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Author(s)	Ling, Loy See
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The Effects of Maternal Oxidative Stress in Pregnancy on Postpartum & Infant Adiposity Development during the early life

Loy See Ling (PhD)

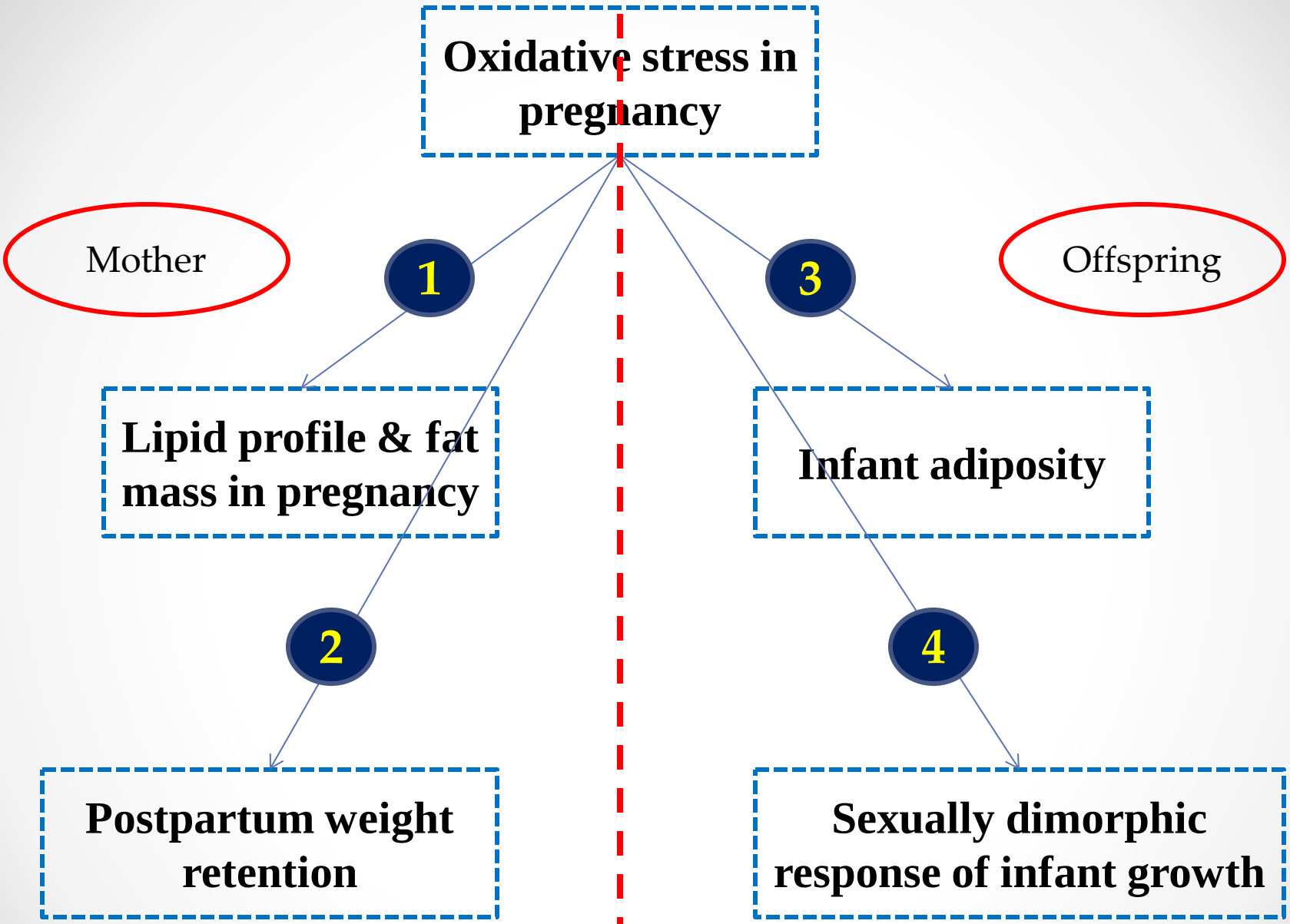
Research Fellow

KK Women's and Children's Hospital, Singapore

Universiti Sains Malaysia, Malaysia

Outline

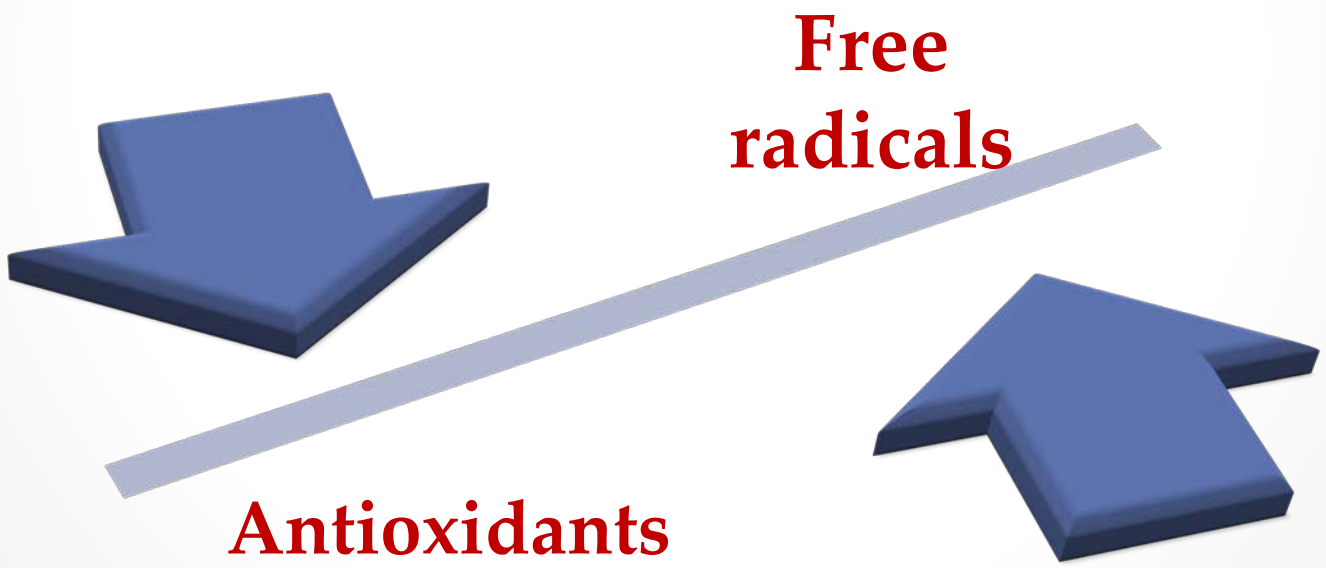
- What is oxidative stress
- Why oxidative stress
- Maternal oxidative stress levels in USM Pregnancy Cohort Study
- Studies on oxidative stress and adiposity in mothers and children


Oxidative stress in pregnancy
Mother
Offspring
1
2
3
4
Lipid profile & fat mass in pregnancy
Infant adiposity
Postpartum weight retention
Sexually dimorphic response of infant growth

What is oxidative stress?

Oxidative Stress

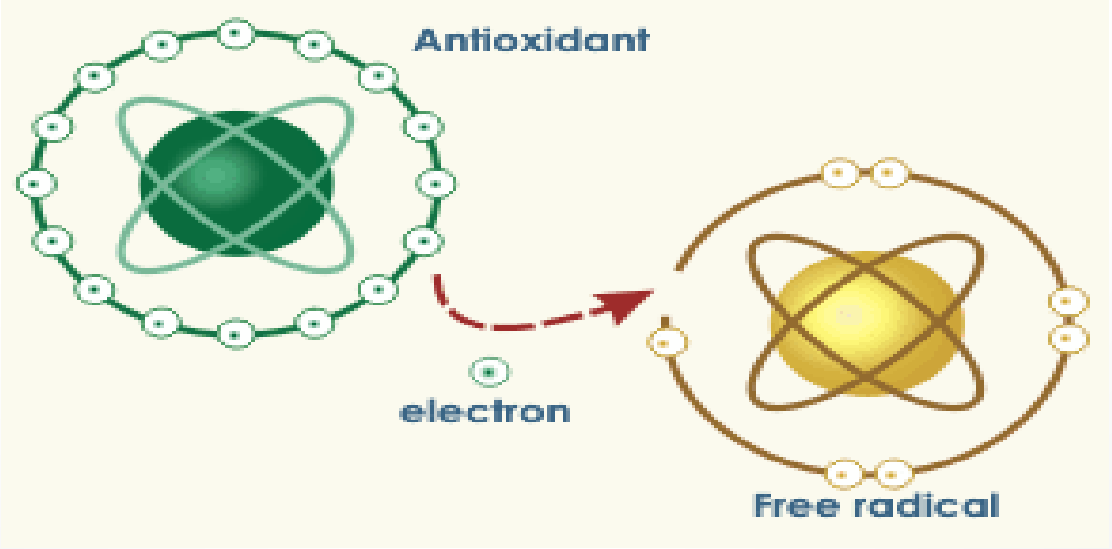
Oxidative stress occurs when **antioxidants are incapable to counterbalance the toxic peroxidation reactions**, due to abundant reactive oxygen species (ROS) production and/or reduced concentration of antioxidants. *(Somogyi et al., 2007; Lázár, 2012).*



Pregnancy & Oxidative Stress

- Pregnancy is an inflammatory state associated with increased free radicals generation *(Furness et al., 2011).*

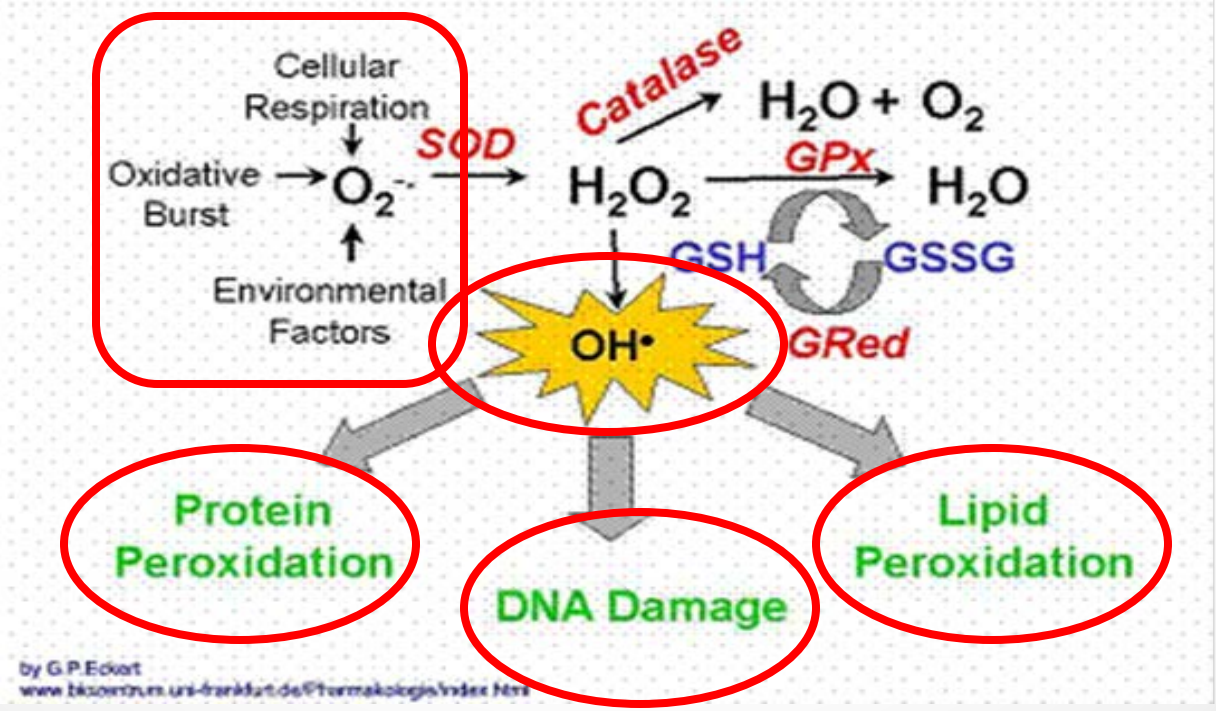
- At homeostatic levels, increase in free radicals is maintained by antioxidant defences *(Burton and Jauniaux, 2011).*



Pregnancy & Oxidative Stress

- However, in pathological state or if a pregnant woman is exposed to stimuli (e.g., nutritional, environmental) that are oxidant and pro-oxidant, uncontrolled production of ROS may occur *(Lázár, 2012).*

- These major perturbations result in an oxidative attack that is capable of damaging biomolecules such as DNA, lipids, and proteins *(Halliwell and Whiteman, 2004).*



Medical Hypotheses (2006) 66, 38–44

ELSEVIER

medical hypotheses

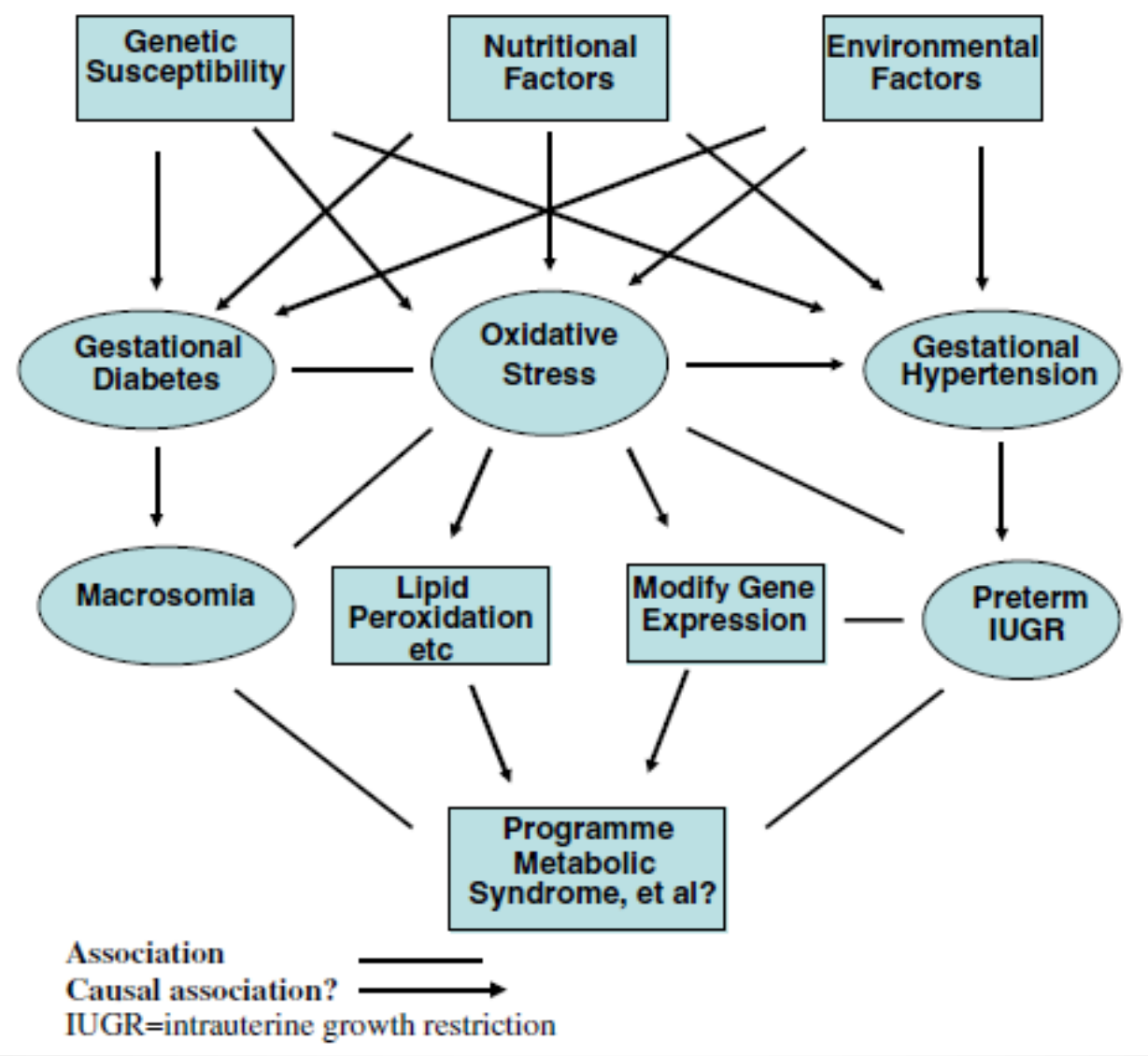
http://intl.elsevierhealth.com/journals/mehy

Tracing the origins of ''fetal origins'' of adult diseases: Programming by oxidative stress?

Z.C. Luo [a,*], W.D. Fraser [a], P. Julien [b], C.L. Deal [c], F. Audibert [a], G.N. Smith [d], X. Xiong [e], M. Walker [f]

[a] *Department of Obstetrics and Gyneocology, Sainte Justine Hospital, University of Montreal, Room-4981, 3175 Cote-Sainte-Catherine, Montreal, Que. Canada H3T 1C5*
[b] *Quebec Lipid Research Centre, Laval University, Canada*
[c] *Department of Pediatrics, Sainte Justine Hospital, University of Montreal, Canada*
[d] *Department of Obstetrics and Gyneocology, Queen's University, Canada*
[e] *Department of Epidemiology, Tulane University, USA*
[f] *Department of Obstetrics and Gyneocology, University of Ottawa, Canada*

Oxidative Stress Programming Hypothesis

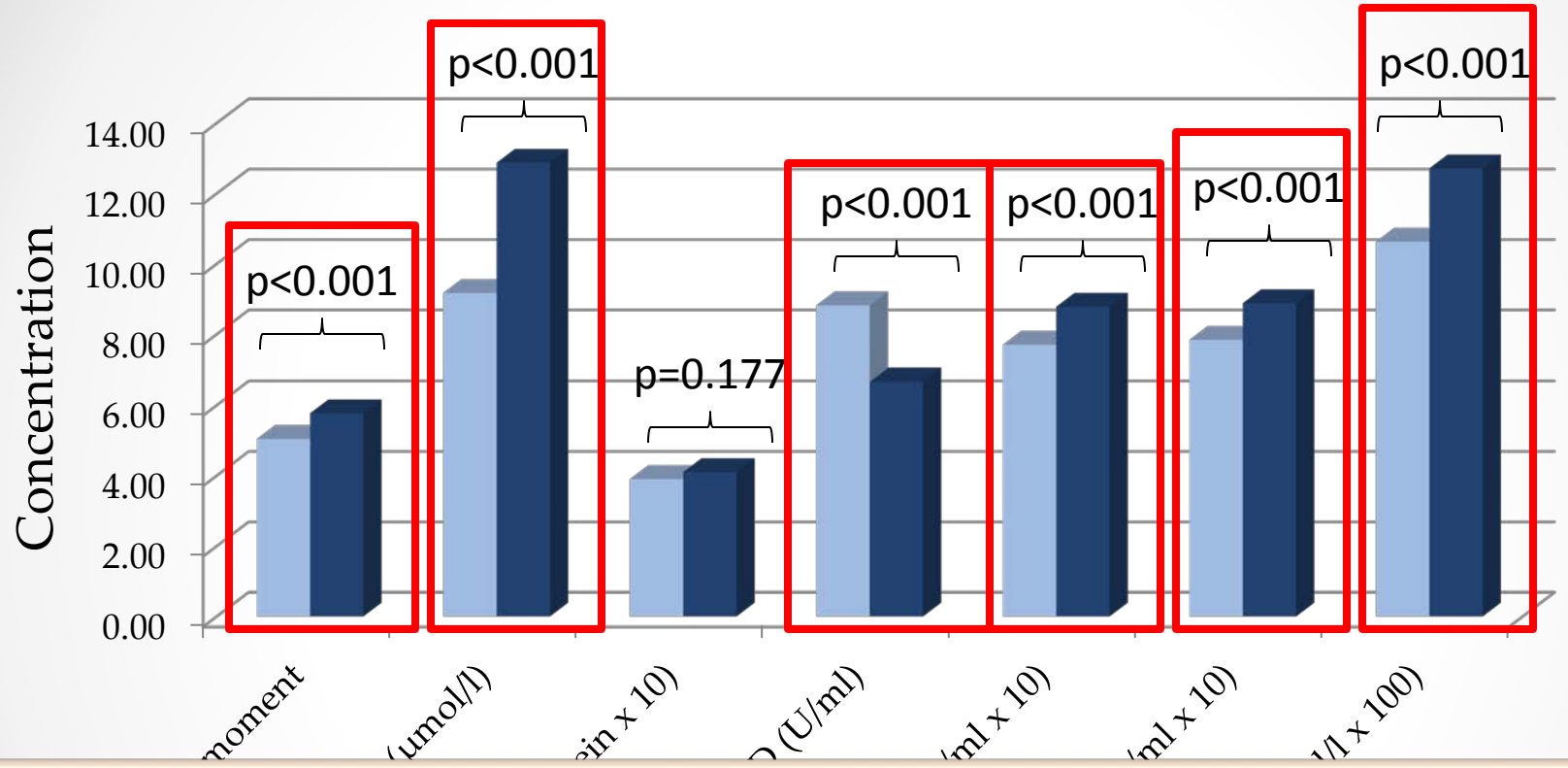


Rationale

- Oxidative stress has been suggested as the **biological plausible mechanism of obesity development**
- Oxidative stress levels are **easily modifiable** during pregnancy and early postnatal periods (which are plausible critical windows)
- The hypothesis, if proved valid, will suggest **new measures** that could be very helpful on fighting the increasing epidemic of the obesity and metabolic syndrome

(Luo *et al.*, 2006)

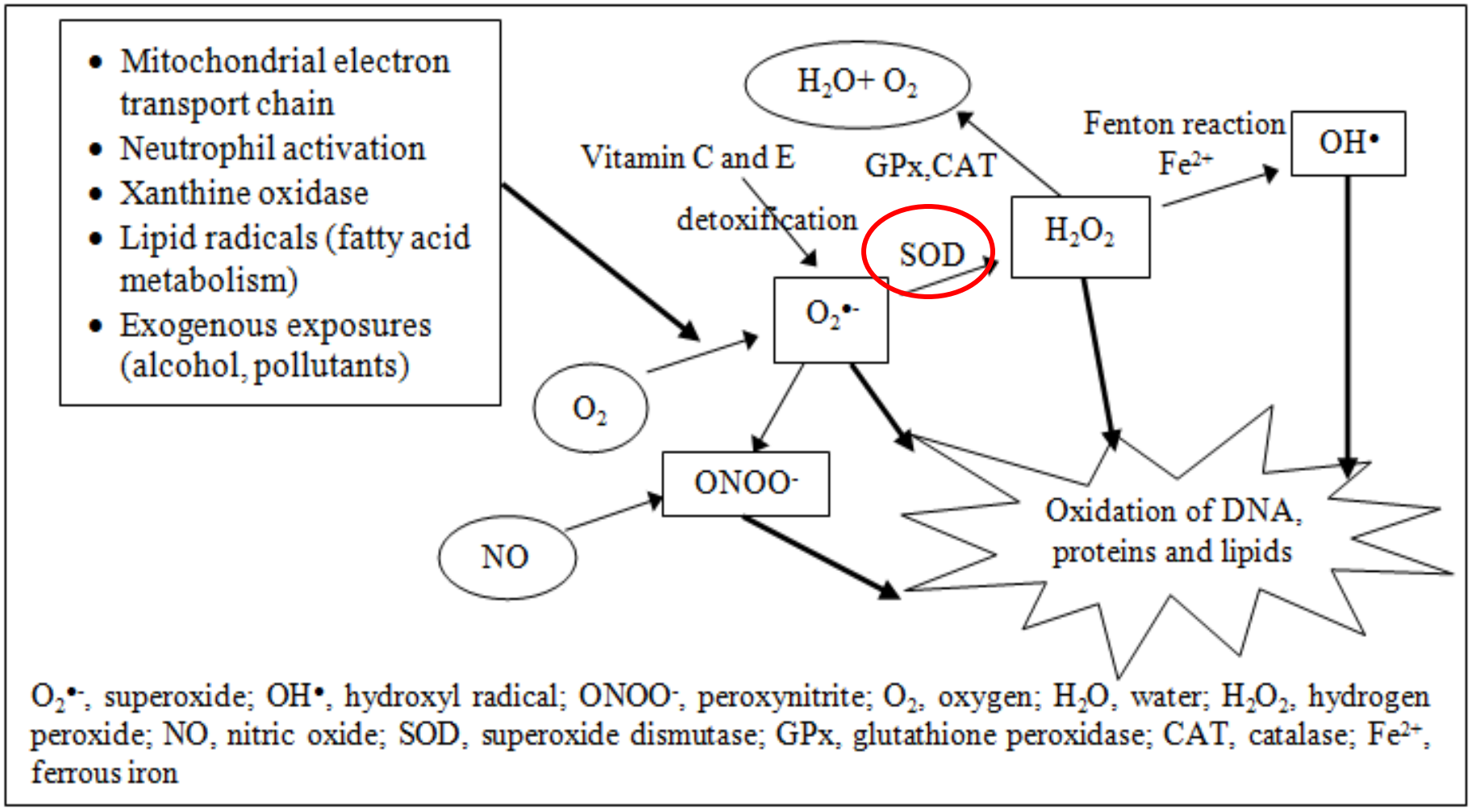
Maternal oxidative stress levels in the second and third trimesters of pregnancy



- Increase in maternal DNA damage level at late gestation could be induced

Diminished activity of SOD indicated high utilization rate of antioxidant enzyme to **control oxygen free radicals attack** (Hung *et al.*, 2010).

against oxidative process to minimize oxidative damage (Chen *et al.*, 2003).



Generation of reactive oxygen species in tissues (adopted from Shoji and Koletzko, 2007; Burton and Jauniaux, 2011)

Part 1:

Maternal Oxidative Stress in Pregnancy and Adiposity Development

1. The association between maternal **lipid profile, adiposity and oxidative stress levels** in pregnancy

2. The mediating effects of maternal oxidative stress in pregnancy on **12 months postpartum weight retention** (PPWR)

Part 1.1:
The association between maternal lipid profile, adiposity and oxidative stress levels in pregnancy

Background

- **Justification**

The metabolic changes and exaggeration of oxidative stress during pregnancy may contribute to the development of maternal obesity. However, the critical window period of this phenomenon to be happened is unclear

- **Objective**

1. To assess changes in **adiposity and lipid profile** in the **second** and **third** trimesters.

2. To correlate total body fat and lipid concentrations with **DNA damage and total antioxidant capacity**.

Study Variables

Second trimester

(18 weeks' gestation)

Third trimester

(34 weeks' gestation)

Anthropometric measurements

- Body weight and total body fat (TBF) → Body composition analyzer (Tanita SC330)

Biochemical analyses

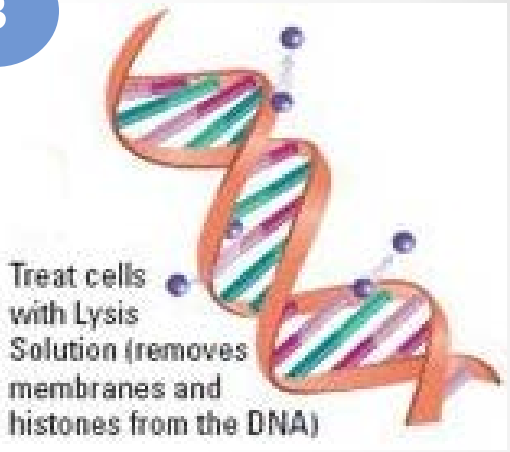
- Total cholesterol (TC), triglyceride (TG), HDL-C, LDL-C
- Total antioxidant capacity (TAC)
- DNA damage
  → Comet assay

Comet Assay Procedure

1

2

3



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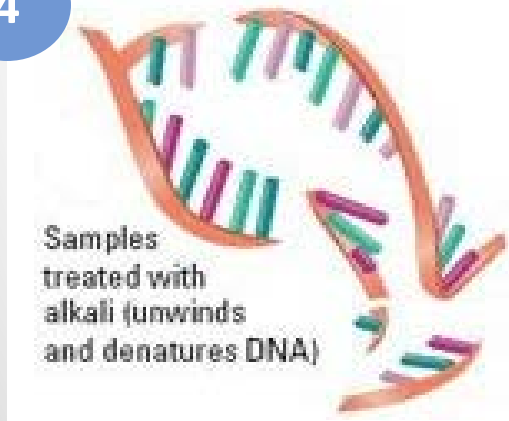

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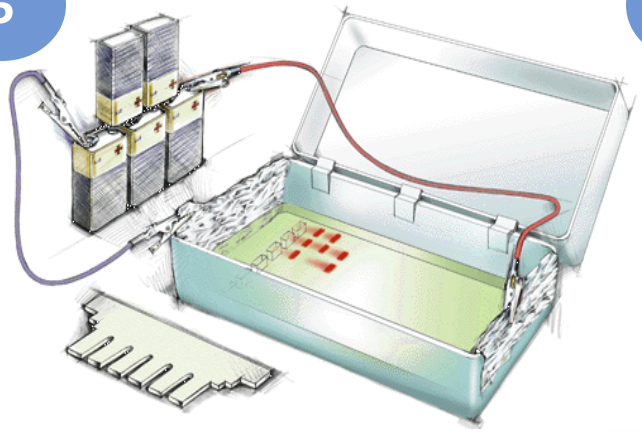

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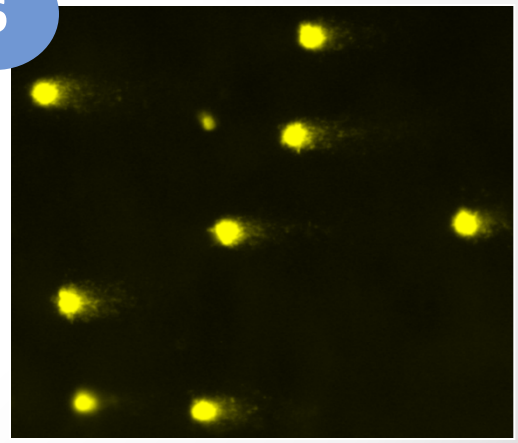
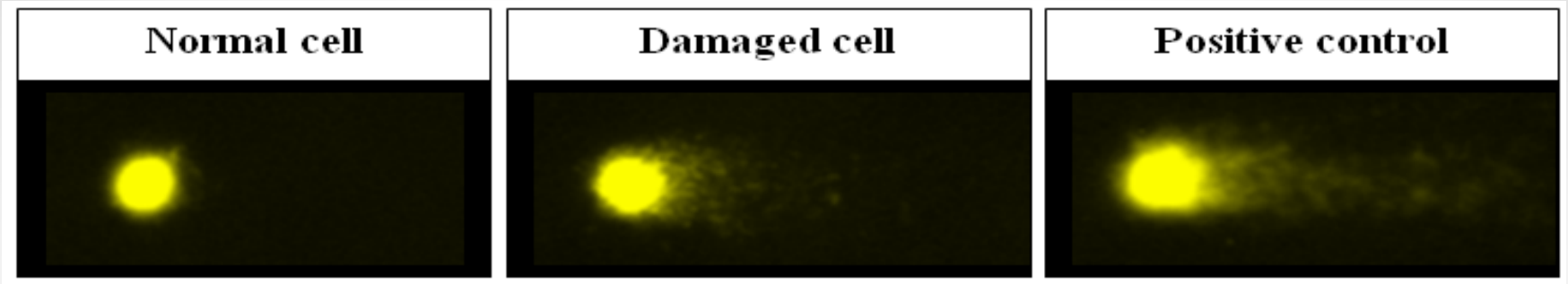
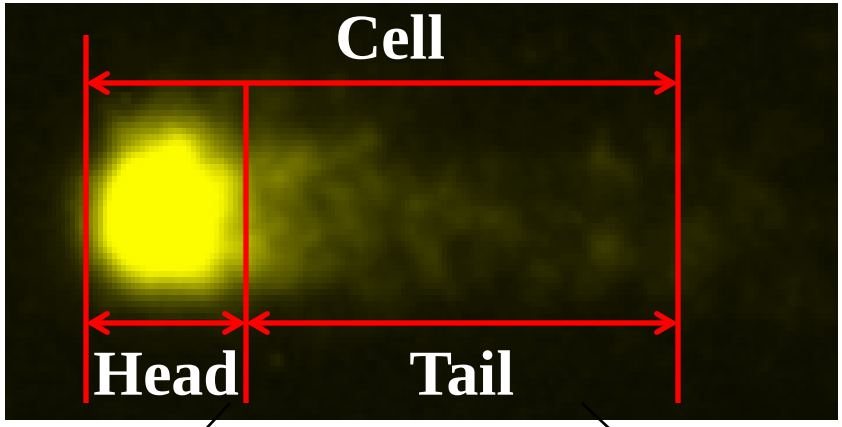


Sample stained with Ethidium Bromide and visualized by fluorescent microscope

(Trevigen, 2013)

Quantification of DNA damage





Markers of DNA damage, measured with Tritek CometScore 1.5

% of tail DNA

Tail moment
(% of tail DNA x tail length)

Statistic Analysis

Performed with **IBM SPSS (PASW) Statistic 20**. Significant level set at $p<0.05$.

Student's paired *t* test

→ compare differences between variables in the 2nd and 3rd trimesters.

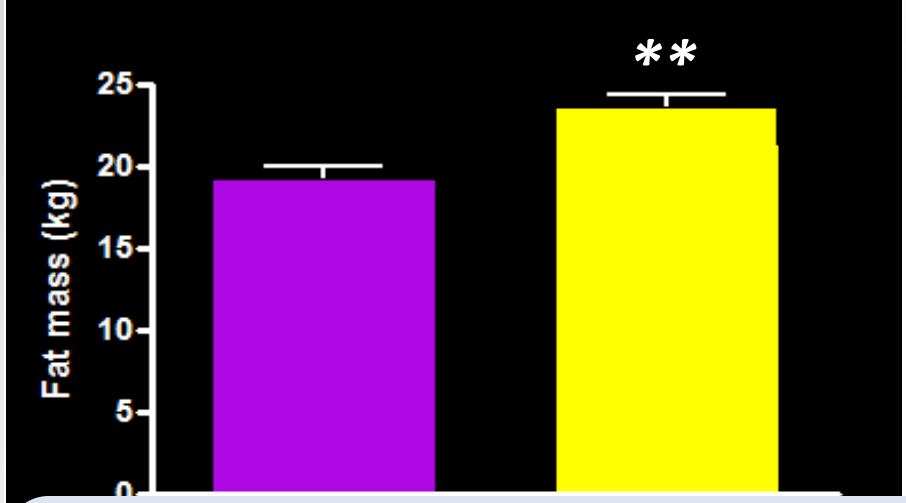
Pearson's correlation test

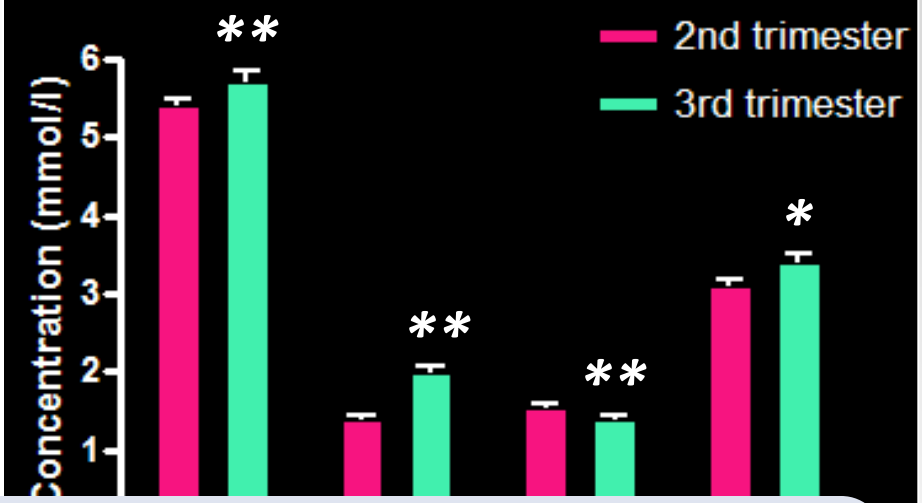
→ to examine the association of maternal adiposity and lipid profiles with oxidative stress markers.

Sample size

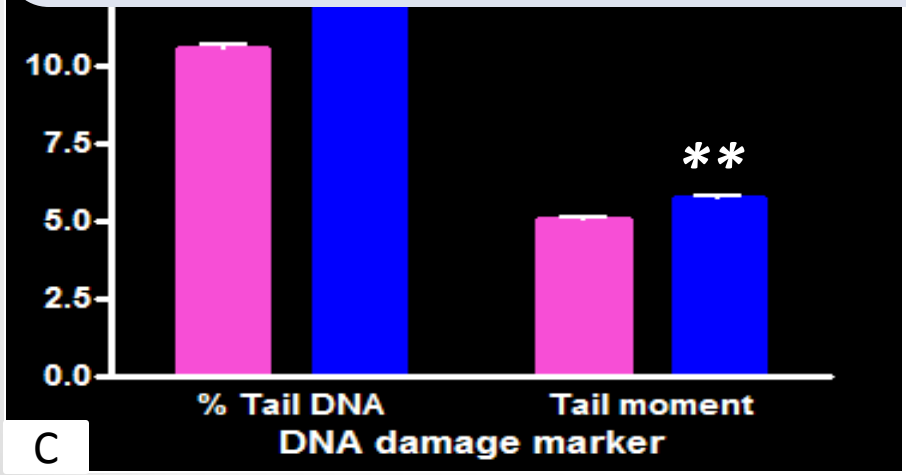
→ 159 mothers

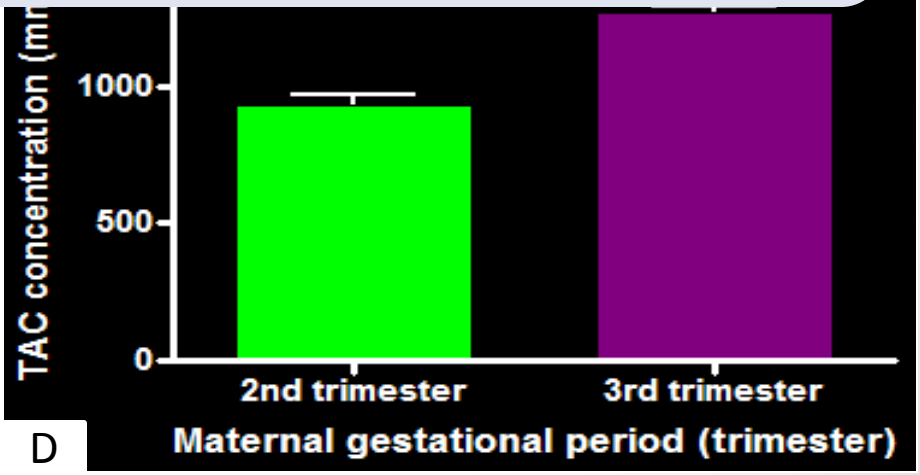
Findings





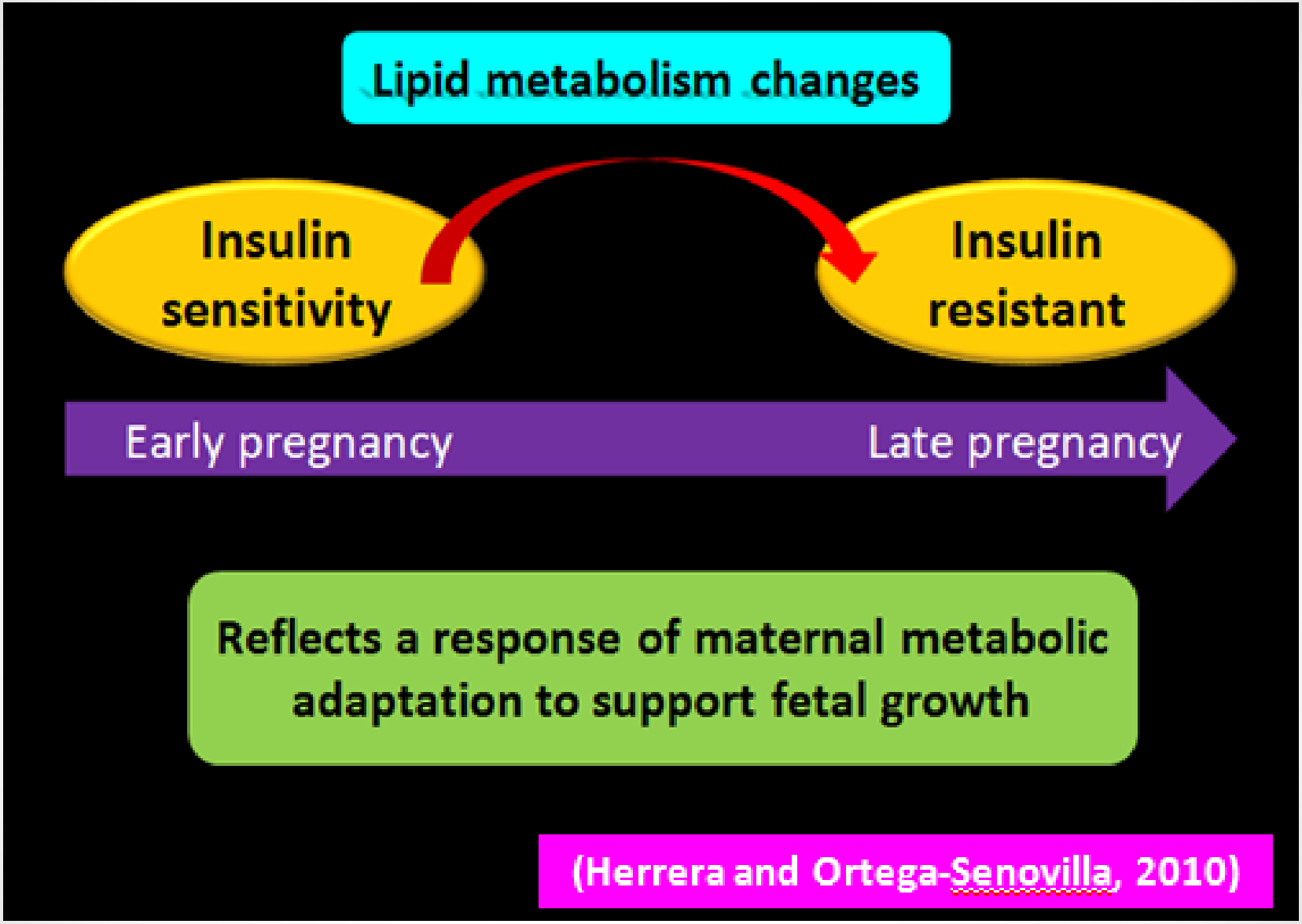
Evidence of mark changes in lipid metabolism and oxidant/antioxidant status during physiologic pregnancy.

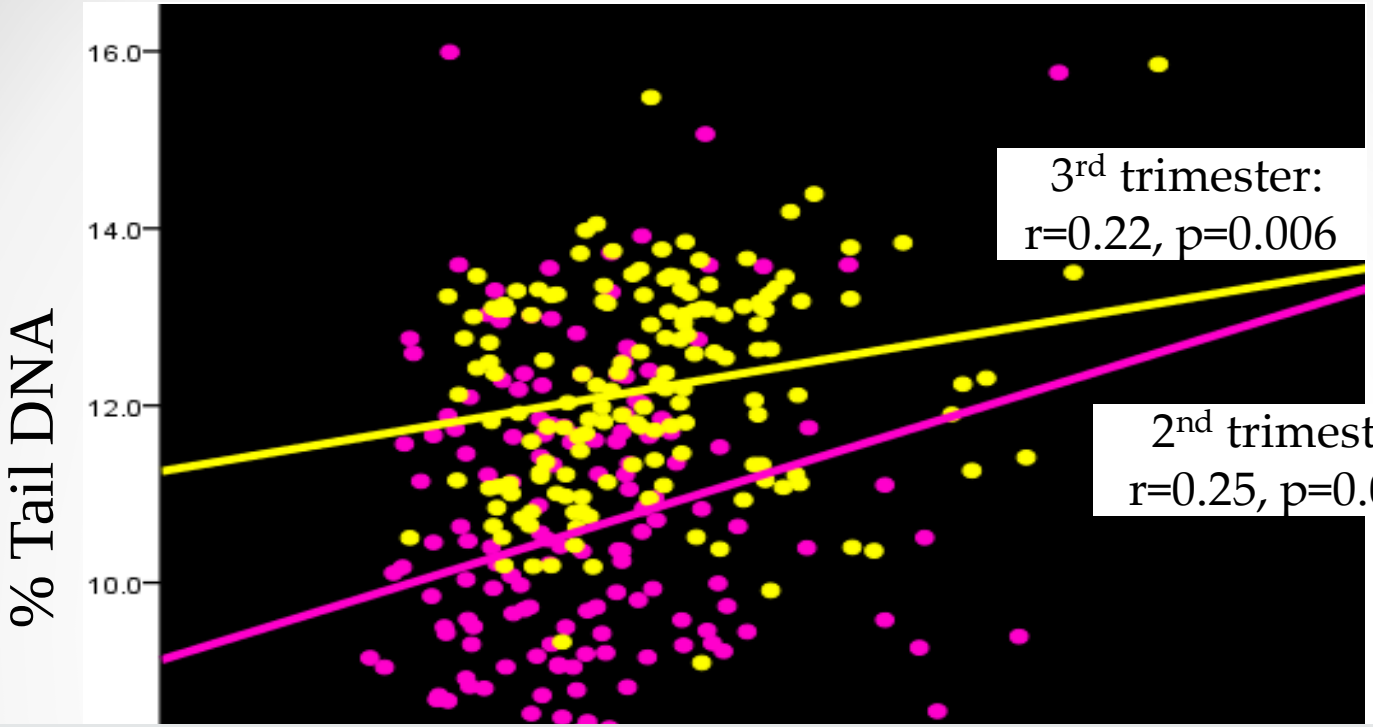




*p<0.01; **p<0.001

Comparison of lipids and oxidative stress markers between the 2nd and 3rd trimesters.

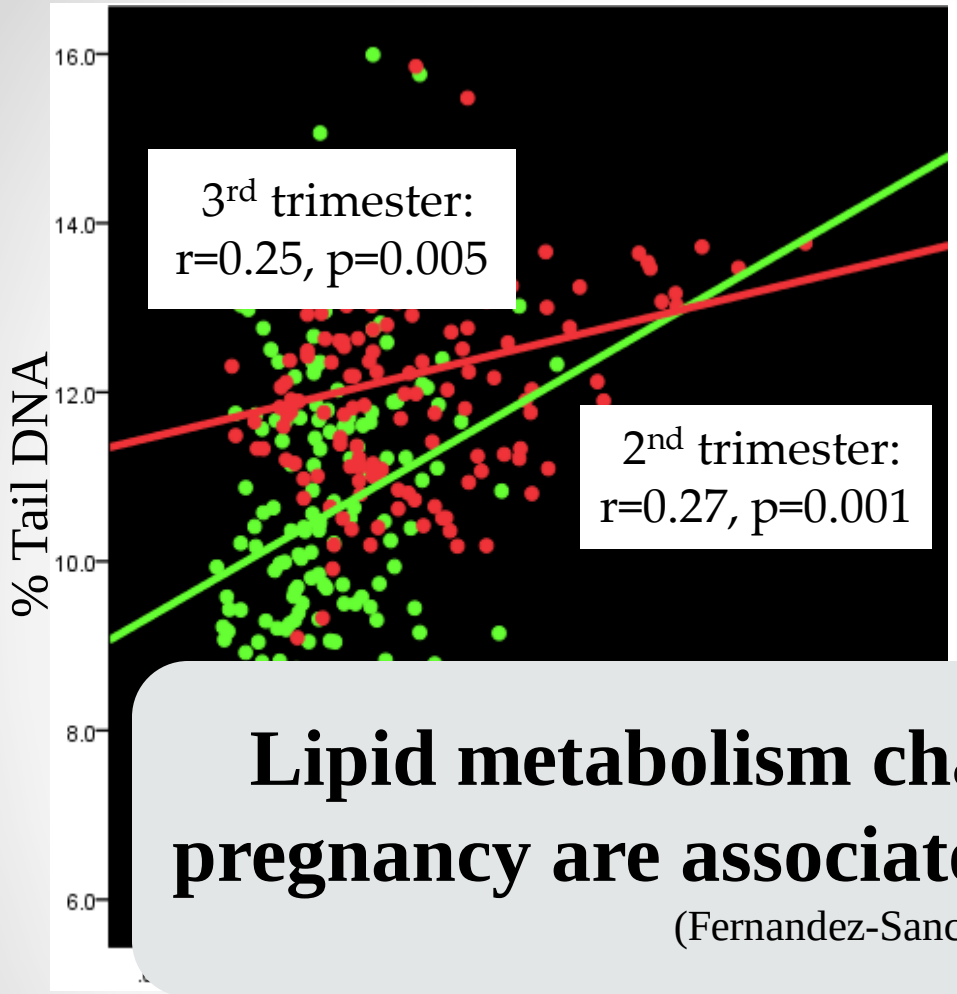

Lipid metabolism changes
Insulin sensitivity
Insulin resistant
Early pregnancy
Late pregnancy
Reflects a response of maternal metabolic adaptation to support fetal growth
(Herrera and Ortega-Senovilla, 2010)

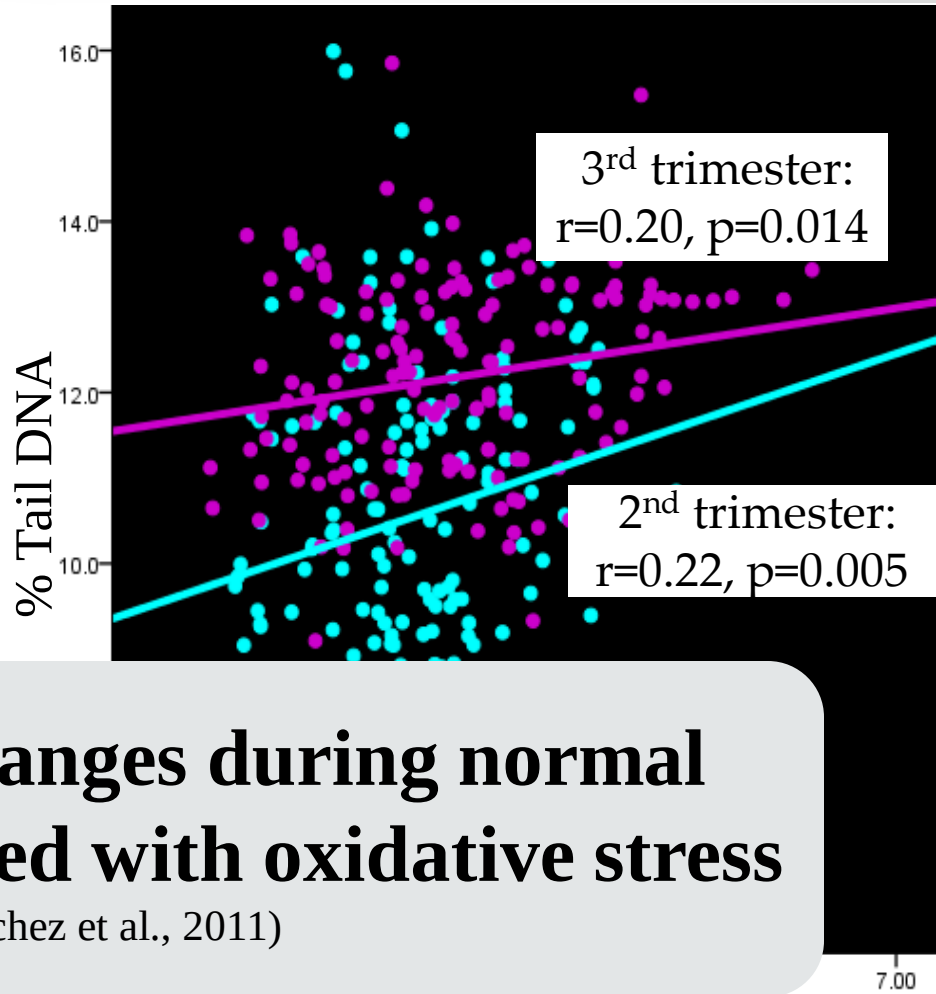


Adipose tissue is a source of oxidative stress production.

(Fernandez-Sanchez et al., 2011)

Pearson's correlation between % Tail DNA and TBF in the 2nd and 3rd trimesters





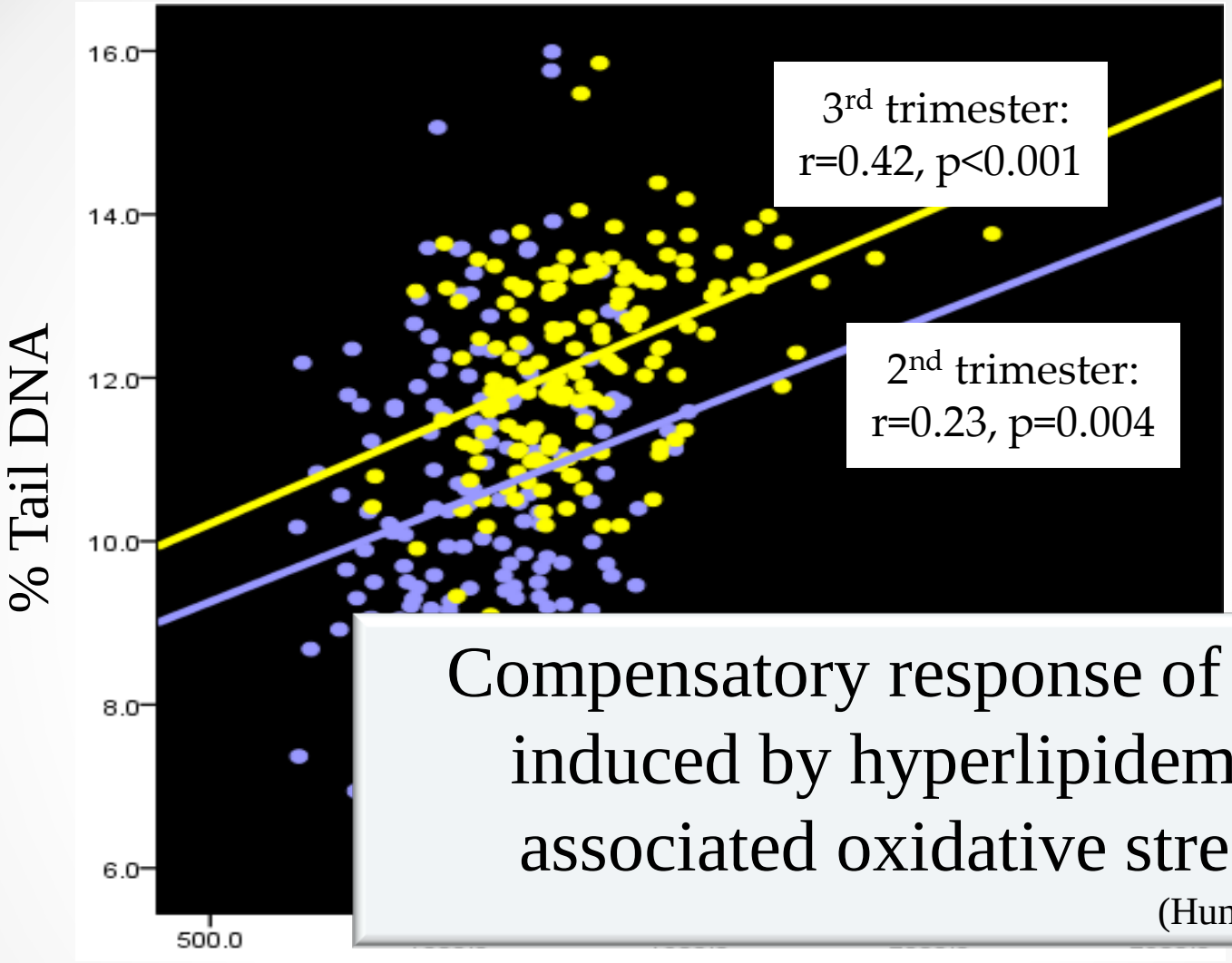
Lipid metabolism changes during normal pregnancy are associated with oxidative stress

(Fernandez-Sanchez et al., 2011)

Serum TG (mmol/L)

Serum LDL-C (mmol/L)

Pearson's correlation between TG, LDL-C and % Tail DNA in the 2nd and 3rd trimesters



Compensatory response of TAC induced by hyperlipidemia-associated oxidative stress.

(Hung et al., 2010)

Pearson's correlation between % Tail DNA and TAC in the 2nd and 3rd trimesters

Conclusion

1. **Lipid metabolism** and **oxidant/antioxidant status** are altered greatly during normal pregnancy.

2. Maternal **dyslipidemia-associated oxidative stress** may have negative health consequences.

3. Higher intake of **antioxidant-rich foods** is recommended to maintain a good antioxidant balance during pregnancy.

Part 1.2:
The mediating effects of maternal oxidative stress in pregnancy on 12 months postpartum weight retention (PPWR)

Background

- **Justification**

On the basis of the maternal oxidative status and the correlation found between oxidative stress and fat mass in pregnancy, oxidative stress may act as the mediator in regulating the relationship between prenatal factor and postpartum adiposity development.

- **Objective**

To examine the **mediating effect** of **oxidative stress** marker between **prenatal factors and 12 months PPWR**

Statistical Analysis

Sample size, n=141

Mediator

Oxidant markers at 18 & 34 weeks' gestation

- DNA damage (TM)
- Lipid peroxidation (MDA)
- Protein oxidation (PC)

Multiple linear regression (SPSS Ver.20)
Partial posterior (PP) approach

Prenatal factors

- Total GWG
- Healthy and Less-Healthy dietary patterns
- Lipid profiles (TC, TG, HDL-C and LDL-C)
- Hair nicotine levels

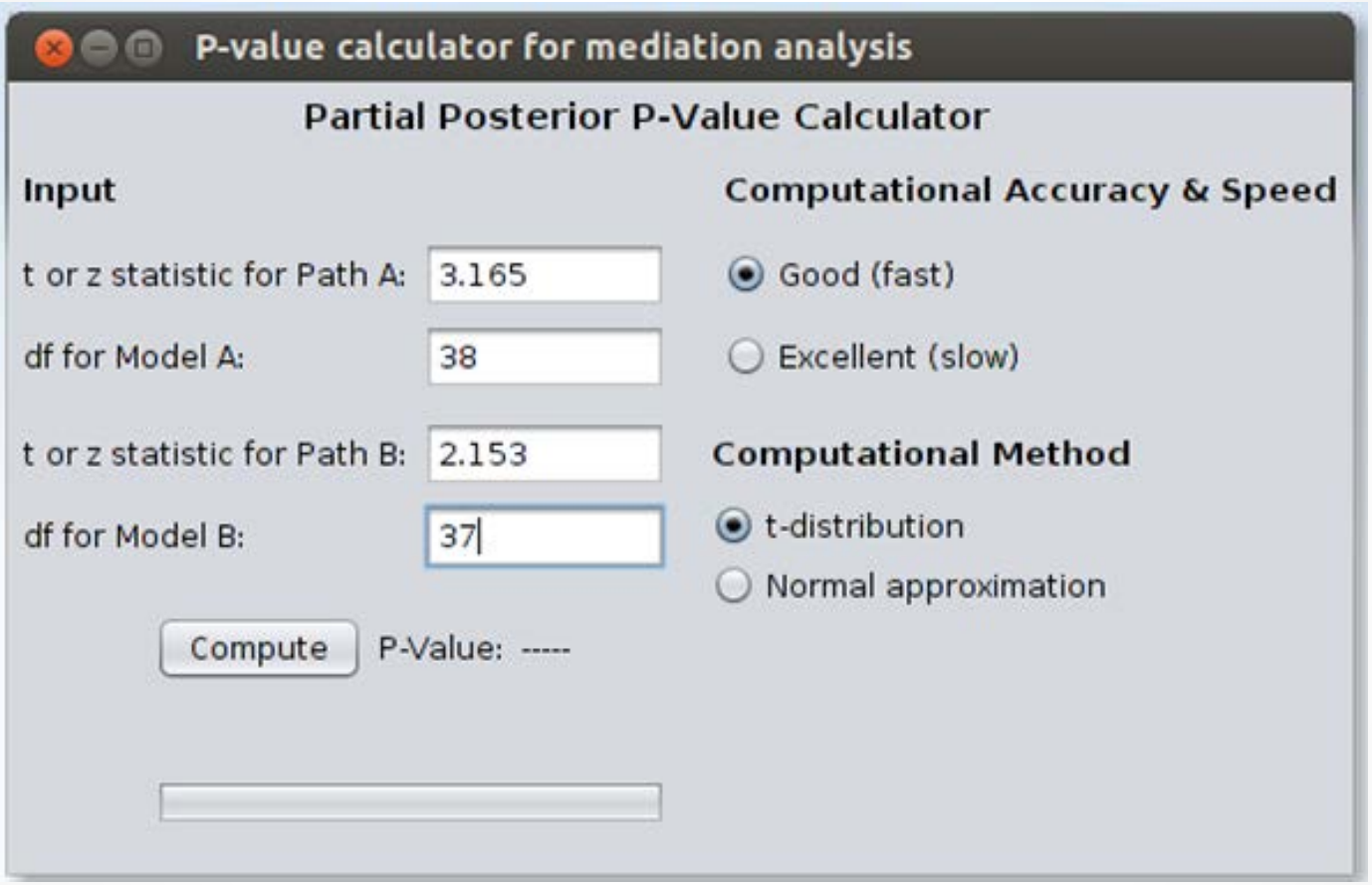
Exposure

12M postpartum weight retention

Outcomes

Confounders:
Parity, prepregnancy BMI, nicotine level, postpartum total energy intake, physical activity and breastfeeding pattern

Mediation analysis



http://www2.psych.ubc.ca/~cffalk/mediation.html#calculator

Biesanz, J.C., Falk, C.F., & Savalei, V. (2010). Assessing mediational models: Testing and interval estimation for indirect effects. *Multivariate Behavioral Research*, *45*, 661-701

Findings

Mediator

Oxidant markers at 18 & 34 weeks' gestation

- DNA damage (TM)
- Lipid peroxidation (MDA)
- Protein oxidation (PC)

No mediation

β= -0.02

PP p-value: 0.469

β= 0.22

Prenatal factors

- Total GWG
- Healthy and Less-Healthy dietary patterns
- Lipid profiles (TC, TG, HDL-C and LDL-C)
- Hair nicotine levels

β=0.30, p<0.001

12M postpartum weight retention

Outcomes

Exposure

Confounders:
Parity, prepregnancy BMI, nicotine level, postpartum total energy intake, physical activity and breastfeeding pattern

Conclusion

- The **complex physiological changes** during pregnancy and obesity development lead to the difficulties in identifying the precise biologic mechanism.
- It could be more likely that multiple pathways via **genetic factors, hypothalamic dysfunction, intestinal gut bacteria and environment** are responsible in affecting obesity predisposition (Das, 2010).
- Huge oxidative stress during gestation that exacerbated by various prenatal factors might reflect a strong likelihood of developing **insulin resistance** in later postpartum life, which can be linked with future metabolic dysfunction and obesity.
- This is supported by the **positive association** that observed between **DNA damage and insulin resistance** in pregnancy.

Part 2:

The Effects of Maternal Oxidative Stress in Pregnancy on Infant Adiposity Development

1. The effects of maternal oxidative stress in pregnancy on infant adiposity development during the **first year of life**

2. Sexually dimorphic responses of infant growth during the **first two years of life** to maternal antioxidant levels in pregnancy

Part 2.1:
The effects of maternal oxidative stress in pregnancy on infant adiposity development during the first year of life

Background

- **Justification**

Although numerous studies have been conducted to examine the causal factors of childhood obesity, the implications of **intrauterine oxidative stress** on **early postnatal adiposity** development remain to be elucidated

- **Objective**

To examine the effect of maternal oxidative stress levels in the **2nd & 3rd trimester** on infant **adiposity development at birth, 2 months, 6 months and 12 months of life**

Statistical Analysis

Sample size, n=153

Exposure variables

Oxidative stress markers at 18 & 34 weeks' gestation:

- **DNA damage**
- **MDA**
- **PC**
- **TAC**
- **GPx**
- **SOD**
- **CAT**

Multiple linear regression

SPSS Ver. 20

Outcome variables

Infant adiposity indicators at 0,2,6 & 12M of age

- **Weight**
- **BMI-for-age**
- **Skinfold thickness**

Confounding variables:

maternal age, prepregnancy BMI, total GWG, prenatal Less-Healthy dietary pattern score, hair nicotine level, gestational age, infant sex, birth weight and breastfeeding patterns.

Findings

Regression coefficients (*p*-values) for infant body weight by prenatal oxidative stress markers in the second trimester of pregnancy

Variables	Body weight (kg)							
	At birth (n=143)		2 months (n=142)		6 months (n=141)		12 months (n=141)	
	Simple regression	Multiple regression[a]	Simple regression	Multiple regression[b]	Simple regression	Multiple regression[b]	Simple regression	Multiple regression[b]
Tail moment	-0.12(<0.001)	-0.12(<0.001)	-0.18(<0.001)	-0.20(<0.001)	-0.20(0.002)	-0.27(<0.001)	-0.19(0.017)	-0.31(<0.001)
MDA, µmol/l	0.02(0.141)	-	0.01(0.833)	-	-0.02(0.457)	-	0.004(0.925)	-
PC, nmol/mg protein	0.18(0.476)	-	-0.19(0.619)	-	-0.22(0.664)	-	0.01(0.991)	-
TAC, µmol/l	0.001(0.334)	-	0.001(0.134)	0.001(0.244)	0.001(0.229)	0.001(0.278)	0.001(0.714)	-
GPx, nmol/min/ml	0.002(0.272)	-	0.004(0.039)	-	0.004(0.109)	-	0.003(0.422)	-
SOD, U/ml[c]	-0.21(0.272)	-	-0.13(0.649)	-	-0.33(0.387)	-	-0.40(0.385)	-
CAT, nmol/min/ml[c]	0.13(0.328)	-	0.09(0.648)	-	0.25(0.329)	-	0.05(0.870)	-

As maternal **DNA tail moment increased**, infant **weights** at birth, 2, 6 and 12 months of age **decreased**.

Regression coefficients (*p*-values) for infant body mass index-for-age by prenatal oxidative stress markers in the second trimester of pregnancy

	Body mass index-for-age (Z-scores)							
Variables	At birth (n=143)		2 months (n=142)		6 months (n=141)		12 months (n=141)	
	Simple regression	Multiple regression[a]	Simple regression	Multiple regression[b]	Simple regression	Multiple regression[b]	Simple regression	Multiple regression[b]
Tail moment	-0.30(0.004)	-0.34(0.003)	-0.09(0.235)	-0.20(0.014)	-0.15(0.053)	-0.26(0.004)	-0.13(0.127)	-0.23(0.011)
MDA, µmol/l	-0.05(0.374)	-	0.01(0.806)	-	-0.001(0.987)	-	0.01(0.727)	-
PC, nmol/mg protein	0.63(0.427)	-	-0.44(0.430)	-	-0.70(0.239)	-	-0.13(0.828)	-
TAC, µmol/l	0.001(0.041)	0.001(0.246)	0.001(0.006)	0.001(0.030)	0.001(0.062)	-	0.001(0.465)	-
GPx, nmol/min/ml	0.01(0.004)	0.01(0.012)	0.01(0.030)	-	0.003(0.322)	-	0.002(0.482)	-
SOD, U/ml[c]	-1.20(0.049)	-	-0.47(0.274)	-	-0.44(0.345)	-	-0.44(0.353)	-
CAT, nmol/min/ml[c]	0.70(0.080)	-	0.07(0.818)	-	0.35(0.249)	-	0.05(0.883)	-

As maternal **DNA tail moment increased**, infant **BAZ** at birth, 2, 6 and 12 months of age **decreased**.

Regression coefficients (*p*-values) for triceps skinfold-for-age by prenatal oxidative stress markers in the second trimester of pregnancy

	Triceps skinfold-for-age (Z-scores)			
Variables	6 months (n=141)		12 months (n=141)	
	Crude	Adjusted[a]	Crude	Adjusted[a]
Tail moment	-0.17(0.066)	-0.21(0.041)	-0.22(0.011)	-0.22(0.030)

Reduced early postnatal growth and adiposity in term infants born to women with **high oxidative stress** during normal pregnancies.

Oxidative DNA damage is mainly arises from the **rapid growing cells** of the **trophoblast columns** which relate to foetal growth (Takagi *et al.*, 2004), it is therefore more sensitive in affecting infant growth and adiposity.

Conclusion

- However, the effects of prenatal oxidative stress on infant growth and adiposity development were **not been well defined** yet in the present study.
- Reduced infant adiposity during the first year of life may be due to the **early effect of oxidative stress on foetal growth restriction**, but not indicating as the final definite results.
- Infants born lighter and thinner may tend to experience **catch-up growth** during their preschool period and subsequently, obesity development (Ong, 2006).

Part 2.2:
Sexually dimorphic responses of infant growth during the first two years of life to maternal antioxidant levels in pregnancy

Background

- **Justification**

Oxidative stress has been suggested as the biological plausible mechanism of obesity development. However, the **gender-specific effects** of prenatal **antioxidant levels** on **infant growth** are unclear

- **Objective**

To examine the longitudinal growth of infants during the **first 24 months of life** in relation to maternal **antioxidant levels in the 2nd and 3rd trimesters** according to **gender**

Statistical Analysis

Boys | **Girls**

Exposure variables

Enzymatic antioxidant markers at 18 & 34 weeks' gestation:

- **GPx**
- **CAT**

Multiple linear regression

SPSS Ver. 20

Outcome variables

Differences in infant growth at 0-6M, 6-12M & 12-24M

- **Weight-for-age Z-score (WAZ)**
- **Length-for-age Z-score (LAZ)**
- **Weight-for-Length Z-score (WLZ)**

Confounding variables:

Maternal age, prepregnancy BMI, smoking exposure, breastfeeding pattern, gestational age and birth size

Findings

Table 1 Adjusted mean changes in **WAZ** during the first 24 months of life in relation to maternal antioxidant levels at **18** weeks' gestation

Variable	Boys						Girls					
	0-6M WAZ change (n=50)		6-12M WAZ change (n=50)		12-24M WAZ change (n=49)		0-6M WAZ change (n=58)		6-12M WAZ change (n=58)		12-24M WAZ change (n=57)	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Log GPx	-0.42 (-2.35, 1.51)	0.663	0.11 (-0.90, 1.12)	0.822	-1.05 (-2.60, 0.50)	0.178	0.99 (-0.64, 2.62)	0.229	-0.17 (-1.05, 0.72)	0.702	-0.54 (-1.91, 0.82)	0.427
Log CAT	-0.95 (-2.12, 0.22)	0.108	0.17 (-0.46, 0.80)	0.587	0.49 (-0.51, 1.48)	0.326	0.39 (-0.38, 1.15)	0.313	-0.35 (-0.75, 0.05)	0.088	**-0.81 (-1.41, -0.22)**	**0.009**

Table 2 Adjusted mean changes in **LAZ** during the first 24 months of life in relat

Higher maternal catalase levels at 18 weeks' gestation were associated with **slower WAZ** and **WLZ gains** from **12-24** months in **girls**, but not in **boys**.

Variable												
											M ange 7)	
												p
Log GPx											32)	0.113
Log CAT											.19)	0.137

Table 3 Adjusted mean changes in **WLZ** during the first 24 months of life in relation to maternal antioxidant levels at **18** weeks' gestation

Variable	Boys						Girls					
	0-6M WLZ change (n=50)		6-12M WLZ change (n=50)		12-24M WLZ change (n=49)		0-6M WLZ change (n=58)		6-12M WLZ change (n=58)		12-24M WLZ change (n=57)	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Log GPx	-0.96 (-3.46, 1.53)	0.440	0.66 (-1.23, 2.54)	0.486	-0.82 (-3.33, 1.69)	0.513	0.51 (-1.17, 2.20)	0.543	-0.19 (-1.34, 0.95)	0.736	0.06 (-1.48, 1.60)	0.935
Log CAT	-0.67 (-2.06, 0.71)	0.331	0.09 (-0.96, 1.14)	0.863	-0.04 (-1.51, 1.43)	0.955	0.37 (-0.44, 1.17)	0.365	-0.51 (-1.04, 0.03)	0.062	**-0.73 (-1.45, -0.02)**	**0.045**

Table 1 Adjusted mean changes in **WAZ** during the first 24 months of life in relation to maternal antioxidant levels at **34** weeks' gestation

Higher maternal catalase levels at 34 weeks' gestation were associated with **slower LAZ** and **WLZ gains** from **6-12** months in **boys**, but not in **girls**.

Variable												
												4M hange 6)
												p
Log GPx												0.172
Log CAT												0.669

Table 2 Adjusted mean changes in **LAZ** during the first 24 months of life in relation to maternal antioxidant levels at **34** weeks' gestation

Variable	Boys						Girls					
	0-6M LAZ change (n=49)		6-12M LAZ change (n=49)		12-24M LAZ change (n=48)		0-6M LAZ change (n=57)		6-12M LAZ change (n=57)		12-24M LAZ change (n=56)	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Log GPx	-1.38 (-3.59, 0.83)	0.214	-0.60 (-1.30, 1.10)	0.481	-1.18 (-3.58, 1.21)	0.324	0.19 (-1.33, 1.71)	0.803	-0.38 (-1.75, 1.00)	0.586	0.51 (-1.09, 2.11)	0.523
Log CAT	0.05 (-1.34, 1.43)	0.945	**-1.16 (-2.15, -0.18)**	**0.022**	0.90 (-0.58, 2.37)	0.226	0.05 (-1.32, 1.42)	0.940	0.20 (-1.04, 1.44)	0.750	0.12 (-1.31, 1.55)	0.863

Table 3 Adjusted mean changes in **WLZ** during the first 24 months of life in relation to maternal antioxidant levels at **34** weeks' gestation

Variable	Boys						Girls					
	0-6M WLZ change (n=49)		6-12M WLZ change (n=49)		12-24M WLZ change (n=48)		0-6M WLZ change (n=57)		6-12M WLZ change (n=57)		12-24M WLZ change (n=56)	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Log GPx	-1.23 (-3.70, 1.24)	0.321	-0.53 (-2.22, 1.15)	0.526	-0.28 (-2.80, 2.23)	0.821	0.06 (-1.49, 1.62)	0.934	-0.45 (-1.50, 0.60)	0.397	0.49 (-0.91, 1.89)	0.481
Log CAT	-0.99 (-2.47, 0.49)	0.184	0.44 (-0.57, 1.46)	0.381	**-1.68 (-3.10, -0.26)**	**0.022**	0.52 (-0.89, 1.94)	0.461	-0.33 (-1.29, 0.64)	0.496	0.50 (-0.78, 1.78)	0.434

Conclusion

- Longitudinal infant growth responses to maternal antioxidant levels during pregnancy **differ** among boys and girls.

- **Catalase** is suggested as a potential biomarker in influencing growth and obesity development in infants at different age intervals.

- These findings are important to provide evidence on planning and implementing effective **preventive strategy** which is **gender specific** in combating childhood obesity epidemic.

Summary

- The influence of maternal oxidative stress levels on **total body fat** during pregnancy was underlined.
- Oxidative stress in pregnancy **did not mediate** the relationships between gestational weight gain and postpartum weight retention.
- High **DNA damage** in the 2nd trimester was related to **reduced infant adiposity** during the first year of life.
- There is a **gender-specific growth response** to maternal antioxidant status in pregnancy

Thank You