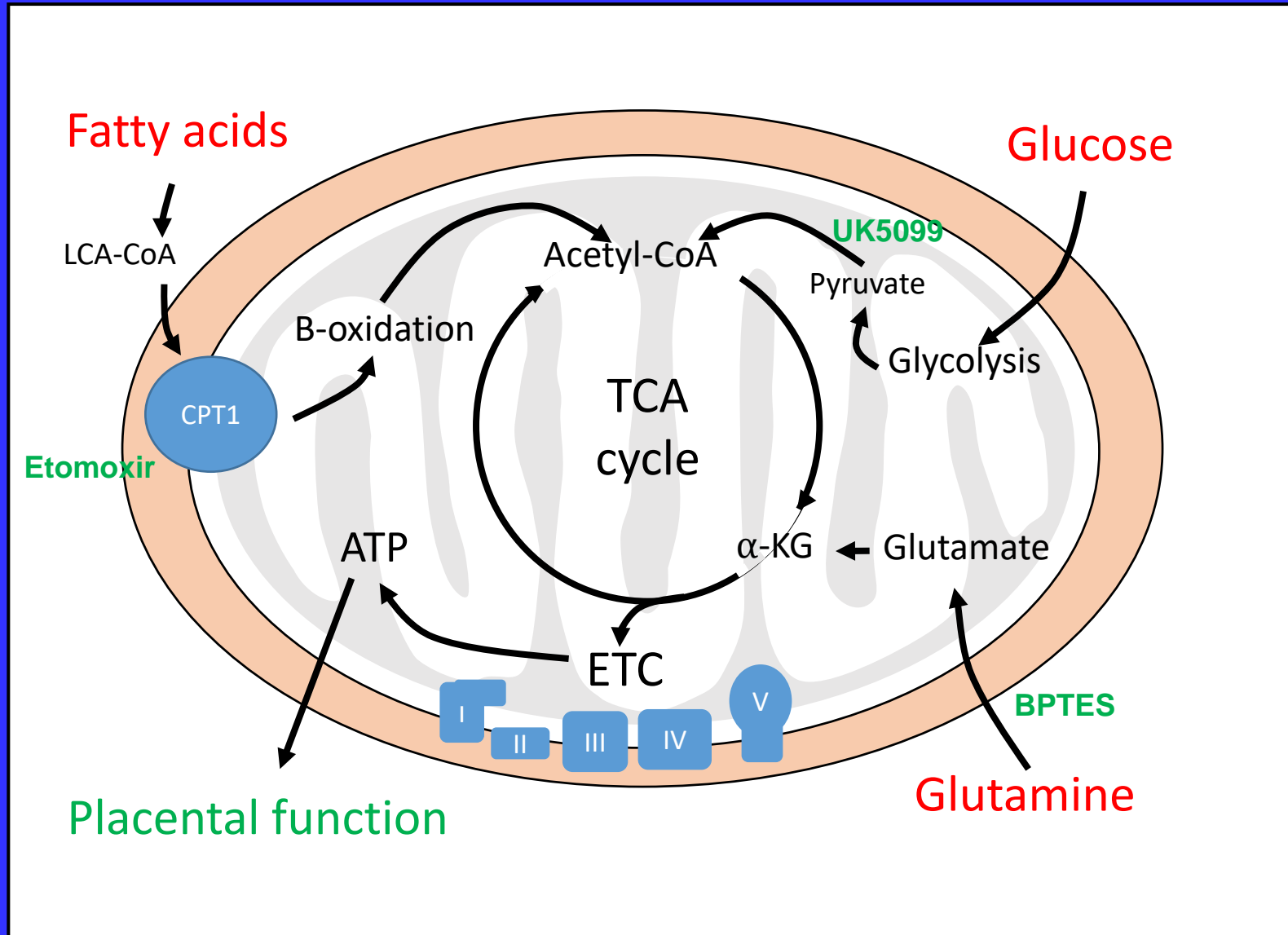
Maternal Metabolic Milieu with Obesity and GDM

- Insulin resistance
- Maternal hyperglycemia, hyperlipidemia
- Inflammation
- Oxidative stress
- Associated with adverse outcomes including stillbirth
- Both program the offspring for disease in later life
- Sexually dimorphic responses

- Increasing maternal adiposity associated with decreased placental mitochondrial respiration and further exacerbated with gestational diabetes.

(Mele et al 2014, Muralimanoharan et al 2016)

Placental Fuel Substrates

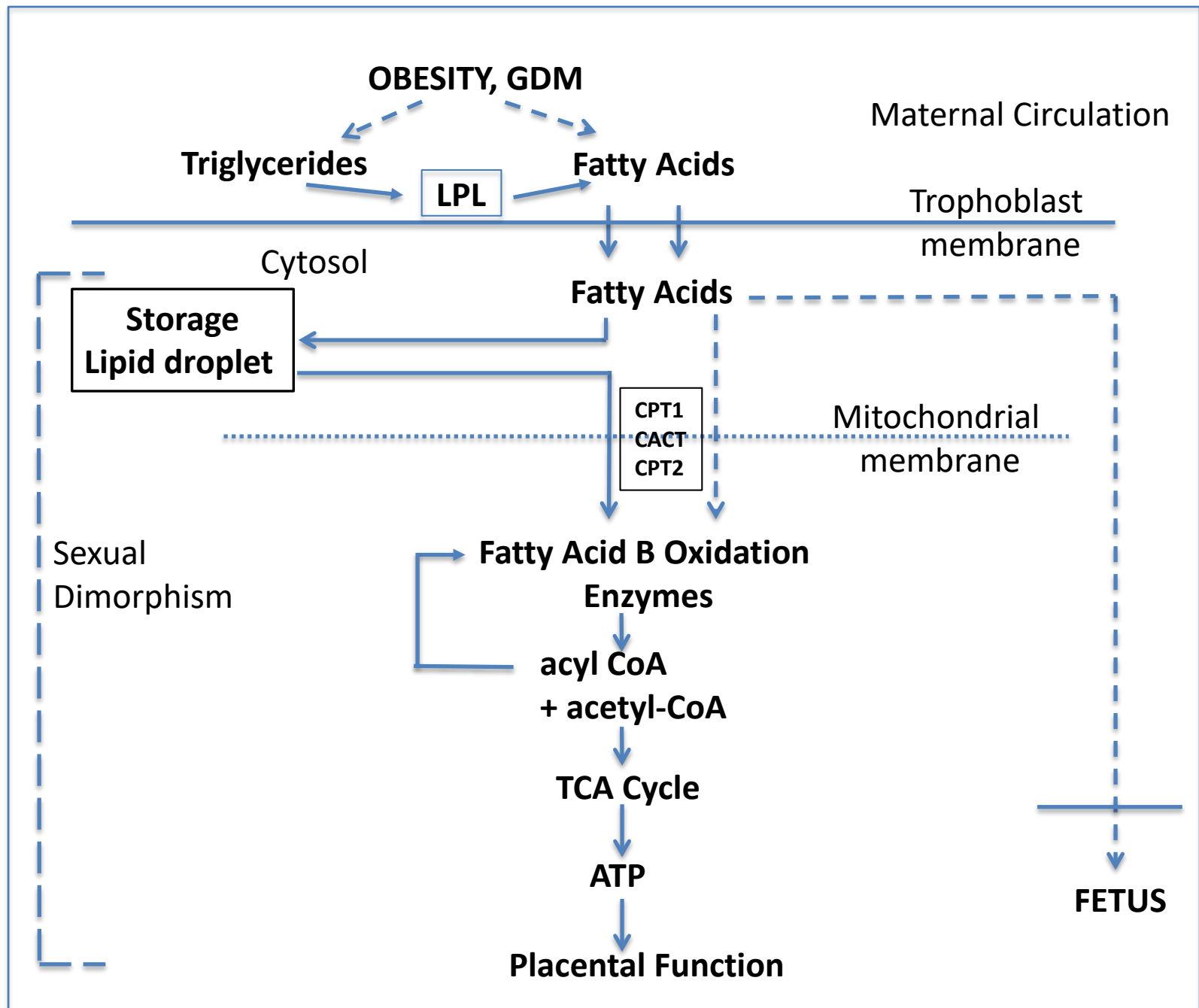


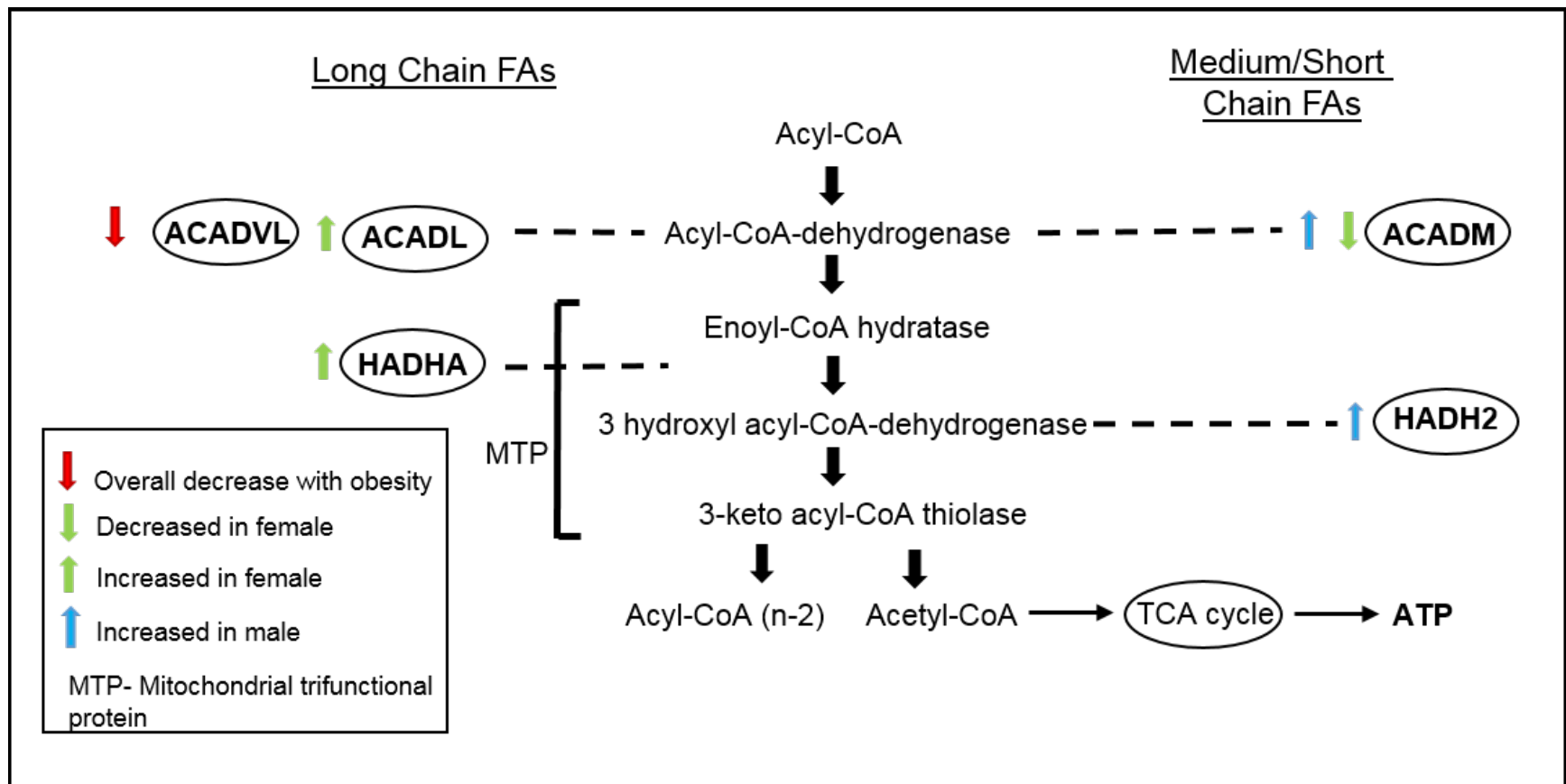
Effect of Obesity and GDM on Fuel Usage by Trophoblast

- In lean women there was no difference in dependency for these three fuels between male and female trophoblast.
- With hyperglycemia and hyperlipidemia of obesity and A2GDM, we find increased dependency on glucose and fatty acids for baseline respiration but only in male placenta.
 - Accompanied by significantly decreased flexibility for both glucose and fatty acids, but also glutamine, i.e. male trophoblast cannot adapt by increasing oxidation of other fuels.
- This may contribute to the increased risk of male for adverse outcomes
- Effect is not due to obesity alone but may reflect the continuum of worsening hyperglycemia and hyperlipidemia from obesity to A2GDM.
- Changes in placental metabolism may affect amount of each substrate available for transfer to fetus and hence fetal growth and development

Fatty Acids and Brain Growth

- Docosahexaenoic acid (DHA, C22:6(n-3)) and arachidonic acid (AA, C20:4(n-6)) are essential brain specific fatty acids (BSFA) important for mammalian CNS development
- Brain growth increases dramatically in the 3rd trimester and post-partum with significant increases in DHA and AA
- The effect of BSFA supplementation in pregnancy on brain size was determined by MRI (n=86, double blind placebo controlled) [Ogundipe et al 2018]
- Males born to the BSFA supplemented group had significantly larger total brain volume, total gray matter, corpus callosum and cortical volumes when compared to placebo group.





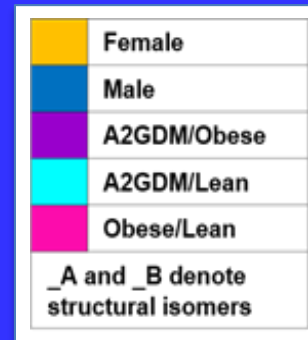
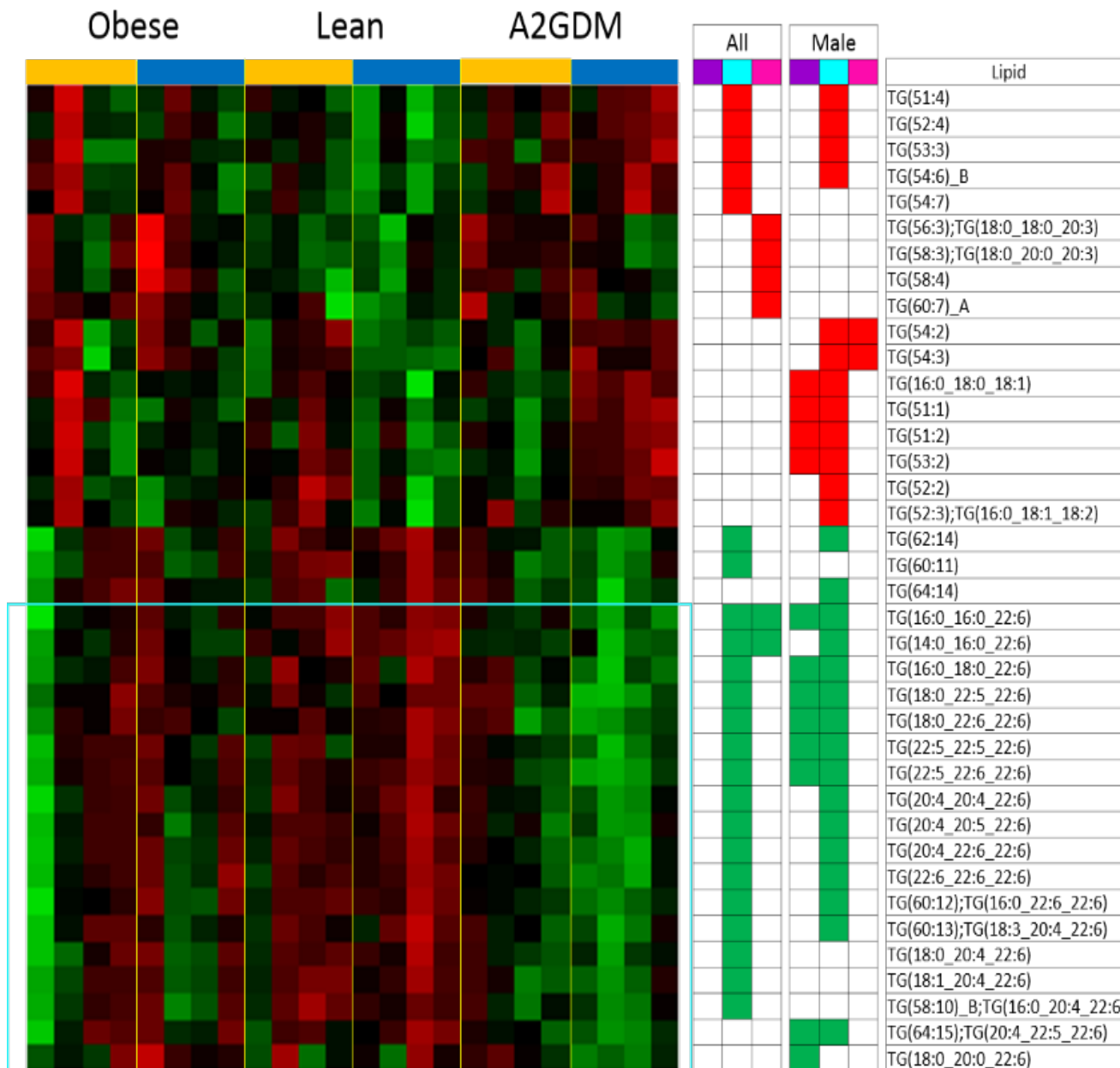
Changes in placental mitochondrial fatty acid β oxidation (FAO) with fetal sex and maternal adiposity.

Changes in protein expression with maternal adiposity or fetal sex are shown.

Enzymes in the β oxidation pathway have preference for either long chain (ACADVL, ACADL, HADHA) or medium/short chain (ACADM, HADH2) fatty acids.

Sexual Dimorphism in Placental Fatty Acid Oxidation

- Sexually dimorphic expression of enzymes involved in fatty acid β oxidation in whole placental tissue.
- With obesity in the male there is increased expression of enzymes preferring the highly available energy substrate medium chain FAs whereas the female placenta increases enzymes preferring long chain FAs.
- This may be an adaptive response to the differing FAs available with obesity
- Or may be an attempt by the male placenta to more easily produce energy from medium chain FA which then alters fatty acid composition and levels available for the fetus for growth and development.



Variable changes in triacylglycerol (TG) were found in male placental villous tissue of A2GDM. This included a significant decrease in TG species containing docosahexaenoic acid fatty acid chains (22:6(n-3))

Amniotic Fluid

↑ MCFA

↑ LC and LC-PUFA

↑ Male, ↑ Female

Placenta

Fatty Acids

↑ MCFA

↑ LC and LC-PUFA

FAO Enzymes

↑ ACADM, HADH2

↑ ACADL, HADHA (LCFA)

Sexual dimorphism in effect of GDM on fatty acid and β oxidation enzymes in placenta and medium chain (MC) and long chain (LC) fatty acids found in amniotic fluid (O'Neill 2018).

Take Home Message(s)

- The placenta directs maternal metabolism to promote fetal growth and development
- The placenta responds to alterations in nutrient supply to ensure fetal survival
- These responses may developmentally program the fetus via epigenetic mechanisms
- At delivery the placenta can be used as a diary of fetal exposures
- There is a sexual dimorphism of function and effect
- Interventional/supplementation studies need to consider sexual dimorphism and effect on adaptation/dysfunction

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