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Department of Nutritional Sciences
Mid-Term Examination
December 11, 2013

Advanced Nutrition
NFS 484H1 F/ 1484H1 F
Duration – 2 hours

General Instructions:

1. This is an open book examination; therefore students may use any aids that have been brought into the examination room.

2. The examination will be marked out of 50; 23 marks for study 1 and 27 marks for study 2.

3. Students must answer all questions in all parts of the examination.

4. All answers should be clearly written in the answer booklets provided. Please provide your answer on the right-hand side of the page only. It will be assumed that the left-hand side of the page is used for note making purposes only and material appearing on this side of the page will not be read or graded.

Background Information

Vitamin D is a fat-soluble vitamin stored in adipose tissue. Exogenous food sources include fortified milk and cereals, as well as flesh of fatty fish such as sardines, salmon, and tuna. Vitamin D is also produced endogenously by the body when the skin is exposed to the sun's UV rays. Promising evidence in the literature has implicated Vitamin D in the prevention and alleviation of diabetes risk factors. In particular, Vitamin D supplementation may play a role in chronic inflammation, glycemic control, and insulin resistance/insulin sensitivity; however, no clear mechanism has been described.

Type 2 Diabetes Mellitus (T2DM) is characterized by prevailing insulin resistance, which causes increased levels of circulating insulin and blood glucose. Previous research has demonstrated vitamin D deficiency in people living with T2DM. South Asian women may be of particular interest since they are at high risk for developing T2DM and have been shown to have a higher prevalence of vitamin D deficiency. The Vitamin D receptor (VDR) gene encodes the nuclear receptor protein for vitamin D3. Decreased expression of VDR may lead to decreased transcription of vitamin D related genes. The underlying mechanism(s) responsible for the vitamin D's role in the prevention and alleviation of diabetes risk factors is not fully understood and appears complex.

Study 1 (Value 23/50)

Study Design

A randomized, controlled, double-blinded intervention was performed to investigate the effect of vitamin D supplementation on insulin resistance (IR) in 235 women of South Asian descent living in New Zealand. All participants were insulin resistant and vitamin D deficient, indicated by homeostasis model assessment 1 (HOMA1) >1.93 and serum 25-hydroxyvitamin D [25(OH)D] concentration <50 nmol/l respectively. HOMA1 is a model which uses a linear equation based on pairing fasting serum glucose (FSG) and fasting serum insulin (FSI) to establish measures of insulin resistance. 4000 IU of vitamin D3 (Cholecalciferol) or placebo was administered daily for 6 months. 25(OH)D, fasting serum blood glucose, fasting serum insulin, and C-peptide were obtained at baseline and 6 months and HOMA2%S, HOMA2%B, and HOMA2-IR were calculated at baseline and 6 months (HOMA%S indicates insulin sensitivity, HOMA2%B indicates beta cell function, and HOMA2-IR indicates insulin resistance). Higher HOMA values indicate more insulin sensitivity, more beta cell function and more insulin resistance, respectively.

Questions

1. Describe the results from Table 1. (6/50 marks)

- **25(OH) D** was significantly higher at 6 months than baseline within the Vitamin D and Placebo groups. The positive change in 25(OH) D was significantly greater in the Vitamin D group than the Placebo group.

- **HOMA2%S** was significantly higher at 6 months than baseline within the Vitamin D group, but there was no significant change from baseline to 6 months in the Placebo group. The change in HOMA2%S was significantly greater in the Vitamin D group than the Placebo group.
- There was no significant change in **HOMA2%B** from baseline to 6 months in either the Vitamin D or Placebo groups and no significant difference between changes in each group.
- **HOMA2-IR** in the Vitamin D group was significantly lower at 6 months than at baseline. There was no significant difference in HOMA2-IR from baseline to 6 months in the Placebo group. The negative **HOMA2-IR** change was significantly greater in the Vitamin D group when compared to the change in the Placebo group.
- **FSI** was significantly lower at 6 months than at baseline in the Vitamin D group, but did not change significantly within the Placebo group. The negative **FSI** change was significantly greater in the Vitamin D group when compared to the change in the Placebo group.
- There was no significant change in **FSG** from baseline to 6 months within either of the groups and no significant difference in the change in FSG between either of the groups.
- There was no significant change in **C-peptide** from baseline to end of study in both Vitamin D and placebo groups. There was no significant difference in **C-peptide** changes in the Vitamin D group when compared to the change in the Placebo group.

2. Describe the results from Figure 1. (2/50 marks)

- Subjects had significantly higher mean serum 25(OH)D and HOMA2%S at 6 months compared to baseline.

3. Using background information and the results from this study, provide a possible explanation for the results seen in fasting serum glucose. Also, if this population is presented with a glucose load how might the response to the load differ between the vitamin D supplemented group and placebo group? (7/50 marks)

- All study subjects were insulin resistant, but did not have diabetes therefore we would not necessarily expect their FSG to be elevated. This explains why there was no significant change in FSG from baseline to end of study in both Vitamin D and placebo groups (Table 1) and there was no significant difference in FSG changes in the Vitamin D group when compared to the change in the Placebo group (Table 1). Also, insulin secretion was not compromised (Table 1), shown by no changes in C-peptide levels (C-peptide is a marker of insulin secretion). Due to this reason we saw no change in FSG and there was no added benefit of Vitamin D

- If presented with a glucose load we would expect:

- At 6 months there was no change in FSG – it remained within normal limits, however, FSI was reduced significantly after vitamin D supplementation.
- This reduction in FSI may be a marker of improved insulin sensitivity resulting in improved uptake of glucose into the cells.
- This was further supported by findings of significantly increased insulin sensitivity (HOMA2%S) and reduced insulin resistance (HOMA-IR). There was no change in Beta cell function (HOMA2%B) or insulin secretion (c-peptide).
- If faced with a glucose load the placebo group would have a higher insulin and higher blood glucose level for a longer period of time (greater area-under-the-curve, AUC) whereas the vitamin D supplemented group would be better able to cope with this challenge, bringing glucose levels back to normal in a shorter period of time. This is due to the observed benefit of vitamin D supplementation on improved insulin sensitivity (HOMA2%S) and reduced insulin resistance (HOMA-IR).

4. Based on the study results, background information and your general knowledge of diabetes risk factors, propose a mechanism by which vitamin D status affects risk of type 2 diabetes. (8/50 marks)

- Type 2 diabetes is characterized by high blood glucose and insulin levels due to insulin resistance (background).
- Studies have shown a high prevalence of vitamin D deficiency in diabetic individuals (background).
- Vitamin D supplementation increased serum Vitamin D levels and subsequently improved insulin sensitivity as seen by HOMA2 %S, and decreased insulin resistance (HOMA2-IR). Fasting insulin levels decreased with Vitamin D supplementation which is expected with resolution of insulin resistance (Table 1).
- Fasting glucose levels stayed the same in the normal blood glucose range. These individuals were insulin resistant but not diabetic so it is expected that blood glucose levels would be normal in a fasting state, but we might suspect that postprandial blood glucose would be elevated for a longer period of time (impaired glucose tolerance). From the results of this study we would suspect that vitamin D supplementation would improve this glucose tolerance through improved insulin sensitivity.
- Vitamin D supplementation did not have an effect on insulin secretion (c peptide and HOMA2% B).
- Therefore Vitamin D supplementation could reduce the risk of type 2 diabetes through improved insulin sensitivity/ reduced insulin resistance.

Table 1: Changes from baseline to endpoint of primary outcomes within vitamin D and placebo groups, and between groups

	Vitamin D (*n* 42)		Placebo (*n* 39)		
	Median	*P* value difference within group	Median	*P* value difference within group	*P* value (difference between groups)
25(OH)D (nmol/l)					
Baseline	21	<0·001	19	<0·001	
End	80		29		
Change: end − baseline	49		8		<0·001
HOMA2 %S					
Baseline	60·6	0·01	65·9	0·69	
End	68·0		60·4		
Change: end − baseline	5·9		−5·9		0·003
HOMA2 %B					
Baseline	163	0·17	144	0·39	
End	152		149		
Change: end − baseline	−11·2		0·6		0·09
HOMA2-IR					
Baseline	1·7	0·03	1·5	0·27	
End	1·5		1·7		
Change: end − baseline	−0·2		0·2		0·02
FSI (mU/l)*					
Baseline	13·2	0·02	11·9	0·27	
End	11·2		13·1		
Change: end − baseline	−1·3		1·1		0·02
FSG (mmol/l)					
Baseline	4·7	0·154	4·9	0·07	
End	4·8		5·0		
Change: end − baseline	0·1		0·1		0·82
C-peptide (nmol/l)					
Baseline	0·81	0·97	0·83	0·11	
End	0·81		0·86		
Change: end − baseline	−0·002		0·07		0·15

Median 25(OH)D, HOMA2%S, HOMA2%B, HOMA2-IR, FSI, and FSG. Columns 2 and 4 are the significance (P-value) for the change within each group from baseline to endpoint (significantly different if p<0.05). Column 5 is the significance of the difference between groups in the change for each variable (p <0.05 is significant). Non-parametric tests were used to compare groups and to compare baseline and endpoint measures within groups.

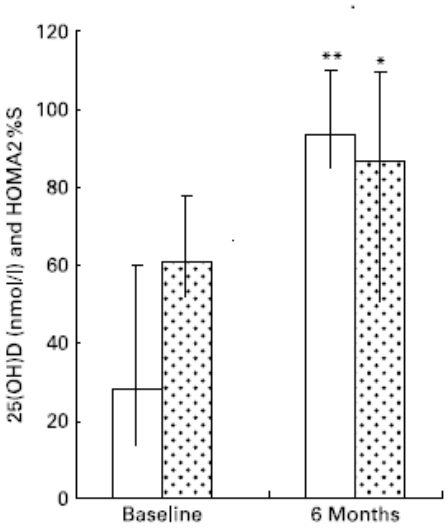

Figure1. Changes in serum 25-hydroxyvitamin D (25(OH)D) (□) and HOMA2%S (▩) over time in subjects Mean value was significantly different from that at baseline: *$P = 0.013$, **$P < 0.001$. Y-axis values include both serum 25(OH)D (mmol/l) and HOMA2%S where 100% is ideal.

Study 2 (Value 27/50)

Background

T2DM may involve an alteration in the expression of glucose transporters, such as GLUT4, which may have an impact on the uptake of glucose by the cells. Vitamin D has been linked to the pathogenesis and prevention of diabetes, but the mechanism is unclear. The present study was designed to investigate the effect of Vitamin D in the overall regulation of muscle cell glucose transporter expression.

Study Design

L6 muscle cells were grown in cell culture medium, and cell lines separated into three main groups: (1) the control (C) group, which were exposed to 8mM of glucose; (2) the IR group, which were exposed to a high glucose concentration (25 mM) and a high insulin concentration (100 nM), as seen in the insulin resistant model; and, (3) the IR + D group, which were exposed to 10^{-7} M calcitriol (the hormonally active form of Vitamin D3) within the conditions of the insulin resistant model (group 2).Subsequently, RNA was extracted and isolated in L6 muscle cells. Real-time polymerase chain reaction was used in order to quantify the nucleic acids extracted from each model. The results from Study 2 are depicted in the graphs below. Results are presented as mean ± SE. Significance was established at $p<0.05$. Figures with different letters indicate that the variables are significantly different from each other.

Questions

5. Describe the results from Figures 2, 3 and 4. (3/50 marks)

Figure 2: GLUT4 expression: Control > IR treated with calcitriol > IR (no calcitriol)

Figure 3: VDR: Control = IR treated with calcitriol > IR (no calcitriol)

Figure 4: IR expression: Control > IR treated with calcitriol > IR (no calcitriol)

6. Using the information presented in both studies, propose a mechanism by which vitamin D supplementation influences glycemic control. (8/50 marks)

- In the Type 2 Diabetes (T2DM) population, there is a high prevalence of vitamin D deficiency. People with type 2 diabetes and insufficient vitamin D stores have lower VDR expression (fig 3), which means there is decreased transcription of vitamin D related genes (background). These genes include GLUT4 genes and insulin receptor genes (Figure 2 and 4). We know both insulin receptor and GLUT4 transporters are required for glucose uptake into the cell (lecture notes), and these mechanisms are both impaired in vitamin D deficient states (Fig 2 & 4). Therefore, there is impaired cellular

glucose uptake which leads to insulin resistance, which is a key feature of T2DM. By this mechanism vitamin D deficiency would increase risk of T2DM.

- Vitamin D supplementation decreases the risk of T2DM in insulin-resistant, vitamin D deficient individuals by increasing VDR expression (Fig 3), which subsequently leads to improved GLUT4 and insulin receptor expression (Fig 2 and 4) and improved glucose uptake into the cells. This leads to improved insulin sensitivity (HOM2%S), decreased insulin resistance (HOMA2-IR), and decreased fasting insulin levels when Vitamin D is given for at least 6 months (Fig 1 and Table 1). Improving insulin sensitivity/decreased insulin resistance may lead to a decreased risk of T2DM. However, it will not decrease the risk of T2DM as much as those who are not insulin resistant (control –Fig 2, 3 & 4)
- Vitamin D supplementation does not affect insulin secretion (Table 1) and therefore does not exert its positive effects through this mechanism.

7. Given what you have learned in Study 1 and 2, the Background, and this course propose three other factors/measures that you would consider including in Study 1. (6/50 marks)

- **HgA1c:** The current Study 1 only includes fasting glucose measurements. Measuring HgA1c will give a better idea of glucose tolerance over time and account for any peaks in glucose from day to day or hour to hour. This may, for example, account for prolonged elevation after eating. I would expect HgA1c to be reduced from baseline levels after vitamin D supplementation.
- **OGTT:** Insulin resistance is traditionally identified by elevated insulin levels and a larger glucose AUC after the administration of a glucose load. This second factor can be measured using an oral glucose tolerance test (OGTT). I would expect this to be improved (i.e. smaller glucose and insulin AUC) after vitamin D supplementation.
- **Hyperinsulinemic-euglycemic Clamp:** During this procedure hyperinsulinemia is induced through a constant infusion of insulin into the vein. Plasma glucose is measured frequently and a glucose solution is infused to keep plasma glucose at a safe level. The rate of glucose infusion is considered to be a reflection of insulin sensitive glucose uptake into the cells and is the gold standard for measuring insulin sensitivity. We would suspect that the vitamin D supplemented group would have an increased rate of glucose infusion due to improvements in insulin sensitivity (Table 1, Figure1).
- **Marker of De Novo Lipogenesis (lipogenic index or imaging):** Insulin resistance is often accompanied by other markers of metabolic dysfunction such as fatty liver as a result of de novo lipogenesis. It may be useful to investigate whether vitamin D supplementation also leads to an improvement in the liver. This could be done by measuring lipogenic index (blood tests that show de novo lipogenesis) or through liver imaging which can show fat deposition in the liver. We may also consider measuring serum triglycerides which would be released in greater amounts during de novo lipogenesis.

- **Exercise vs. Vitamin D:** In this course we learned that exercise can cause GLUT4 transporters to move to the cell membrane of muscle cells leading to increased glucose uptake. This seems to be similar to the role of vitamin D supplementation in improving glucose management through the activation of GLUT4. It may be interesting to include four groups in Study 1: a placebo group, a vitamin D supplemented group, an exercise intervention group, and an exercise + vitamin D supplementation group. This would help us to identify if there is a benefit of one intervention over the other and if including both has a greater benefit than either alone.
- **Diet vs. Supplementation:** It would be ideal to avoid the need for daily supplement pills to achieve the same benefit of vitamin D. A third group in Study 1 that included a vitamin D rich diet intervention instead of supplements in a capsule form could help to identify if dietary changes alone could have the same positive effects.
- **Sun Exposure:** As discussed in the background, vitamin D can be produced endogenously with exposure to UV rays from sunlight. It may be interesting to measure estimated daily length of sun exposure and latitude to see if increased sun exposure is associated with some of the benefits of exogenous vitamin D seen in study 1 and 2.

8. Think back to the course assignment regarding the Sandy Lake First Nations community of Northern Ontario, where some individuals had a single nucleotide polymorphism at amino acid 319 (G→S) in the *HNF1A* gene which caused a decrease in pancreatic insulin production. A recent health screening program in the Sandy Lake community has found that the prevalence of vitamin D deficiency is very high. (10/50 marks)

A. What specific characteristics of the community have contributed to the development of Vitamin D deficiency? Limit to only 2-3 sentences.

B. Discuss the potential effect of vitamin D supplementation on homozygous and wild type genotype and what effect this would have on T2DM risk. Make sure to compare and contrast the impact of vitamin D supplementation in both the GG (wild type) and SS (homozygote for the SNP) groups (you do not need to consider the GS heterozygote group in your answer).

(A)

- Individuals are not hunters and gatherers anymore (Assignment 1) and do work that is more likely to take place indoors leading to decreased sun exposure and therefore vitamin D production in the skin (Background).
- We have also been told that the diet of these individuals has changed significantly (Assignment 1) which may include changes in the amount of vitamin D rich foods consumed including fish and dairy (Background).
- This population lives in Northern Ontario were UV levels (Background) are insufficient to support adequate vitamin D production during several months of the year and due to changes in diet this decreased endogenous source of vitamin D is not being compensated by increased exogenous vitamin D intake.

(B) Vitamin D supplementation would be beneficial in reducing Type II Diabetes risk in the Sandy Lake community through improved glycemic control in both the wild type and homozygous groups.

- Wild Type: Although this group does not have the reduced insulin production that we see in the homozygous individuals they do have increased diabetes risk, largely secondary to an obesogenic Western diet (1st Assignment). The recovery of vitamin D adequacy in the GG individuals would aide in improved glycemic control through the increased activation of GLUT4 glucose transporters (Figure 2) and insulin receptors (Figure 4), reducing any apparent insulin resistance (often present in obesity even without diabetes) (Table 1) and help to bring postprandial blood glucose levels back to normal through increased insulin sensitivity (Table 1 & Figure 1). Notably, as we saw in Figures 2, 3, and 4 insulin sensitivity is unlikely to improve so much that it returns to normal therefore this population will have a decreased diabetes risk from a pre-supplemented state, but risk will still be elevated in comparison to a healthy population.

- Homozygote: The vitamin D supplementation will likely have an even greater benefit to the SS group. The reduced insulin production in this group leads to poor ability to reduce blood glucose. With a recovery of good vitamin D status we may see increased expression of vitamin D receptors (Figure 3), an improvement in GLUT4 transporter expression (Figure 2) and insulin receptor (Figure 4) activation and a reduction in markers of insulin resistance (HOMA2-IR, FSI in Table 1) and improvement in insulin sensitivity (HOMA2%S in Table 1 and Figure 2). This could mean that although these individuals are producing less insulin, a recovery of vitamin D status may increase the ability of the cells to respond to the circulating insulin and therefore take in glucose, reducing hyperglycemia and the resulting T2DM risk in the homozygote individuals. As with the GG group diabetes risk will not return to that of a healthy population as insulin sensitivity will not be fully recovered (Figures 2, 3, 4) and insulin production will be impaired. Therefore, T2DM risk will be greater than that of the wild type group.

- Other Factors: As both groups will still be at a relatively increased risk of T2DM, a more inclusive approach to disease prevention is suggested. I would therefore not only provide vitamin D supplementation, but encourage intake of higher vitamin D foods so that they may at some point not require supplementation, but also encourage low glycemic index carbohydrates for a reduced postprandial glucose AUC, increased exercise to help move GLUT4 to the muscle cell membranes, and overall weight loss strategies to reduce diabetes risk.

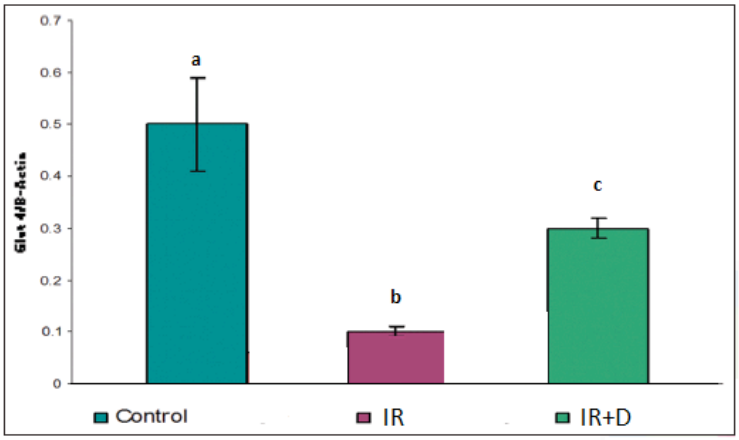

Figure 2: GLUT4 expression (Control = Normal glucose (8 mM), IR = high glucose (25 mM) + high insulin (100 nM) (Insulin Resistant model), IR+D= Insulin resistant model treated with 10^{-7} M calcitriol)

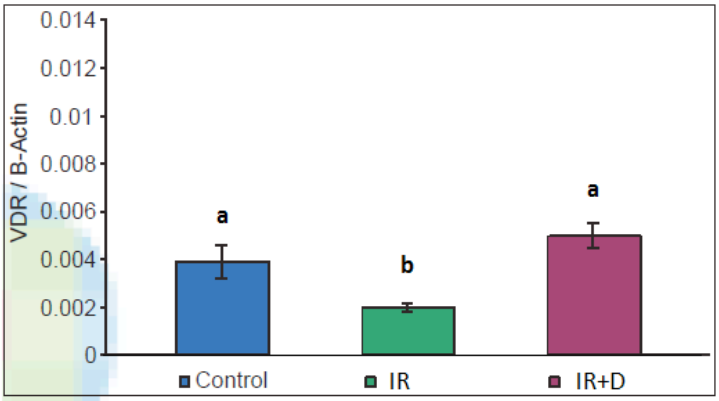

Figure 3: Vitamin D receptor (VDR) expression (Control = Normal glucose (8 mM) IR = high glucose (25 mM) + high insulin (100 nM) insulin resistant model, IR+D = insulin resistant model treated with 10^{-7} M calcitriol

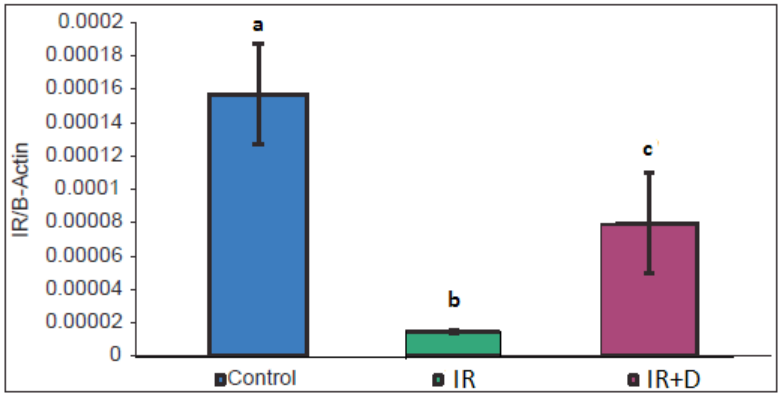

Figure 4: Insulin receptor (IR) expression (Control = Normal glucose (8 mM), IR = high glucose (25 mM) + high insulin (100 nM) (Insulin resistant model), IR+D= Insulin resistant model treated with 10^{-7} M calcitriol)