| Q1 | Q2 | Q3 | Total |
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Name Student ID number

NUTR*4320 – Nutrition and the Metabolic Control of Disease
Midterm Examination – February 25, 2014

Answer **ALL** of the questions in the space provided.

Define **ALL** abbreviations upon first use.

**Each question is worth 15 marks and the total marks for the examination is 45.**

1. Amelia was adopted at age 2 months from an orphanage in Bolitin Romania. Cathy and John Gustavsson brought her back to Mississauga, Ontario where they currently reside. At her adoption screening Amelia was severely underweight, had microcephaly and was suffering from daily seizures. She is fair skinned and blue-eyed. There is virtually no information on her family background or her prenatal or post-natal experiences. Keen to give their daughter the best possible life in Canada, Cathy and John have come to the Hospital for Sick Children in Toronto, to determine the reasons for Amelia's ill health and what can be done to help her.

a) Do you have a preliminary diagnosis for Amelia?
Classic Phenylketonuria (PKU). (1 mark).

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b) Assuming full access to a clinical research laboratory, what tests would you do to confirm your diagnosis and the form of the disease? What would the outcomes of these tests show?
First measure blood phe levels using fluorometry or HPLC/MS. ( 1 mark). This would likely show a phe concentration higher than 1.2 mM (1200 µM). (1/2 mark). Then we would do a $BH_4$ loading test (1/2 mark) of 20 mg/kg/day and measure the changes in blood phe (1/2 mark). If the decrease in $BH_4$ was greater than 30% this would be considered $BH_4$ Responsive PKU. (1/2 mark). If the decrease in blood phe in the loading test is less than 20%, this would indicate it is probably a defect in the phenylalanine hydroxylase (PAH) enzyme. (1/2 mark). If the PKU is sensitive to $BH_4$ it could be either a defect in PAH or $BH_4$ synthesis or regeneration (1/2 mark). To distinguish, you would measure neurotransmitters; A defect in $BH_4$ would show abnormal levels that improved with $BH_4$ treatment. (1/2 mark).

Alternatively the students may choose to do PCR to identify mutations in either PAH or $BH_4$ synthesis or regeneration. (1 mark). They may also do *in vitro* and *in vivo* testing. This would start with collecting a blood sample or liver biopsy and measuring the conversion of phe to tyr in the absence and presence of $BH_4$. (1 mark). If it was a defect in PAH it would show little to no activity in the absence or presence of $BH_4$. (1/2 mark). If it was a $BH_4$ defect the activity would look like control in the presence of $BH_4$ and low in its absence. (1/2 mark)

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c) What pathological mechanisms might be at work to produce the current symptoms Amelia is expressing and what other deficits might you expect to find upon examination and follow-up?

Small head circumference is typical of untreated PKU (1/2 mark), where brain growth and myelination are severely inhibited in the presence of high phe and phe metabolites such as phenylpyruvate and phenylactate (1 mark). The impaired growth is likely a result of both intrauterine growth retardation and phe toxicity in the early post-natal period (1/2 mark). The seizures likely result from abnormal brain morphology and impaired production of catecholamines and other neurotransmitters because of an inability to synthesis tyrosine and thus dopamine, epinephrine and norepinephrine and possibly serotonin (1 mark). The excess phe competes with tyr and other large neutral amino acids for uptake into the brain so they cannot be converted.(1/2 mark). Also phenylpyruvate interferes with synthesis of homogentisate from existing tyr leading to impaired synthesis of the fatty acid desaturase cofactor alphatocopherol quinone (1 mark) leading to impaired eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) synthesis and impaired cognitive function including mental retardation (0.5 marks).

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d) What treatment strategy would you propose to Cathy and John? What are Amelia's long term prospects with respect to growth and development?

Unfortunately a lot of permanent damage has been done already because of the 8 weeks exposure to high phe (1/2 mark). Thus while she may not achieve a totally normal IQ (100) she may get close (1/2 mark). However, further impairments can be prevented with the introduction of a low protein diet with just sufficient phe to meet the basic requirement (200-500 mg/d) (1/2 mark). This would be achieved by introduction of a PKU formula supplemented with tyr, branched chain amino acids, large neutral amino acids and EPA and DHA (1 mark). She should avoid negative N or energy balance to prevent mobilization of protein stores leading to excess phe in the circulation (1/2 mark).

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e) Assuming Amelia largely "recovers" from her current problems are there any issues for her future or those of her potential children to be concerned about?

Because Amelia has Classic PKU she must stay on the diet for life. (1/2 mark). This will avoid the loss of cognitive functions she may have gained during treatment and higher integrative/special functioning (1 mark). Lifetime phe restriction will also avoid Maternal PKU Syndrome which will occur if her offspring are exposed to high fetal phe during development. (1/2 mark). Maternal PKU Syndrome leads to permanent and severe mental retardation and cardiac defects, regardless of the genotype of the baby. (1 mark). Because she carries two mutant copies of PAH or a $BH_4$ enzyme she may also want to consider prenatal genetic screening so that in the event that the fetus is also PKU, dietary management can begin immediately at birth. (1/2 mark).

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2. Fourteen year-old Trevor is the youngest of Philip and Martha's three children (16 year-old Kaitlin and 18 year-old Michael). Kaitlin has type-I Diabetes Mellitus that was diagnosed at age 6 and has been well controlled since that time. Trevor recently started high school and seems to be having some trouble adjusting. He is easily irritated and appears to be losing a significant amount of weight. In addition to the usual acne associated with puberty, Trevor appears to have developed some sort of eczema or dermatitis. He constantly complains of stomach aches and never feels like eating.

a) Do you have a diagnosis for Trevor?

/1 Celiac Disease. (1 mark).

b) What are the likely contributing factors that have resulted in disease presentation at this particular time? What are the etiological factors, both genetic and environmental that contribute to the phenotype? Describe the sequence of events (including pathophysiology) that most likely led to Trevor's current symptoms. What tests would you do to confirm your diagnosis?

This is latent onset celiac disease because it did not present during the first few years of life. (1/2 mark). It has likely been precipitated by the stress associated with changing schools and that puberty is in full swing (1 mark). He may also have changed his diet to include more prolamine-rich foods but this is not necessarily the case (1/2 mark). Because Trevor's sister has type-I diabetes which is autoimmune in nature (1/2 mark), this suggests that he has genetic susceptibility probably at the Class II MHC (or HLA) locus; most commonly he would have the DQ2 or DQ8 haplotype (1 mark). In addition to needing the permissive genetic background, Trevor would also have to been exposes to gluten in the recent past (1/2 mark). The hypothesis goes that some kind of irritation in the gut (possibly stress hormones) or low grade inflammation due to infection or dietary components, causes a weakening in the connections between mucosal cells leading to increased permeability of the gut wall (1 mark). This allows partially digested gluten peptides to cross the mucosal cell layer and enter the villus where they come in contact with antigen presenting cells (usually macrophages or dendritic cells) where the peptide is engulfed and then presented on the APC cell surface in the context of Class II antigens. (1 mark). If there is a T-cell hanging around that has the capacity to respond to this antigen presenting cell at just the right time, it will generate a proliferative and activation response in the T-cell leading to production of cytokines and eicosanoids that are proinflammatory (i.e. TNFα and INFγ) (1 mark). These cytokines etc stimulate matrix metalloproteinase production by resident cells which induce the mucosal cells to lose their connections to each other and go through apoptosis leading to shedding of the mucosa and villus shortening (1/2 mark). The tissue tries to compensate by increasing proliferation leading to crypt hyperplasia (1/2 mark). B cells are recruited as are other immune cells and exacerbate the reactions, B cells begin by making anti-gliadin antibodies and migrate to the peripheral lymphoid organs to produce IgA and other immunoglobulin species (1/2 mark). The mucosal shedding causes additional damage leading to exposure of the endomysium and allows tissue transglutaminase to be released and available for autoimmune antibody production through other T and B cell population expansion (1/2 mark). Loss of the mucosa causes generalized malnutrition leading to weight loss and secondary lactose intolerance which could contribute to the stomach aches (1/2 mark). The dermatitis is an expression of celiac disease in the skin. These lesions would contain IgA and this could be measured or a blood titre of high IgA anti-endomysial or anti-tissue transglutaminase would confirm diagnosis (1/2 mark). Could do a duodenal biopsy but this is not necessary. (1/2 mark).

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b) What is the treatment protocol for Trevor? How would you monitor compliance? What risks does non-compliance pose?

Trevor must absolutely avoid all possible sources of gluten or related prolamines (tritical family species) including wheat, barley, rye (1 mark). The gluten free diet (GFD) can usually include oats, corn, rice and other starches (1 mark). Trevor must read labels carefully as lots of vitamins, medications, sauces and spices contain hidden gluten (1/2 mark). You could monitor compliance by having him complete a food diary and following the antibody titre for anti-TG IgA, which should dramatically decrease after a few months of the GFD (1 mark). Non-compliance would worsen the symptoms of malnutrition (1/2 mark) and lead to increased risk of anemia, (1/2 mark) and high risk of osteoporosis. (1/2 mark). Non-compliance also leads to a dramatic elevation in the risk of life-threatening Enteropathy Associated T-cell lymphoma and small intestinal carcinomas (1 mark).

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3. African American Malcolm Smith plays linebacker for the Seattle Seahawks of National Football League. Malcolm is 24 years old and weighs 230 lbs. Despite long hours of training and a relatively high level of fitness both in the on- and off-seasons, Malcolm suffers from hypertension and has a family history of obesity and type II diabetes. His diet consists of very high levels of meat protein and simple carbohydrates such as bread, pasta and rice. As one of the members of his healthcare team, you suggest to Malcolm that he may want to increase his dairy consumption. He tells you that this will be difficult as he is allergic to milk.

a) What is the likelihood that Malcolm is allergic to milk? What is more likely the reason for his symptoms when he consumes dairy? What might those symptoms look like? What genotype is Malcolm likely to carry? What mechanisms typically produce symptoms?

Unlikely that he is allergic to milk; this is less common than intolerance to lactose which is the major sugar of mammalian milks (1 mark). If he were allergic, this would have been present since early after birth and means he would not have consumed milk at all, rather than probably just decreasing his consumption after weaning. (1/2 mark). Malcolm most likely experiences typical symptoms of lactose intolerance when consuming high lactose dairy products. This would include stomach ache, cramping, diarrhea and possibly nausea 5-60 min after ingestion (2 marks). Because of his African American heritage, Malcolm most likely has the LAC*R/LAC*R genotype ( 1 mark) such that he would have had high lactase expression during infancy and gradually lost expression as he entered childhood and adolescence (1 mark). Undigested lactose is osmotically active and attracts water into the lumen of the intestine increasing the luminal volume (1 mark). It is also used as a fuel by resident microorganisms producing end products such as short chain fatty acids, $CO_2$ and other gases that cause stretching of the smooth muscle wall (1 mark). The increase in stretch and lumen volume causes peristalsis which produces the cramping and diarrhea associated with lactose consumption (1 mark). The gases could also produced increased flatulence (1/2 mark).

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b) Assuming you had access to a full research laboratory what tests might you do to find out whether Malcolm has a problem with milk or one of its components? What are the limitations of this technique? Describe the procedures.

If it was an allergy, a skin test would show an immune reaction. (1 mark). The test for lactose intolerance is the hydrogen breath test. (1/2 mark). If you give a large lactose load and measure $H_2$ in the breath over the next few hours, and it exceeds 20 ppm, this is considered lactose maldigestion. (1/2 mark). The limitation of this test is that it overdiagnoses lactose intolerance compared to doing the same test with milk. (1/2 mark). It is thought that milk is a more appropriate challenge since it presents lactose in the form to which one would normally be exposed. (1/2 mark). Milk exposure results in slower gastric emptying and contains components that are thought to improve the metabolism of the lactose. (1/2 mark).

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c) Why might you be suggesting dairy to Malcolm, as part of a normal healthy diet for him in particular? If he does currently have "problems" with milk, how might you resolve this problem? How does this approach work?

Persons of African American decent are often genetically predisposed to have higher incidence of hypertension and related cardiovascular disease and type II diabetes. (1/2 mark). In particular the DASH (dietary approaches to stop hypertension) diet contains foods low in fat, high in fruits and vegetables and considerable dairy (between 1 and 3 servings per day) so this is likely why you are recommending it to Malcolm (1 mark). It is thought that the typical diets of this population, and persons who more frequently have lactose intolerance are low in dietary calcium and that calcium deficiency contributes to higher risk of hypertension and obesity related disorders (1/2 mark). Research has shown that most persons, even those with the LAC*R/LAC*R genotype can tolerate milk products when given as part of a meal or meals (1/2 marks). To resolve his problem, very gradually add milk products to his normal diet regimen. You would start with low lactose foods like yogurt and gradually increase the "dose" of dairy over a period of weeks (1/2 mark). You could also start with some LactAID which provides an exogenous source of lactase to help with lactose breakdown (1/2 mark). Gradually exposure will give the microflora a chance to adapt and allow the "favourable" organisms to increase in population. These new species help because they either do not produce the "toxic" metabolites (SCFAs, $C0_2$, methane) (1/2 mark), alter the pH of the gut and/or provide β-galactosidase to breakdown lactose so that the monomeric sugars glucose and galactose can be absorbed across the mucosa via glucose transporters (1/2 mark).

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