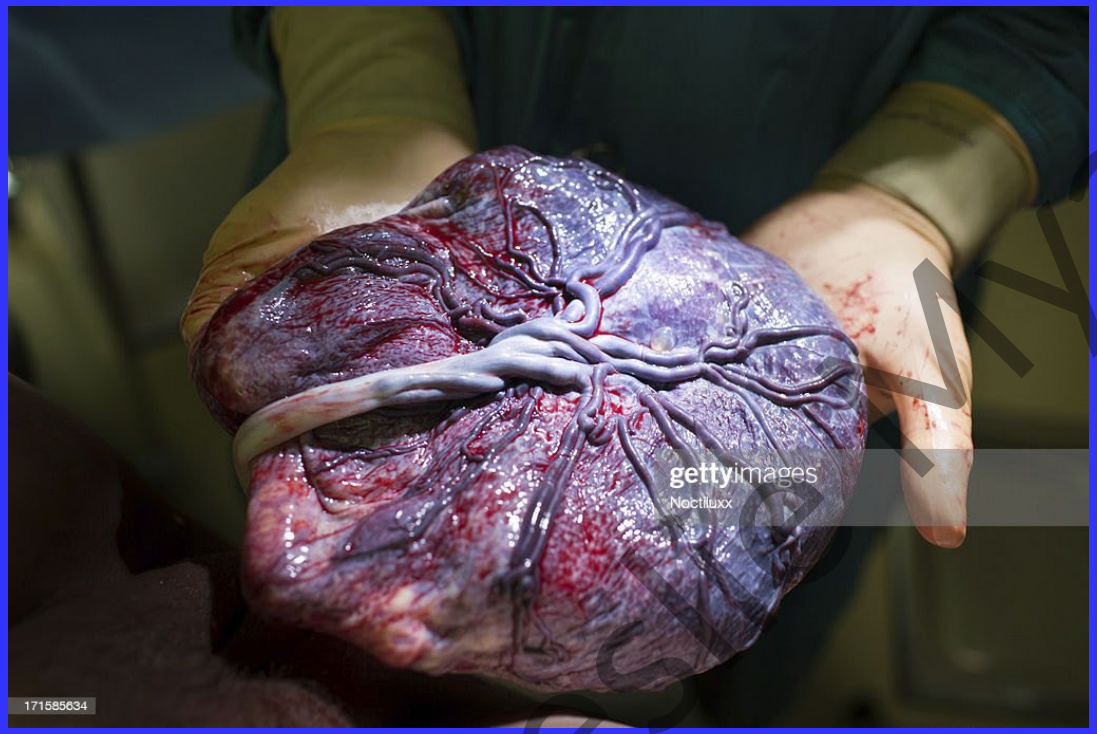
The Placenta: More than a Conduit



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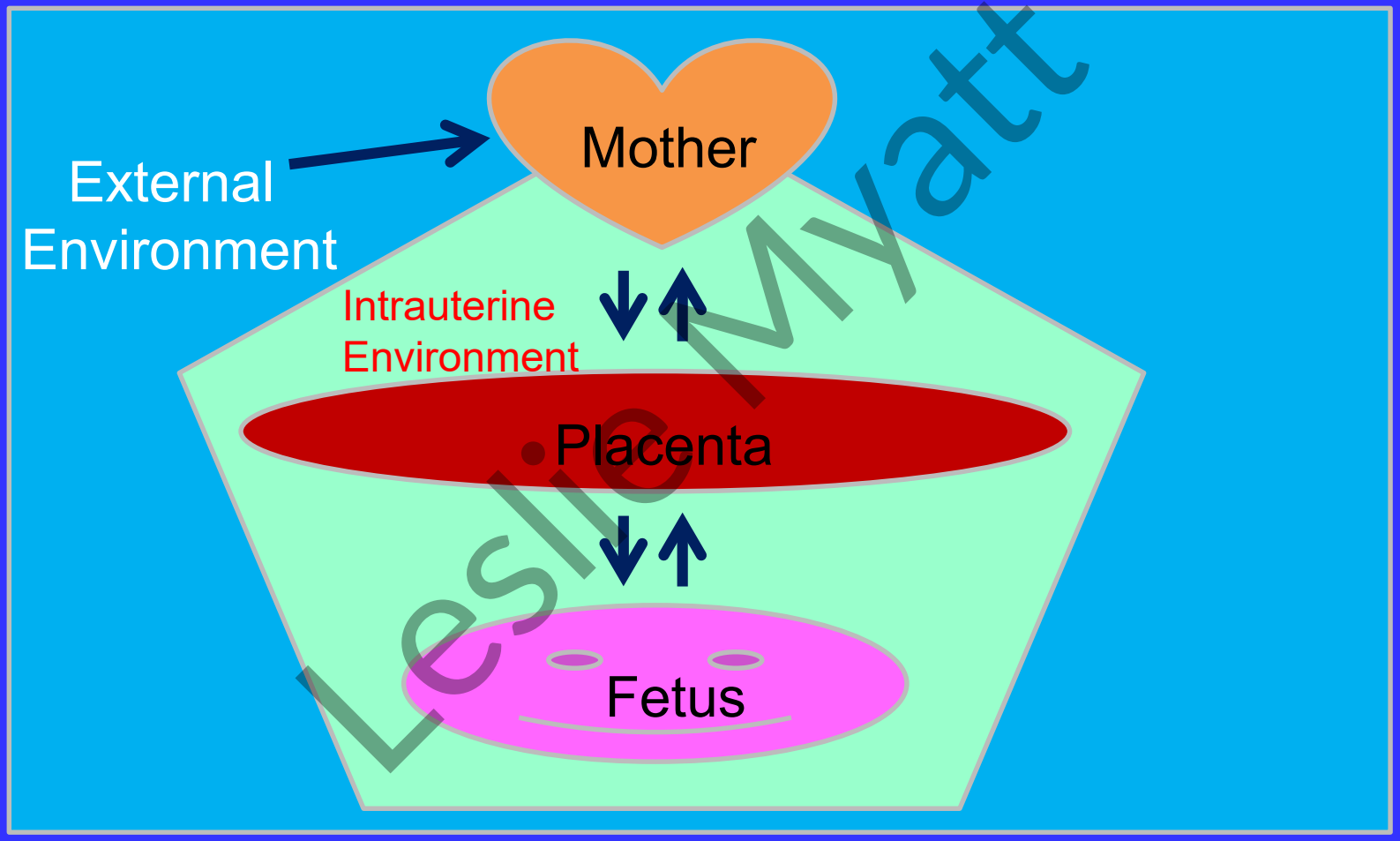
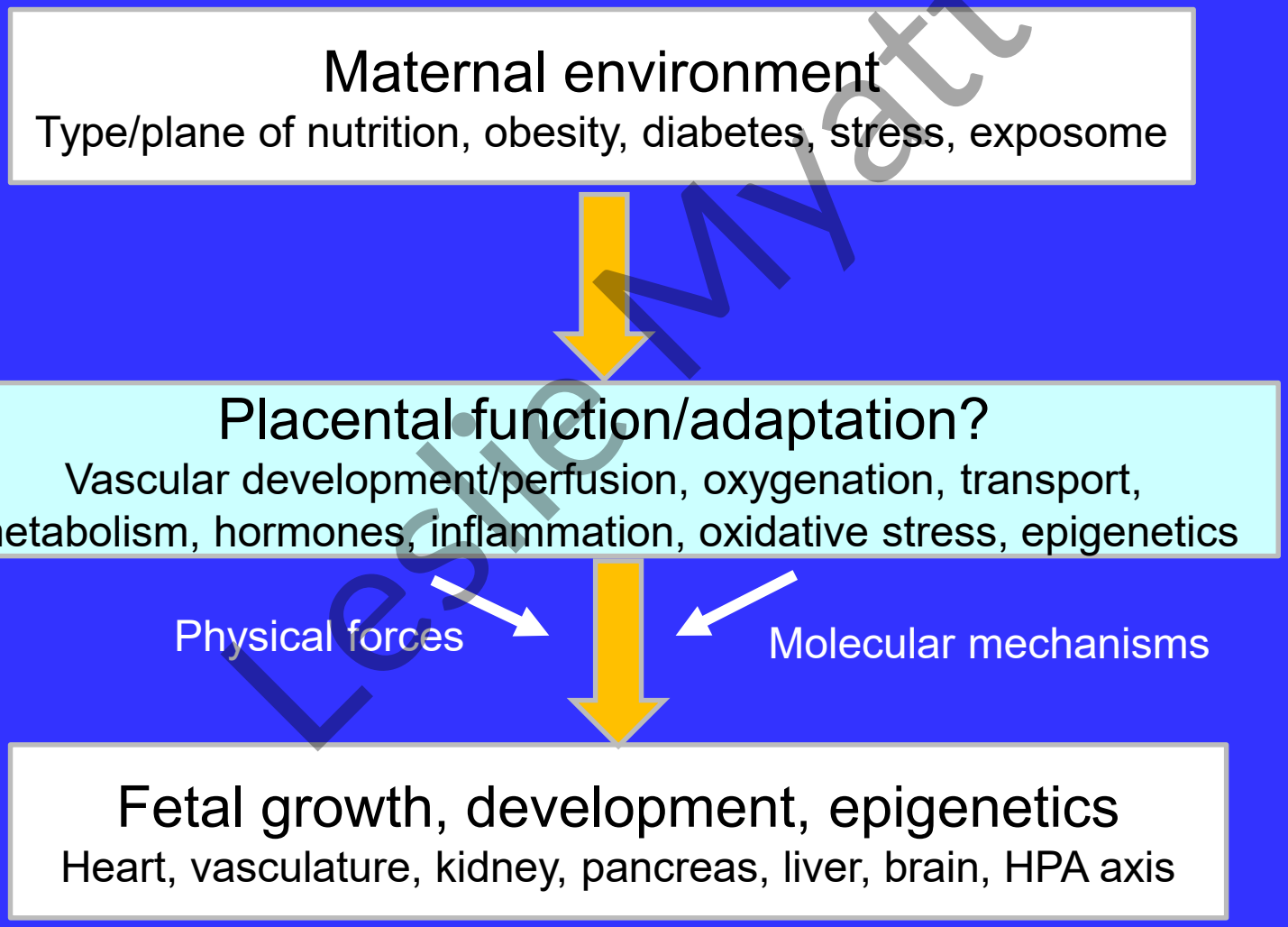
The Placenta is the Center of the Perinatal Universe



Role of the placenta in fetal programming



Placental function and metabolism

- Second only to brain in number of transcripts expressed
- Not simply a conduit, but regulates nutrient composition and supply from mother to fetus in several ways
- Source of hormonal signals regulating maternal metabolism and hence type and amount of substrate available to it
- Takes up, transfers, stores substrates to buffer transfer
- Placental metabolic activity 6x higher/unit weight than fetus
- Consumes substrates to provide energy for anabolic activity

  e.g. sheep placenta consumes 50% of oxygen, 75% of glucose supplied to uterus, - may limit fetal supply
- 1/3 of placental O_2 consumption for generation of peptides, 1/3 to maintain membrane cation gradient for transport
- Placental adaptation (metabolic flexibility) occurs to try and ensure fetal survival in adverse conditions

Sexual Dimorphism in Fetal Outcomes

- Male fetuses are larger but have more adverse outcomes:
  - preterm birth, PPROM, placenta previa, preeclampsia, lagging lung development, macrosomia, late stillbirths, poorer maternal B cell function and increased risk of GDM.

- Differences in fetal programming of metabolic syndrome based on sex of fetus.

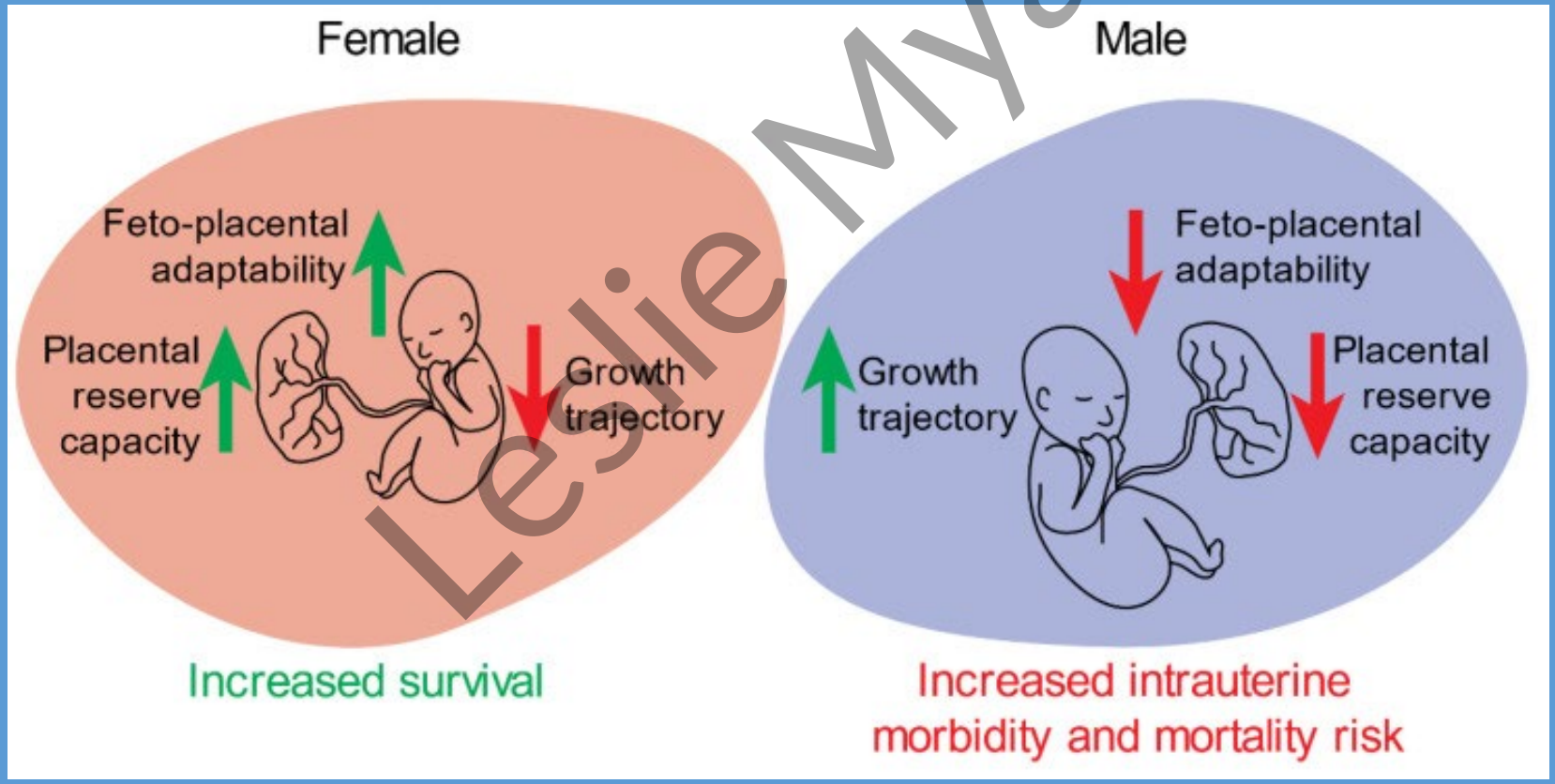
- "Boys live dangerously in the womb" *(Eriksson et al 2010)*

Q.'s What are the mechanisms?

Are boys more reckless or have girls evolved a protective mechanism?

Sex-specific in utero adaptation

- Males grow larger than females in utero but suffer more adverse outcomes as a consequence
- There is sexual dimorphism in placental function to accommodate this



Meakin AS et al 2021

Evidence for Sexual Dimorphism in Placental Function

- Differences in gene expression, 1st trimester and term
  - immune genes expressed at higher level in female placenta (JAK1, IL2RB, Clusterin, LTBP, CXCL1, IL1RL1, TNFR)
- Sexual dimorphism in placenta gene expression linked to failure of X-linked inactivation (Gong et al JCI, 2018)
- Inflammatory, hypoxia, apoptosis and autophagy responses
- Antioxidant defenses, expression of antioxidant enzymes
- Lipid uptake and metabolism
  - Fatty acid transporters
  - Fatty acid oxidation
- Response to maternal adiposity and inflammatory status
- microRNA expression in normal pregnancy
- Aromatase expression with preeclampsia

Maternal Metabolic Milieu with Obesity and GDM

- Insulin resistance, hyperglycemia, hyperlipidemia
- Inflammation, oxidative stress
- Sexually dimorphic responses

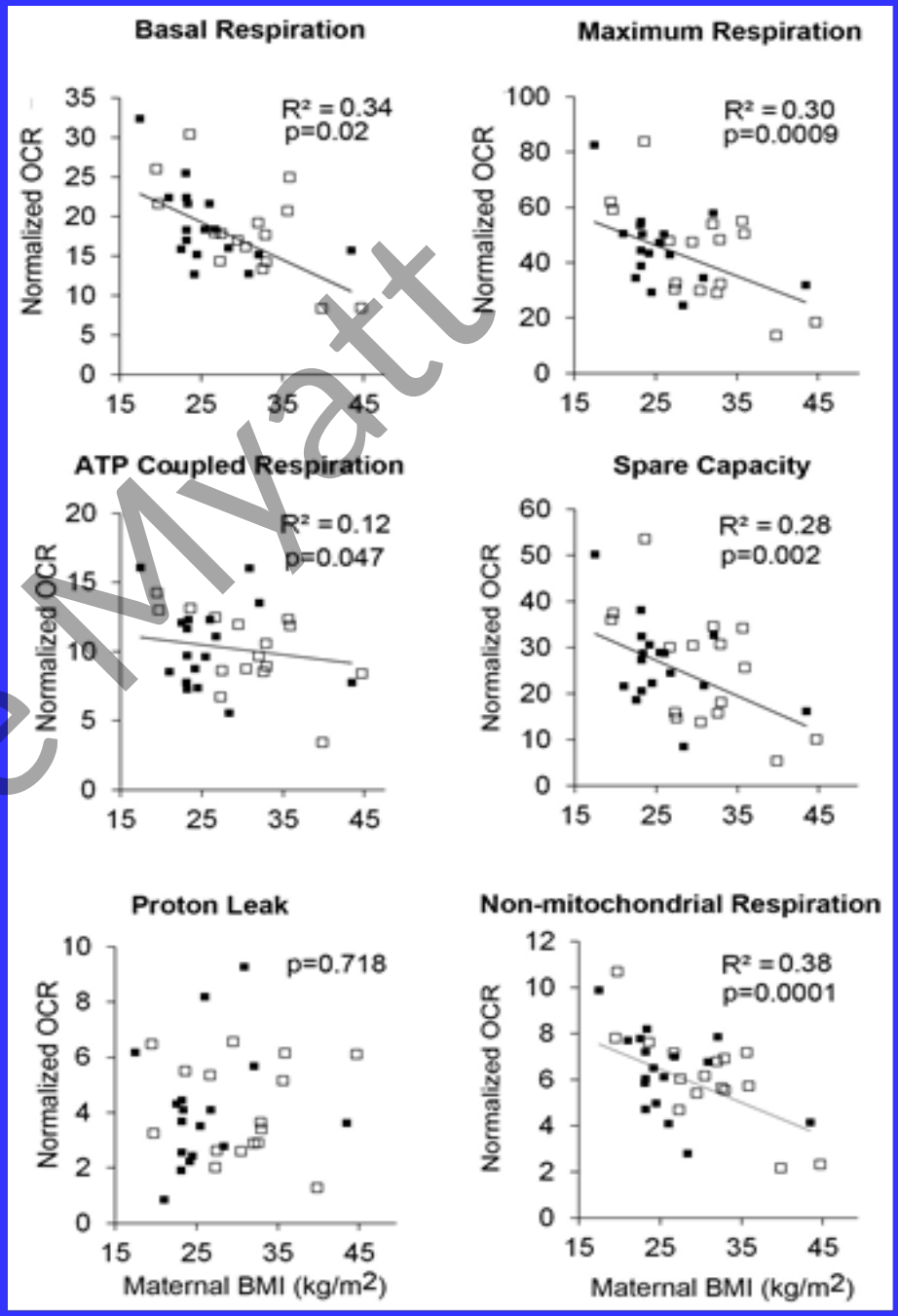
Is there metabolic reprogramming i.e. changes in cellular bioenergetics to adapt to environmental conditions?

A hallmark of cancer –Warburg effect (aerobic glycolysis), altered mitochondrial metabolism

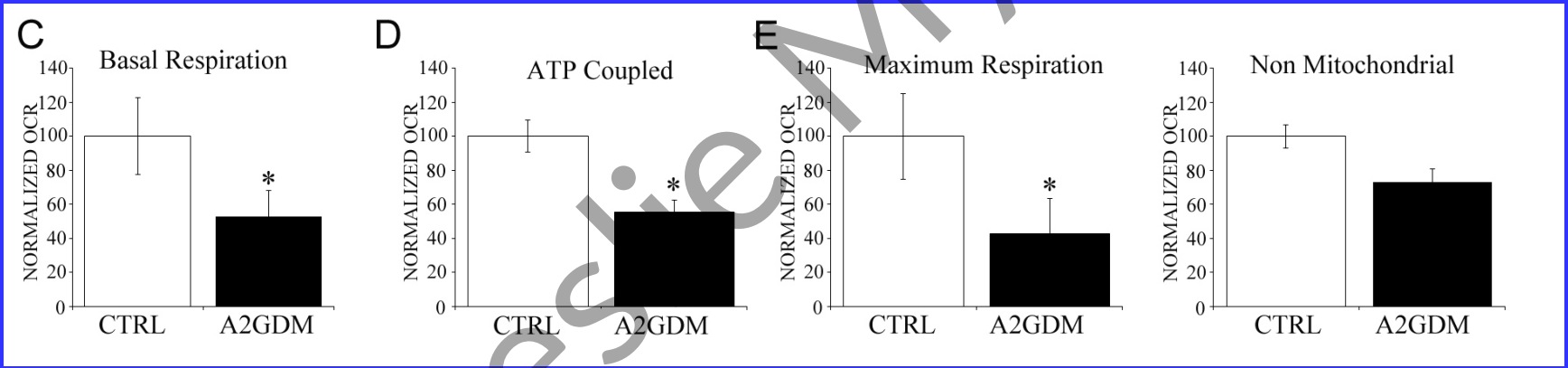
Effect of increasing maternal BMI on mitochondrial respiration

N=33 separate cultures from placentas of females (open circles) and males (closed circles).

Mele J et al 2014



Effect of Obesity and A2GDM on Mitochondrial Respiration in Trophoblast

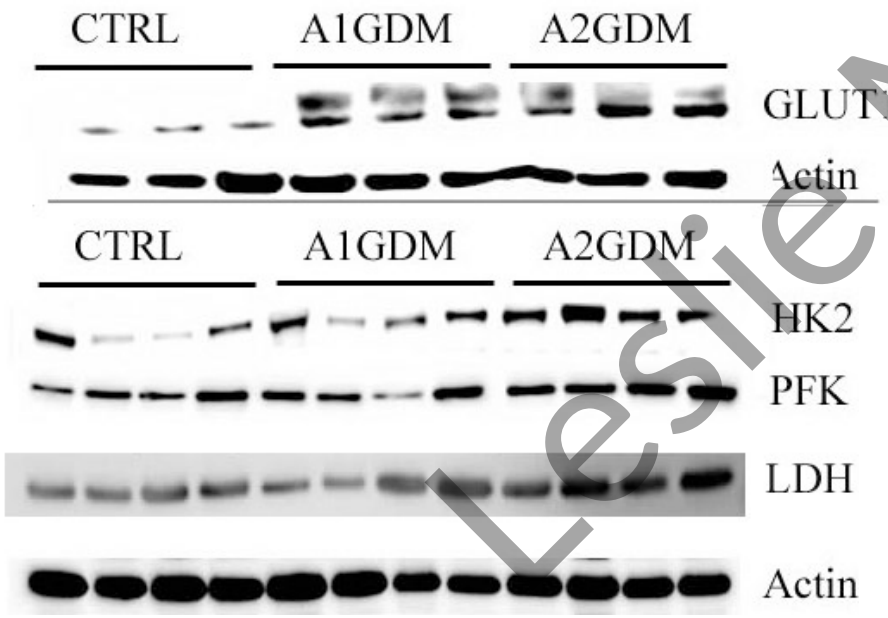


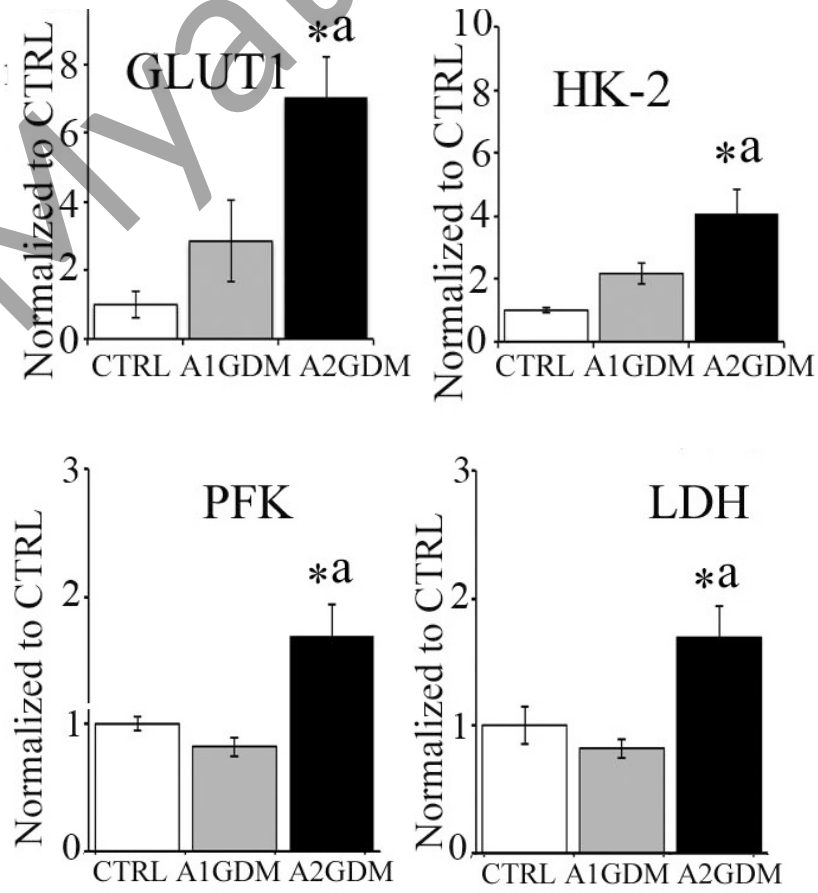
N=6, mean ± SEM

No additional effect of A1GDM vs obesity

Mele J et al 2014

Protein Expression of Markers of Glycolysis in Villous Tissue with GDM



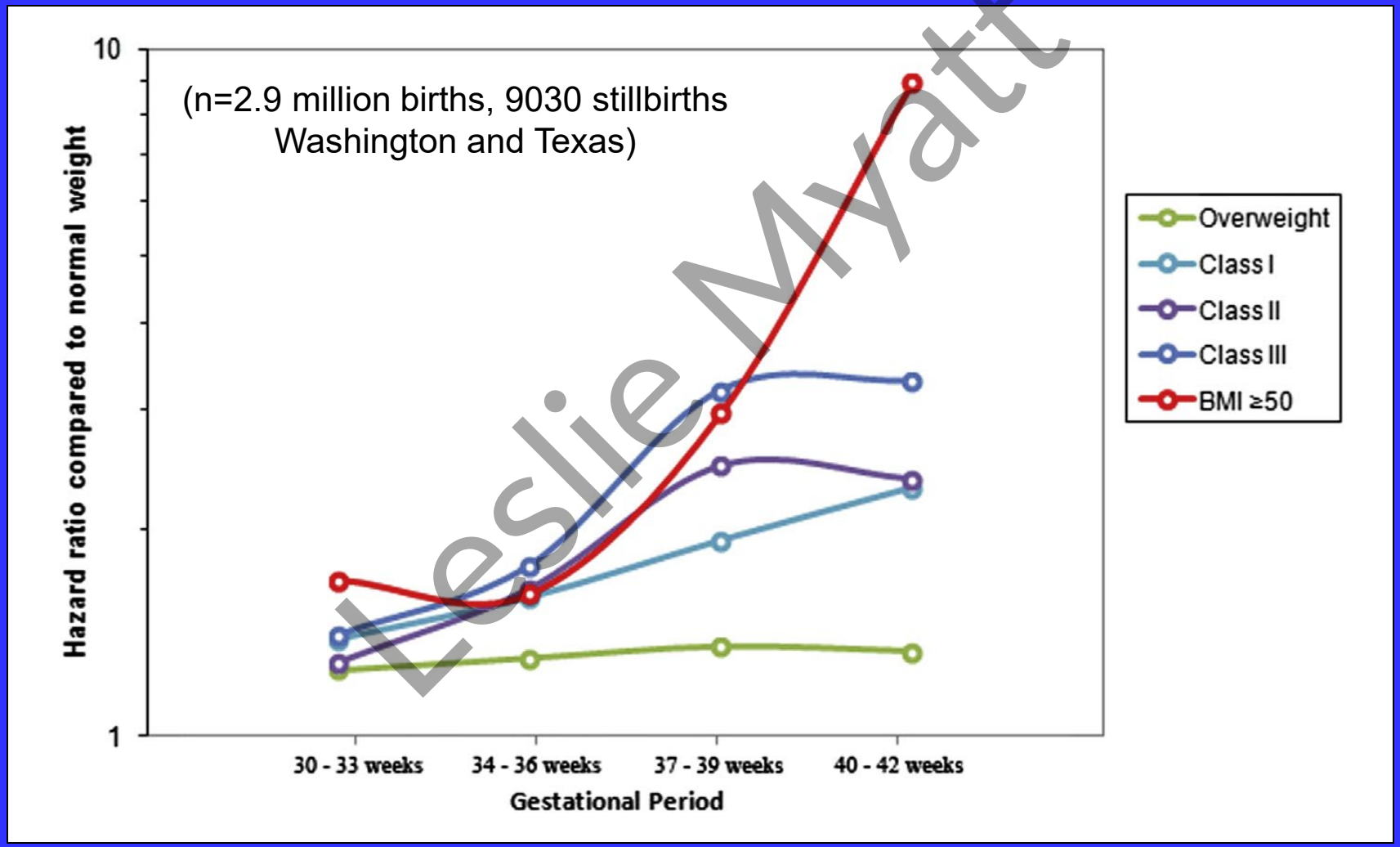


Mele J et al 2014

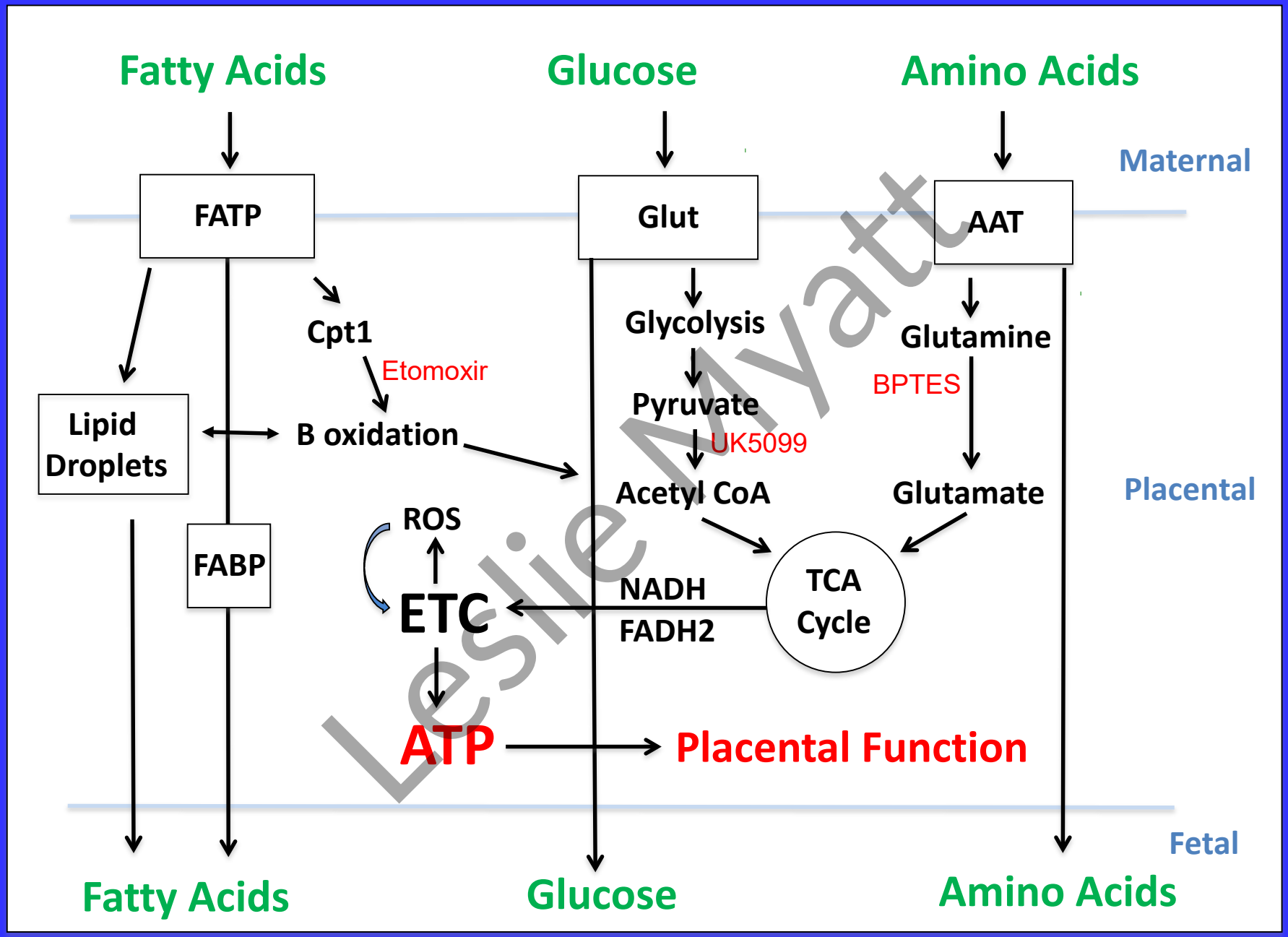
Clinical Implications

- Mitochondrial respiration compromised by increasing maternal adiposity and by A2 GDM
- But all these pregnancies went to term with good outcomes!!
- **However there may be a cost in later life for offspring and mother!**
- Does compromised mitochondrial respiration matter?
- Is there sufficient placental reserve?
- What happens if there is additional insult or reserve is exceeded?
- Could this be associated with stillbirth??

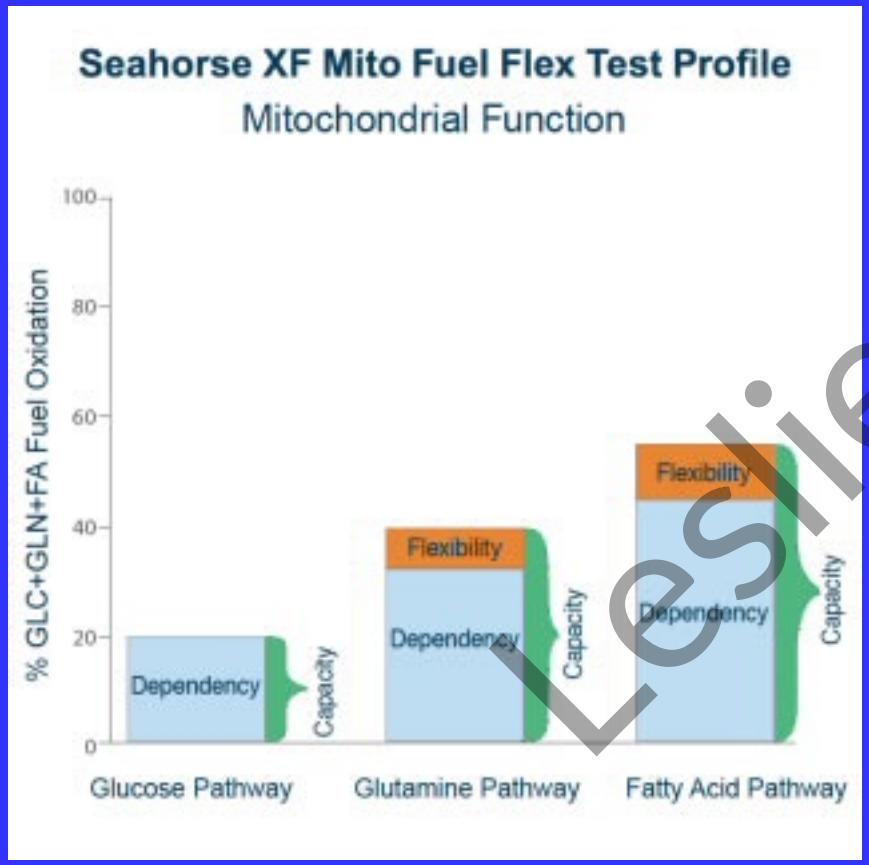
Risk of Stillbirth by Gestational Period and BMI



Yao R, et al . Am J Obstet Gynecol 2014;210:457.e1-9.


Fatty Acids
Glucose
Amino Acids
Maternal
FATP
Glut
AAT
Cpt1
Etomoxir
Glycolysis
Glutamine
BPTES
Lipid Droplets
B oxidation
Pyruvate
UK5099
Acetyl CoA
Glutamate
Placental
ROS
FABP
NADH
FADH2
TCA Cycle
ETC
ATP
Placental Function
Fetal
Fatty Acids
Glucose
Amino Acids
Leslie Myatt

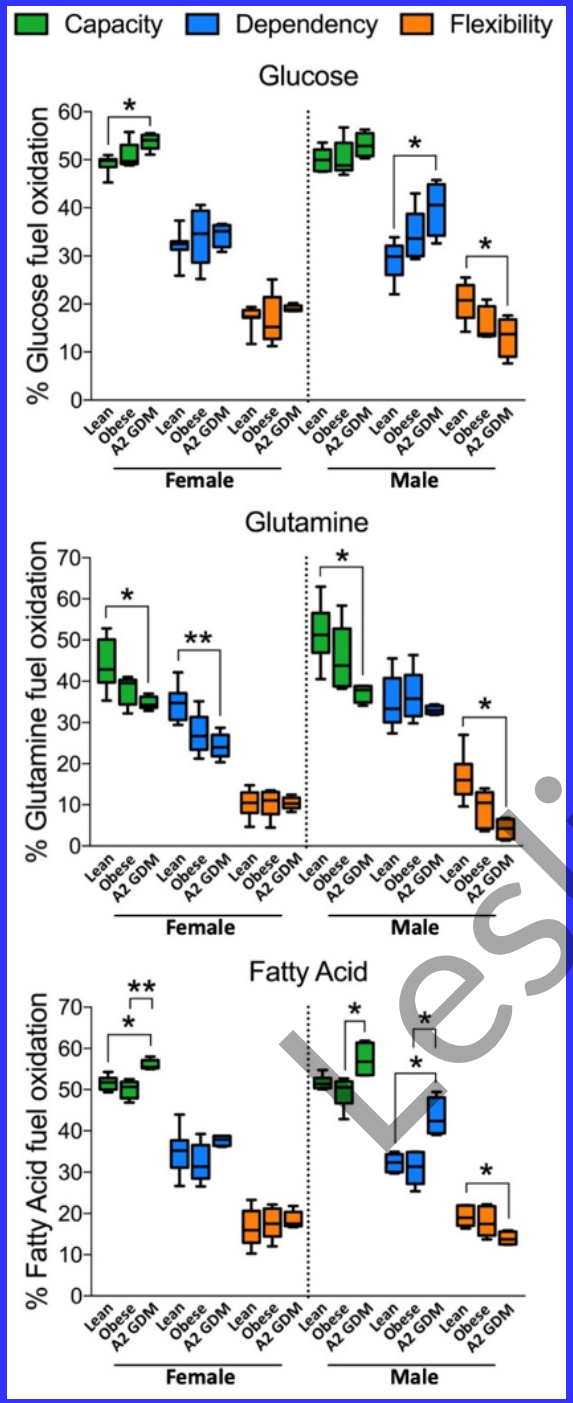
Mitochondrial Fuel Flex Assay



Fuel Dependency The measurement of cells' reliance on a particular fuel pathway to maintain baseline respiration.

Fuel Capacity The ability of a cell's mitochondria to oxidize a fuel when other fuel pathways are inhibited.

Fuel Flexibility The difference between fuel capacity and dependency, that is, the ability of cells to increase oxidation of a particular fuel to compensate for inhibition of alternative fuel pathway(s).



Sexual Dimorphism in Effect of Maternal Metabolic State on Fuel Flex Parameters

Mean ± SEM
*p< 0.05
** P<0.01
Kruskal-Wallis test with Dunns post hoc test
Lean n=6M, 7F
Obese n=5M, 5F
GDM n=4M, 5F

Wang E et al 2019

Sexual Dimorphism in Fuel Flex

- In lean women no difference in dependency for three fuels between male and female trophoblast (35% glutamine and FAs, 30% glucose).
- With hyperglycemia and hyperlipidemia of obesity and A2GDM, in male placenta only, we find increased dependency on glucose and fatty acids for baseline respiration.
- This is accompanied by significantly decreased flexibility for use of both glucose and fatty acids, but also glutamine, i.e. male trophoblast cannot adapt by increasing oxidation of other fuels.
- Decreased flexibility seen in male and not female trophoblast may contribute to the increased risk of the male for adverse outcomes
- Dependency and flexibility for glucose and fatty acids change incrementally from lean to obese women and are further exacerbated with BMI-matched A2GDM suggesting that the effect is not due to obesity alone but may reflect the continuum of worsening hyperglycemia and hyperlipidemia from obesity to A2GDM.

Examples and Consequences of Placental Metabolic Reprogramming

- High altitude hypoxia has been inferred to lead to increased placental anaerobic glycolysis at the expense of mitochondrial respiration to spare oxygen to support the fetus, this results in increased glucose utilization in the placenta with consequent decreased glucose delivery to the fetus leading to growth restriction.

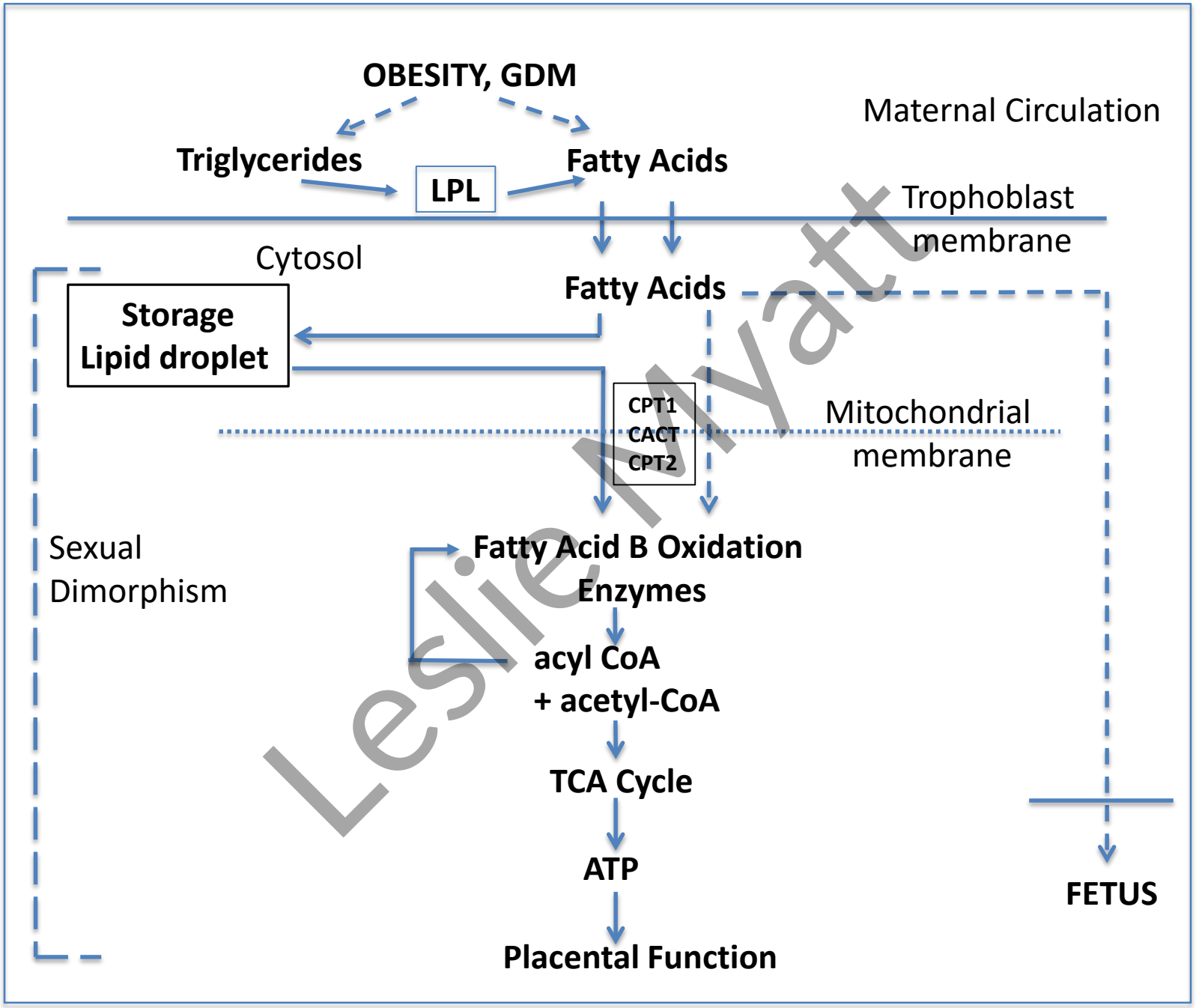
- Recent findings using a four vessel sampling technique in humans has shown that the placenta consumes 30% of glucose taken up from maternal blood and that placental consumption modulates maternal to fetal glucose transfer and fetal glucose consumption such that high placental use of glucose limits fetal glucose delivery and consumption.

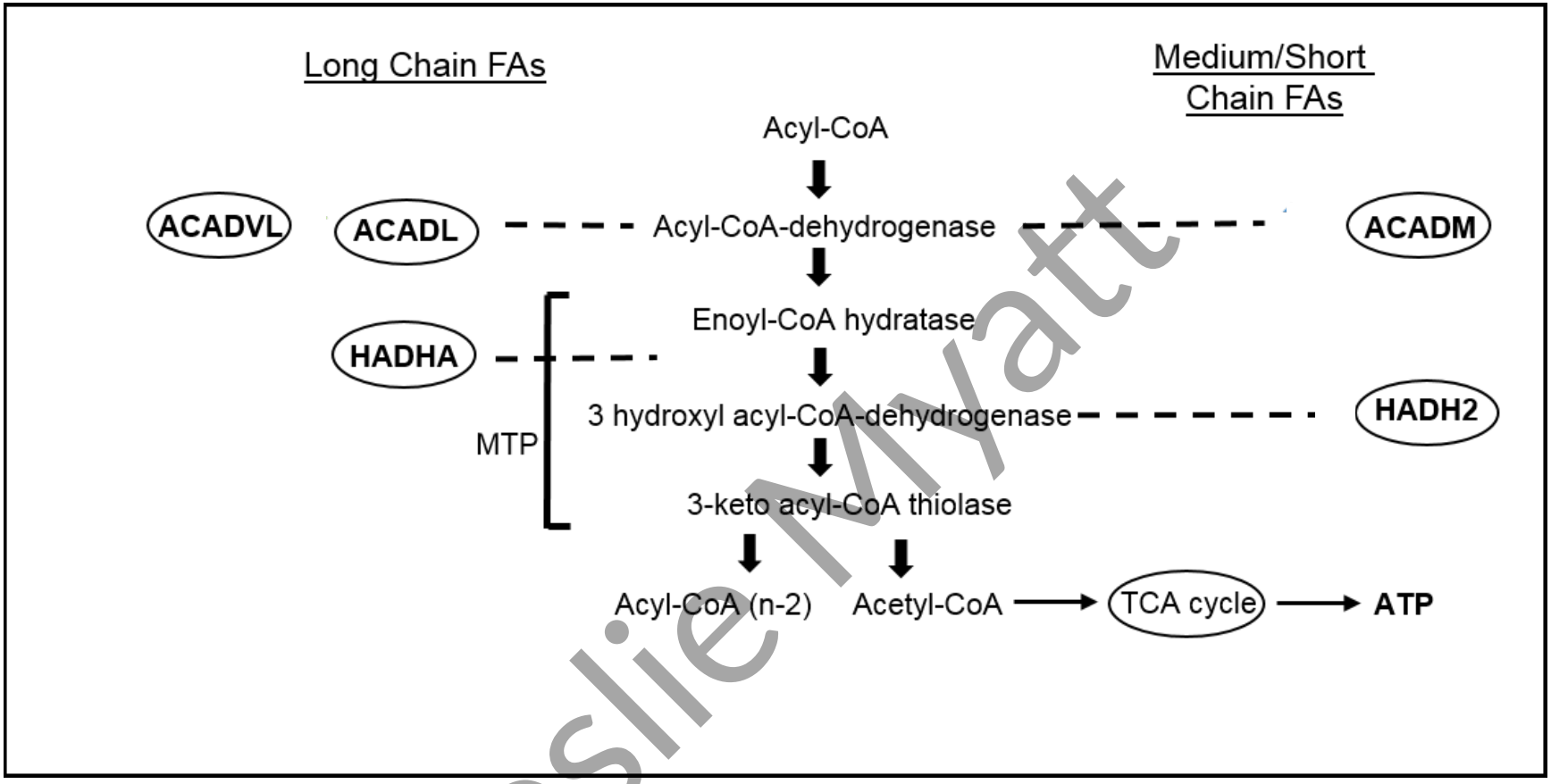
- Studies in the isolated perfused placental cotyledon coupled with computational modeling showed that placental metabolism also influences fatty acid transfer to the fetus with the vast majority of fatty acids taken up being incorporated into placental lipid pools.

- Hence the placenta is not simply the passive conduit for substrate transport to the fetus but its metabolism influences substrate supply to the fetus.

Fatty Acids and Brain Growth

- Docosahexaenoic acid (DHA, C22:6) and arachidonic acid (AA, C20:4) 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.


OBESITY, GDM
Maternal Circulation
Triglycerides
LPL
Fatty Acids
Trophoblast membrane
Cytosol
Fatty Acids
Storage Lipid droplet
CPT1
CACT
CPT2
Mitochondrial membrane
Sexual Dimorphism
Fatty Acid B Oxidation Enzymes
acyl CoA + acetyl-CoA
TCA Cycle
ATP
Placental Function
FETUS
Leslie Myatt

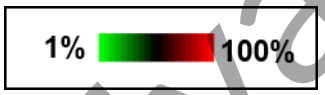


Placental mitochondrial fatty acid *β* oxidation (FAO)

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

Lipidomic Analysis

In total 436 lipids were quantified.

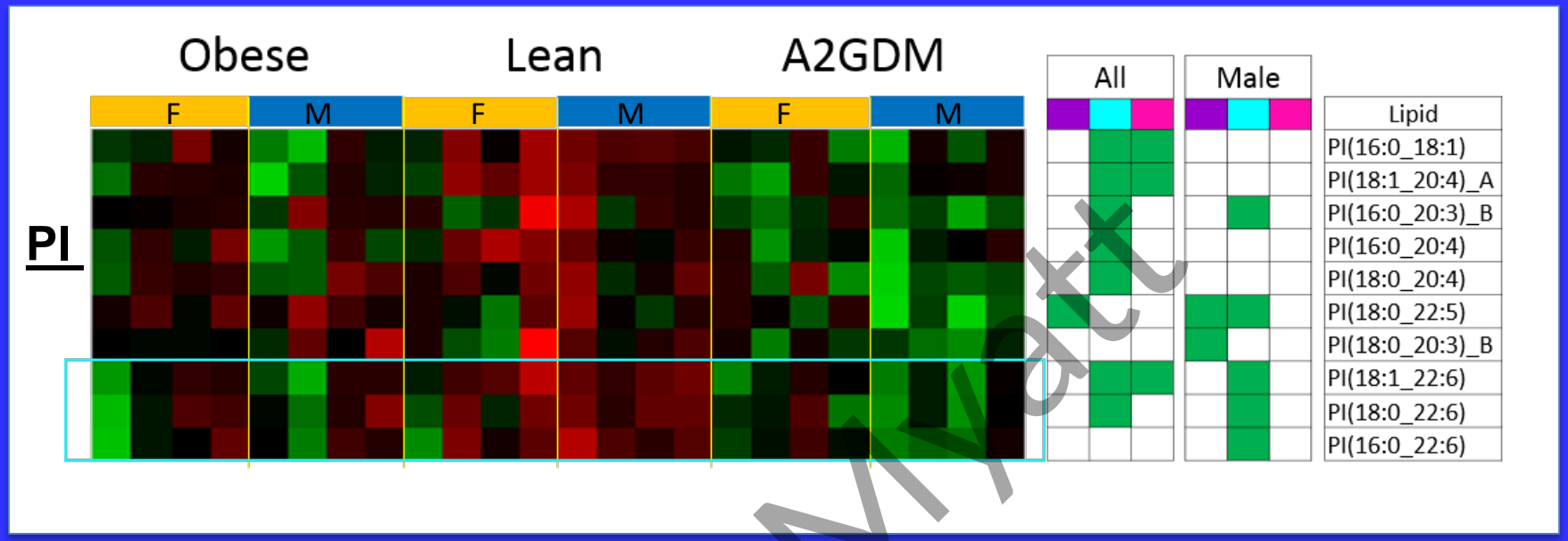
Data are displayed as heatmaps 

species containing docosahexaenoic acid fatty acid chains (22:6)

>85 lipids changed significantly (= Increased, = Decreased) comparing A2GDM to Obese, A2GDM to Lean and Obese to Lean (adjusted $p<0.05$, ANOVA with Tukey test correction).

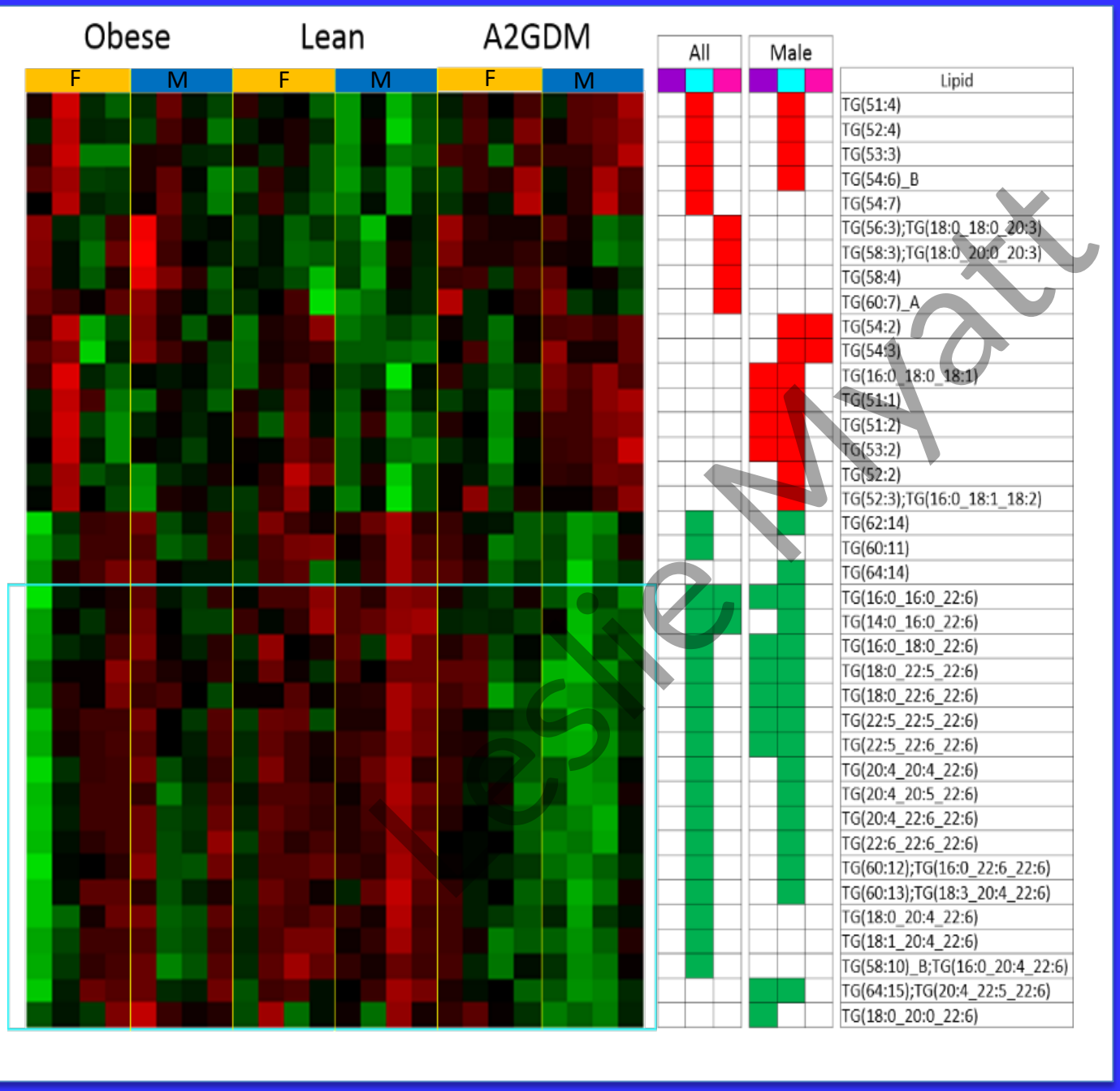
Key and comparisons

Female
Male
A2GDM/Obese
A2GDM/Lean
Obese/Lean
_A and _B denote structural isomers



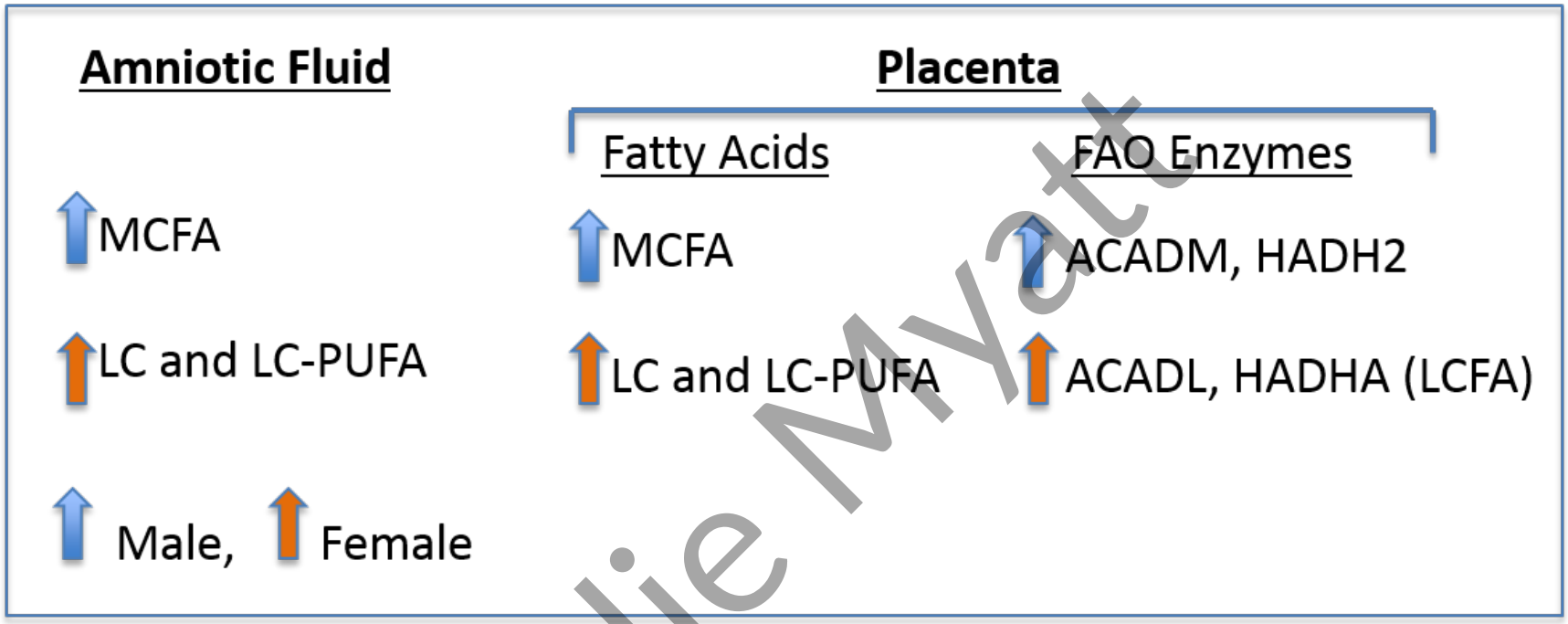
A significant decrease in phosphatidylinositol (PI) species containing docosahexaenoic acid fatty acid chains (22:6) was found in **male** villous tissue of A2GDM

Female
Male
A2GDM/Obese
A2GDM/Lean
Obese/Lean
_A and _B denote structural isomers



Female
Male
A2GDM/Obese
A2GDM/Lean
Obese/Lean
_A and _B denote structural isomers

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)



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).

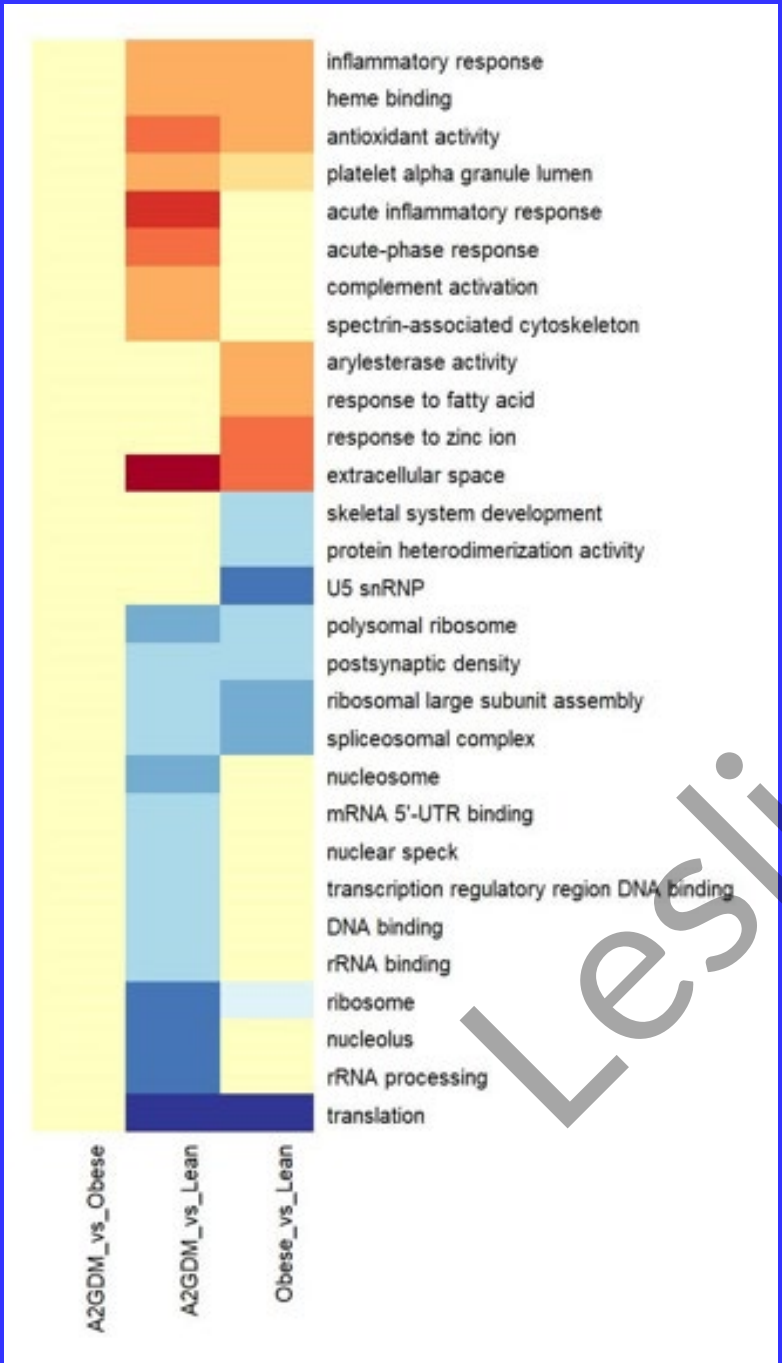
Proteomic Analysis

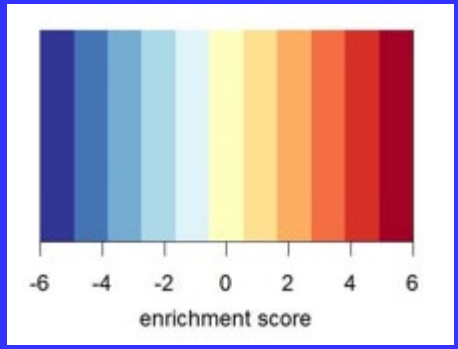
- DAVID analysis recognized 2980 proteins to create functional annotation clusters of pathways
- Significant differences in expression of proteins ($p<0.05$) were identified comparing specimens between the different groups

	Number of Proteins	
Comparison	**Upregulated**	**Downregulated**
Obese vs Lean	**53**	**143**
A2GDM vs Lean	**57**	**220**
A2GDM vs Obese	**34**	**44**

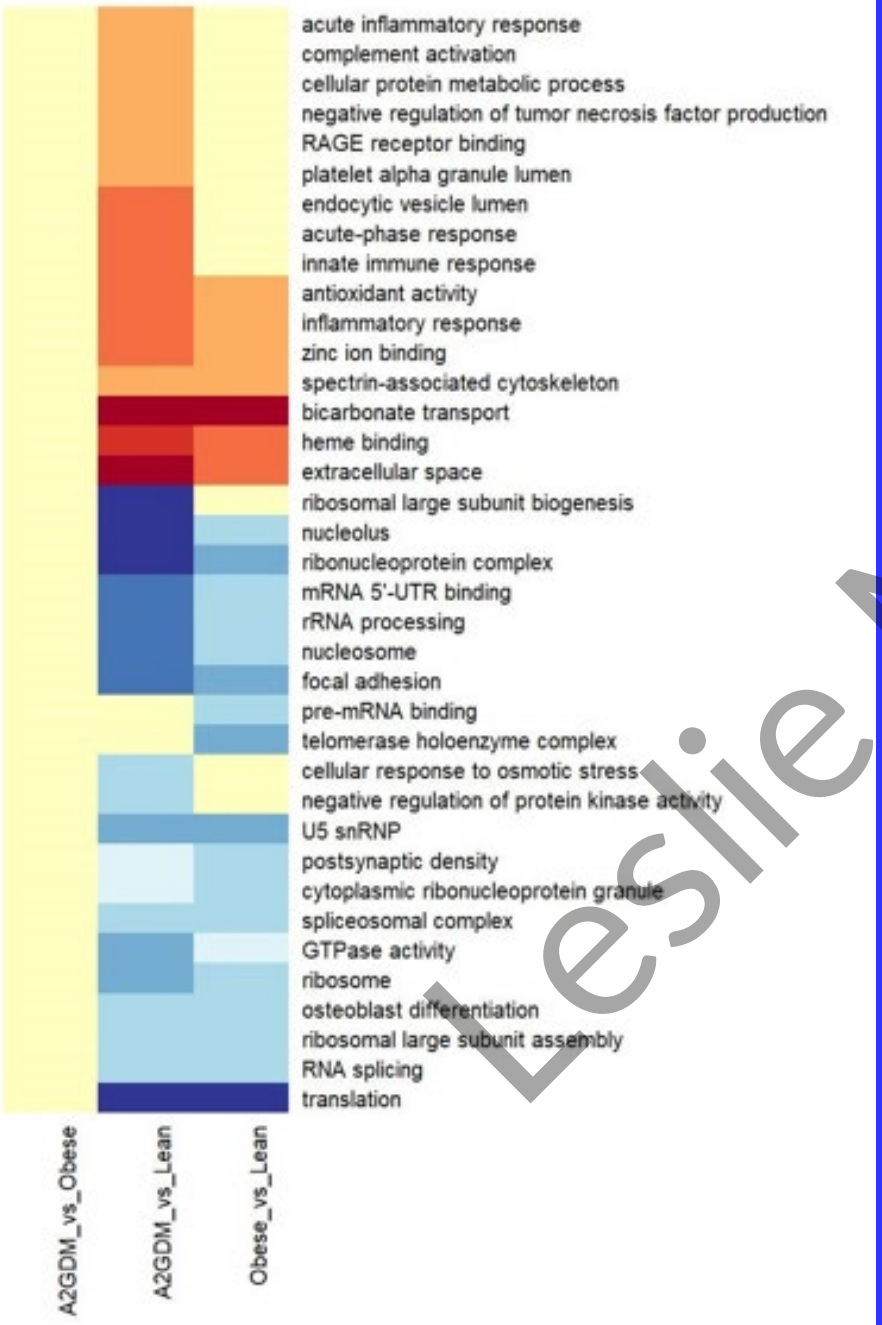
- The differentially expressed proteins were used to find significant gene ontology (GO) pathways, identify proteins involved in each pathway and calculate an enrichment score (log10 p-value from Fisher's exact test) and create heatmaps

Enriched Pathways M+F Combined





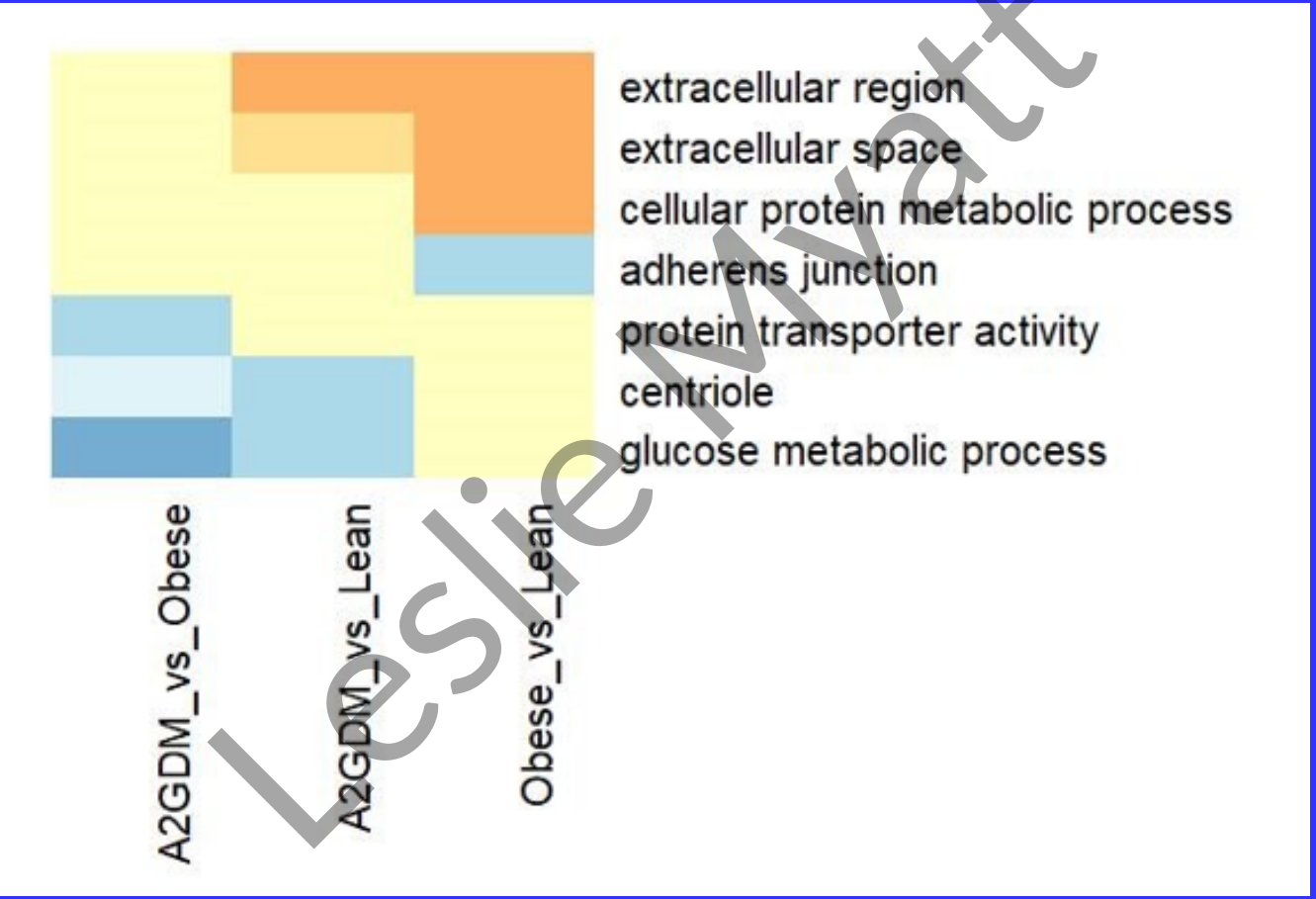
Enriched Pathways Male



Driven more by inflammation vs hyperglycemia or hyperlipidemia?

Are these adaptive changes to maintain or enhance growth but with risk of demise?

Enriched Pathways - Female



Driven by worsening hyperglycemia and hyperlipidemia?
Are they built upon pre-existing differences in gene expression related to female fetal growth and survival?

Female Placenta

Upregulated

- extracellular region
- extracellular space

Downregulated

- protein transporters
- centrioles
- glucose metabolic pathways

This may be driven by worsening hyperglycemia and hyperlipidemia with GDM

Male Placenta

Upregulated

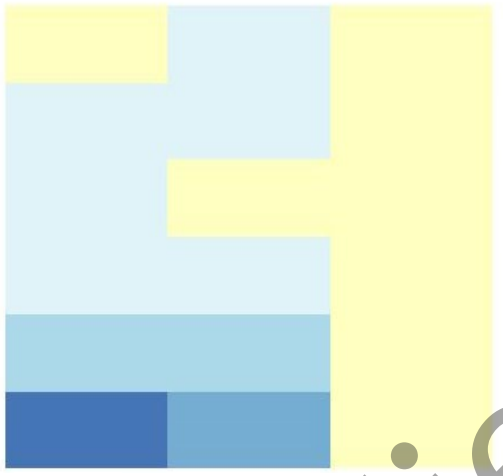
- cytoskeleton
- extracellular space
- heme binding
- inflammatory response
- antioxidant activity
- complement activation
- bicarbonate transport

Downregulated

- translation
- mRNA and rRNA processing
- ribosomal subunit biogenesis
- RNA splicing
- nucleosome
- focal adhesion

These changes may be driven more by the inflammatory milieu rather than hyperglycemia or hyperlipidemia.

Female Placenta: Glucose metabolic process



Aldo-keto reductase family 1 member A1

GDH/6PGL endoplasmic bifunctional protein

Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial

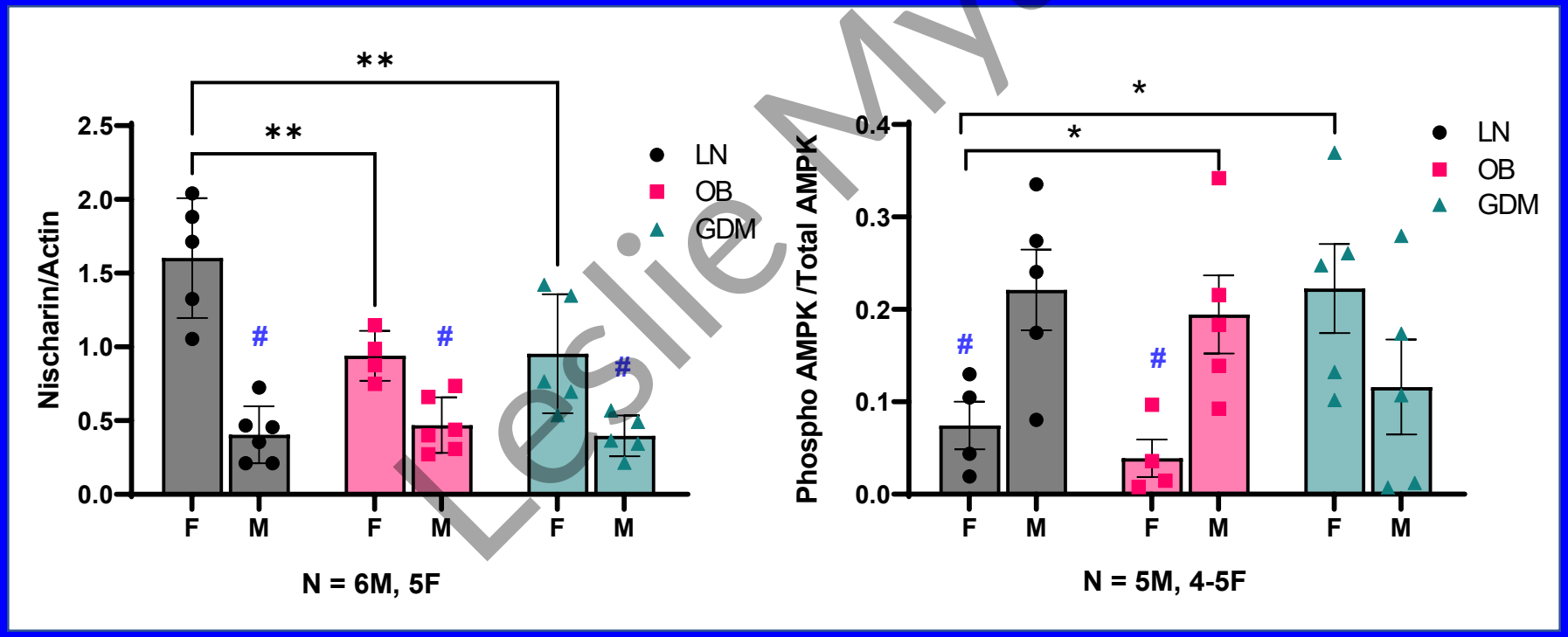
5'-AMP-activated protein kinase catalytic subunit alpha-1

Phosphoglucomutase-like protein 5

Nischarin

- Nischarin inhibits activity of AMPK - major regulator of cellular energy homeostasis.
- Mouse ko model: females show disruption in insulin signaling, develop insulin resistance, and decreased glucose tolerance, males have increased glucose tolerance
- Humans: mRNA levels of Nischarin inversely corelated to obesity

Nischarin expression and phospho AMPK in placentas of lean, obese and GDM groups



Mean ± SD. Significant difference between clinical groups, '**' $p<0.001$. '*' $p<0.05$ (ANOVA), between M and F in each group '#' $p<0.05$ (T test)

Acknowledgements

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