NFS 484/ 1484 Assignment 1

Due: 4:30 pm on October 9, 2014

Background:

The developmental origins of health and disease hypothesis (DOHaD) states that nutrition and environmental exposures during the critical periods of prenatal and postnatal development may influence developmental pathways and induce permanent changes in metabolism and susceptibility to chronic diseases, such as the metabolic syndrome. The metabolic syndrome constitutes a cluster of symptoms, such as insulin resistance and central adiposity, which predispose individuals to type 2 diabetes (T2DM) and cardiovascular disease (CVD), leading causes of death in developed countries. Despite extensive epidemiological data, the underlying biological mechanisms of the DOHaD are poorly understood. One hypothesis, the "thrifty phenotype" hypothesis, postulates that maternal undernutrition during pregnancy or the early postnatal period alters fetal organ development. An undernourished fetus will ensure its survival by directing nutrients towards survival organs, such as the brain and heart, while compromising nutrient delivery to other organs, such as the pancreas and kidney. The reallocation of nutrients may lead to the failure of compromised organs, increasing an individual's risk of developing components of the metabolic syndrome. Epigenetic regulation is another potential biological mechanism that is currently being studied. Early exposures may induce long term changes in gene expression via epigenetic regulation, thereby influencing disease risk. A particular gene of interest is the PPARγ gene which encodes the nuclear receptor protein, PPARγ, a type of nuclear regulatory protein involved in the transcription of genes regulating glucose and fat metabolism. PPARγs lower the risk diabetes and hyperlipidemia because they lower blood levels of glucose and triglycerides, without increasing pancreatic insulin secretion.

Study 1: (Value 18/50 marks)

An *in vivo* study was conducted to evaluate the effects of maternal nutrient restriction in early gestation on the glucose-insulin dynamics and adiposity of the offspring in adulthood. Pregnant ewes were subjected to a nutrient-restricted (NR: 50% National Research Council (NRC) recommendations (50% food restriction)) or a control (C: 100% NRC recommendations) diet during gestational days 28-78 [gestational length approx 150 days, so NR or C during much of the first half of pregnancy]. Both the NR and C groups were then fed a 100% NRC recommended diet from gestational day 79 throughout the remainder of gestation and lactation. Female lambs from each litter were reared on a 100% NRC recommended diet for 6 years. At 6 years of age, ewes from both maternal treatment groups were adapted from the standard diet to a highly palatable experimental diet, which increased intake over an 11 week intervention period. The experimental diet was offered *ad libitum* {food available at all times in unrestricted amounts}. Fasted baseline plasma glucose and insulin concentrations were measured before and after the 11 week feeding intervention. There were no significant differences in total food intake, fasting plasma glucose, and insulin levels between the two groups; however, the NR offspring had a greater % increase in body weight and a greater efficiency of body weight gain (kg BW gain/kg feed consumed) relative to the C offspring. A frequently sampled intravenous glucose tolerance (FSIGT) test was also performed prior to and following the 11 week feeding intervention. Insulin sensitivity (SI; higher numbers reflect greater tissue glucose uptake in response to insulin), acute insulin response to a glucose load (AIRg: insulin release in first 10 minutes after a glucose (250 mg/kg body weight) injection) and disposition index (DI: measure

of the ability of the pancreatic β-cells to increase insulin secretion in the face of decreased insulin sensitivity, with a higher DI indicating greater insulin secretion in response to a glucose load over both first and second phase insulin secretion (chronic excess insulin secretion can lead to pancreatic exhaustion and subsequent glucose intolerance)) were measured. Blood samples were collected every two weeks during the feeding period and plasma insulin and insulin to glucose ratio were measured. To determine the impact of maternal nutrient restriction on gene expression, mRNA expression of peroxisome proliferator-activated receptor γ (PPARγ: a transcriptional regulator that modulates glucose and fat metabolism) in hepatic tissue was measured.

Table 1: Glucose and insulin dynamics of 6-yr-old female offspring born to control (C) and nutrient restricted (NR) mothers in response to the *ad libitum* feeding period

Measure	C - Control (n= 4)	NR- Nutrient Restricted (n= 4)	P-value
Insulin sensitivity (SI), $X10^{-4}$ $mIU^{-1}\cdot1\cdot min^{-1}$	9.3 ± 1.5	5.7 ± 0.7	0.049
Acute Insulin response to glucose (AIRg), $mIU\cdot l^{-1}\cdot min$	32.9 ± 7.7	144 ± 23.0	0.001
Disposition Index (DI)	322 ± 99.1	727 ± 77.0	0.009

Data are expressed as means ± SE. SI, AIRg and DI were unaffected by time over the 11 week feeding period and were therefore presented as an average measure obtained before and after the feeding trial. P-values denote significant differences between NR and C offspring.

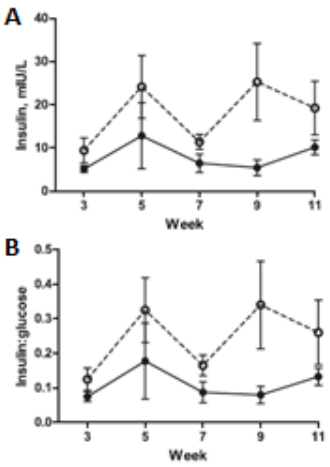

Figure 1: Plasma (A) insulin and (B) insulin to glucose ratio of control (C) female offspring (closed circles) and nutrient restricted (NR) offspring (open circles) during *ad libitum* feeding. Insulin and insulin to glucose ratio were greater overall in NR than C offspring (P=0.001).

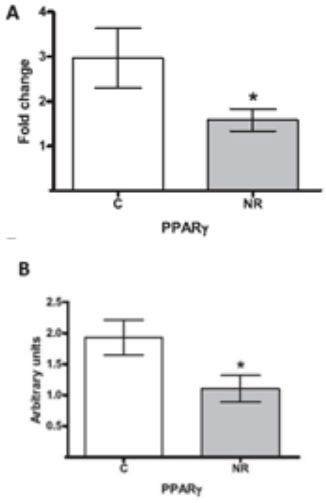

Figure 2: Relative mRNA expression (A) and protein levels (B) of PPARγ, in livers from aged female offspring of control (C, white bars) and nutrient restricted (NR, gray bars) dams. *P<0.05

Questions:

1. Describe Table 1 and Figure 1. (4/50)

2. Describe the relative mRNA expression and protein abundance of hepatic PPARγ in C and NR ewes (Figure 2). (2/50)

3. Drawing on the data in Table 1 and Figure 1, discuss group differences (NR vs. C) that you would expect to see in the cystolic/plasma membrane GLUT4 transporter locations following the glucose load. Be certain to include in your answer how you are using the data in the table and figure to defend your answer. (4/50)

4. Based on the background information, study results, lecture material and your basic understanding of the regulation of energy metabolism, discuss how maternal undernutrition during pregnancy can increase the offspring's risk of developing obesity and type 2 diabetes mellitus in adulthood. (8/50)

Study 2: (Value 32/50 marks)

A retrospective observational study was conducted to assess the effect of early life nutrition on metabolic phenotype and gene expression in childhood. 237 children (aged 2-9 years) were recruited to take part in the study. Type of feeding during infancy (breast feeding or formula feeding) was recorded by parental recall. Each child underwent a physical assessment and a blood sample was drawn. Weight and height were recorded for calculation of BMI. HOMA-IR (an indicator of insulin resistance - the higher the number, the more insulin resistant the individual [reverse measure to insulin sensitivity]) and levels of circulating triglycerides were measured. Additionally, PPARγ mRNA expression was measured in peripheral blood cells.

Table 2. Odds ratio of being overweight (BMI>75th percentile), having high HOMA-IR (>75th percentile) or high circulating triglycerides (>75th percentile) in breastfed vs. formula-fed children. Data represent the number of subjects (n) and the percentage of subjects within each group (%) >75th percentile. Odds ratios (OR) and corresponding P-values (*P*) for each comparison are indicated.

	Formula- Fed children (n=110)	**Breastfed children (n=127)**	
	n (%)	**n (%)**	**OR (*P*)**
BMI >75 percentile	34 (30.9)	26 (20.5)	0.575 (0.045)
HOMA-IR > 75th percentile	23 (25.6)	20 (19.4)	0.702 (0.041)
Triglycerides > 75th percentile	34 (32.4)	20 (17.2)	0.435 (0.007)

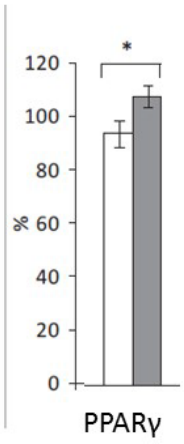

Figure 3. Expression levels in total blood cells of PPARγ in formula-fed (white bar) and breastfed (gray bar) children. Results are mean ± SEM. *P*<0.05 by Student's t-test.

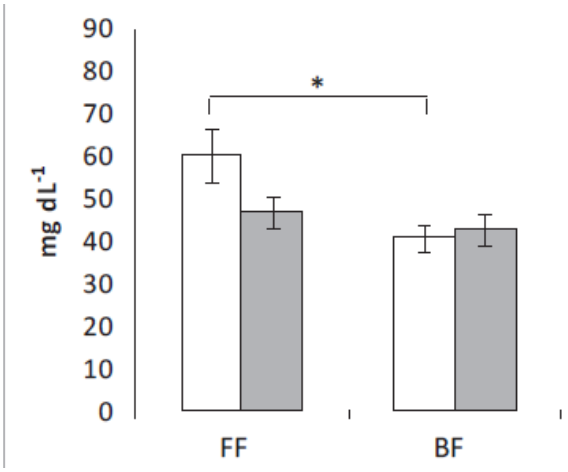

Figure 4. Triglyceride levels in children with high (grey bars) and low (white bars) expression levels of PPARγ in blood cells. Results are mean ± SEM. *P*<0.05 by Student's t-test.

Questions:

5. Describe the results from Table 2. (2/50 marks)

6. Describe the results presented in Figure 3. (1/50 marks)

7. In no more than 2-3 sentences and drawing on assignment background information, discuss how PPARγ expression in Study 2 may have influenced levels of circulating triglycerides in response to type of feeding during infancy (Figure 4). (2/50 marks)

8. What are the major similarities and differences in the designs of study 1 and 2? What inferences can be made from the results of the different study designs with regards to nutrition during prenatal and postnatal development? [Do not include species difference in your answer and assume that observations in ewes in study 1 would be reflected in a human study.] (3/50 marks)

9. Drawing on the results of studies 1 and 2, predict the effect of maternal undernutrition on the offspring's body weight and composition (BMI and % body fat) at the end of the study and the factor(s) that may be mediating this? (4/50 marks)

10. Based on all of the information provided in this assignment, what role do maternal and infant nutrition play in the etiology of the metabolic syndrome? Be certain to discuss the metabolic effects of maternal and infant nutrition reported in studies 1 and 2 in your answer (8/50 marks)

11. You are a nutritional consultant working in an impoverished area. You have been tasked with the job of implementing a program to improve the health of children in this community. You are given two options: 1) provide nutritious and adequate meals to pregnant mothers; or 2) provide high quality infant formulas which more closely resemble the nutrient content of breast milk compared to the lower quality brands currently being used. Which program would you implement? Defend your answer using the data and background information in this assignment, as well as, information from class lectures. [There is no *a priori* correct answer and either option can be defended. You will be graded on your ability to take a stance and defend it based on the totality of material in this assignment and general knowledge acquired during class lectures.] (12/50 marks)