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Original research

Effect of a vitamin and mineral supplementation on glycemic status: Results from a community-based program

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ABSTRACT

Aims: Diet is a major risk factor for type 2 diabetes mellitus. As cofactors necessary for enzyme function of all metabolic pathways, vitamins and minerals have the potential to improve glucose metabolism. We investigated the effects of a nutrient intervention program on glycemic status.
Methods: We used a form of natural experiment to compare Pure North program participants (n = 1018) that received vitamin D alone (Vital 1) or vitamin D in combination with other nutrients (Vital 2) during two different time periods. Changes in 25-hydroxyvitamin D [25(OH)D], high-sensitivity C reactive protein (hs-CRP), glycated hemoglobin (HbA1c) and glycemic status were characterized over one and two years.
Results: Serum 25(OH)D concentrations increased significantly in both Vital 1 (to 111 ± 49 nmol/L) and Vital 2 (to 119 ± 52 nmol/L) over one year. HbA1c and hs-CRP were significantly reduced over time in Vital 2. Higher 25(OH)D levels after one year were associated with larger decreases in HbA1c and hs-CRP in Vital 2. At one year, 8% of Vital 2 and 16% of Vital 1 participants progressed from normoglycemia to prediabetes/diabetes, whereas 44% of Vital 2 and 8% of Vital prediabetes/diabetes subjects regressed to normoglycemia.
Conclusions: Vitamin D combined with other nutrients was associated with a reduced risk of progression to diabetes and with an increased rate of reversion to normoglycemia in high risk participants. The results suggest that nutrient supplementation regimes may provide a safe, economical and effective means for lowering diabetes risk. Further examination of this potential via randomized controlled trials is warranted.

Introduction

World-wide, 347 million people have diabetes mellitus, with type 2 diabetes constituting 90% of cases [1,2]. Diabetes is one of the most common chronic diseases in Canada, with a 70% increase in prevalence between 1999 and 2009 [3]. The prevalence of type 2 diabetes is highest in older persons, but over 50% of the affected population are of working age [3]. The economic burden associated with direct and indirect medical costs is estimated at $12.2 billion annually [4]. There are known modifiable lifestyle risk factors for type 2 diabetes that provide the opportunity for intervention and prevention [5]. The possibility of modifying the risk of diabetes by improving nutrient levels may offer a simple, safe and scalable strategy to reduce the burden of this prevalent chronic disease.

While lifestyle interventions tend to be the primary focus of diabetes prevention strategies, other strategies are also used, such as bariatric surgery or the use of pharmacological agents [6]. High intensity

programs, such as the US Diabetes Prevention Program, have demonstrated up to a 58% reduction in relative risk of diabetes [7]. However, such studies often have protocols that are labour intensive, expensive and severely limited in their capacity to be implemented in a community setting [8].

The development of an inexpensive and more easily implemented intervention program for the prevention of diabetes could play a role in improving the health of individuals worldwide. The use of nutritional supplements as part of such an intervention strategy is an area that merits investigation. The development of diabetes is preceded by abnormalities in glucose homeostasis leading to insulin resistance, glucose intolerance and eventually the development of type 2 diabetes [9]. It has been postulated that daily variation in glucose homeostasis may be aggravated by inadequate nutrient composition as many micronutrients are necessary cofactors for the proper function of enzymes involved in energy metabolism. The role of dietary supplements in glucose control has been investigated in basic research and observational studies as a

means to address inadequate nutrition and chronic disease [10,11]. Nutrients with proposed benefit in glucose homeostasis include vitamin D, vitamin K, calcium, magnesium, zinc, chromium, and omega-3 fatty acids [12,13]. Vitamin D, in particular, appears to play a significant role in the progression of diabetes, with studies linking low serum 25(OH)D levels with both insulin resistance and β-cell dysfunction [14,15]. However, randomized clinical trial evidence for individual nutrients, including vitamin D, is inconsistent; some trials show benefit while others report null results which may only reflect the differences in study design [16,17]. It is also possible that a combination of nutrients is required to derive benefit. In this study we characterize the effect of a nutritional intervention program, utilizing a natural experiment in which two supplement groups occur, on glycemic status over one and two years.

Patients and materials and methods

Study design

This study is a form of a "natural experiment" [18] where two groups were retrospectively identified, Vital 1 (Dec. 2008 to Mar 2010) and Vital 2 (Mar. 2010 to May 2012). We compare the effect of the different interventions received by the two groups on glycemic status and development of type 2 diabetes mellitus.

This was a retrospective database analysis. This study focused on participants who joined the program between December 2008 and May 2012 and was approved by the research ethics board at the University of Calgary (E-24890). Participants provided written informed consent for the use of their data for research.

The Pure North community-based program (intervention)

Pure North S'Energy Foundation is a not-for-profit wellness program based in Calgary, Alberta, Canada, that focuses on the prevention of chronic disease. The Pure North program offers lifestyle advice, education and nutritional supplements to participants. There is no inclusion/exclusion criteria for entering the program and the program does not substitute for conventional health care. The nutritional supplements provided by the Pure North program are selected to address common problems such as vitamin D deficiency.

The core tenant of the program is to achieve optimal nutritional status with a focus on physiological levels of vitamin D. All participants are encouraged to achieve a 25(OH)D level above 100 nmol/L (< 250 nmol/L); levels that can be naturally attained through regular sun exposure [19], are safe [20,21], and associated with a reduced risk of many chronic diseases including bone disease [22,23], depression [24], autoimmune disease and cancers [25,26].

Because of large inter-individual response differences to a given dose of vitamin D3, dosages were adjusted accordingly for the individual to achieve the target serum 25(OH)D level by the treating health care professional. Vitamin D3 doses were often above the upper level of intake, 4000 IU/d, to achieve the target. Vitamin D3 intake recommendations ranged from 1000 to 20,000 IU/d [26] under medical supervision.

In the program, each participant meets with a health care professional (Medical Doctor, Naturopathic Doctor or Nurse Practitioner) who provides lifestyle advice appropriate for the individual participants' health goals and current diagnoses. Dietary advice is provided as deemed appropriate, such as the DASH diet for patients with cardiovascular disease or risk factors like hypertension or hyperlipidemia. There is generally a focus on increasing vegetable and fruit intake and reducing processed foods as only 50% of the Canadian population is consuming more 5 servings of vegetables and fruit daily and the recommendation is for 7–10 servings daily. Exercise that is appropriate for the participants' health is recommended to ensure cardiovascular health and muscle strength. This applied to both the Vital 1 and Vital 2 groups.

Intervention groups

We exploit changes in the program to compare two groups that differed in their supplement intake: Vital 1 and Vital 2. The group construction was based on dates when the program difference occurred. Supplement composition was the only major difference in the program experienced by Vital 1 and Vital 2; other aspects of the program remained consistent between the two groups.

Vital 1

The Vital 1 multivitamin supplement (Table S1) was introduced to the program in December 2008 and was given in combination with a liquid vitamin D_3 supplement (1000 IU/drop; Ddrops®, Toronto, ON). Vital 1 contained 200 mg of niacin, twice daily, for a total of 400 mg/d of niacin. An informal survey of participants in the Vital 1 cohort suggested a very low compliance with the multivitamin as a result of the flushing produced by the niacin. The lack of change in serum vitamin B12 levels despite an increase in serum 25-hydroxyvitamin D [25(OH)D] levels was consistent with the low level of compliance with Vital 1 (Fig. 1), but supported compliance with vitamin D_3 supplements. Thus, the Vital 1 cohort is referred to as the "Vitamin D_3 only group."

Vital 2

Over time the Vital 1 multivitamin was reformulated, niacin was reduced to 15 mg, and the new multivitamin was named Vital 2. The Vital 2 formulation (Table S1) was introduced in March 2010. At the same time other supplements were incorporated creating the "full program." The core supplements of the full program were vitamin D_3 (1000–20,000 IU/d as needed to achieve target levels), Vital 2 (multivitamin), and omega-3 fatty acids (400 mg EPA and 200 mg DHA). Additional supplements were recommended on a discretionary basis, in response to deficiency or clinical indication, including vitamin C, magnesium, caprylic acid, PGX (PolyGlycopleX®) and probiotics. These were reported by participants and used for covariates in statistical models where appropriate.

When building the dataset, not all of the participants included in the Vital 1 and Vital 2 groups at one year are included at two years (lost to follow-up). As such, this results in slightly different baseline values and we have presented baseline versus one year and baseline versus two years for each Vital cohort separately to allow a direct comparison
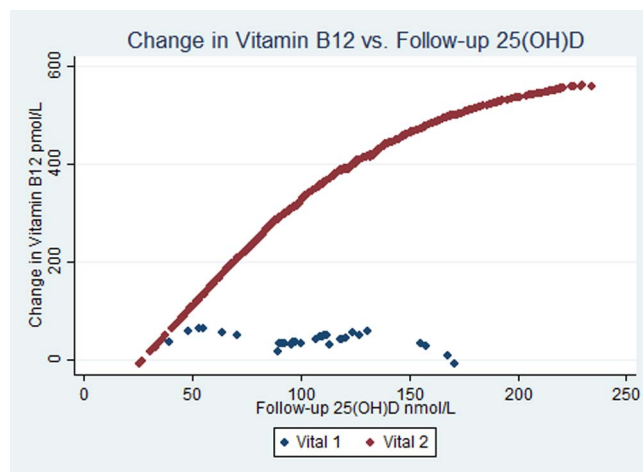

Fig. 1. Establishing Groups Vital 1 versus Vital 2. Comparison of Vitamin B12 and 25(OH)D changes at one year in each multivitamin cohort. In Vital 1 participants only vitamin D increases over one year suggesting that they are not taking the multivitamin containing vitamin B12. The proportional increase in vitamin D and B12 in Vital 2 suggests that Vital 2 participants are consuming both vitamin D and the multivitamin (containing vitamin B12) and supports the assumption of two groups.

Table 1
Baseline Characteristics of Pure North Program Participants for Vital Cohorts that Remained in the Program for each Time to Follow-up.

Characteristic	One year follow up		Two year follow up	
	Vital 1	Vital 2	Vital 1	Vital 2
	N = 223	N = 790	N = 101	N = 269
Male – No. (%)	135 (61%)	393 (50%)*	65 (64%)	123 (46%)*
Age – yr.	40.1 ± 8.2	41.1 ± 8.9	40.2 ± 7.8	41.8 ± 9.1
BMI	27.8 ± 5.3	27.1 ± 5.4	29 ± 5.9	26.4 ± 4.8*
HbA1C-%	5.51 ± 0.39	5.64 ± 0.35*	5.55 ± 0.43	5.68 ± 0.51*
Hs-CRP (mg/L)	2.08 ± 2.0	1.60 ± 1.78	2.13 ± 2.0	1.53 ± 1.7*
25(OH)D (nmol/L)	79 ± 35	87 ± 45*	77.5 ± 33	91.8 ± 48*

Values are mean ± standard deviation or mean (standard error).
Please note: Baselines differ for each year of follow-up due to discontinuation in the program.
*Indicates significant difference between Vital cohorts (two sample *t*-test, p < .05).

between baseline and follow-up (Tables 1-3).

Dataset construction and measurements

Participants included in the dataset analyzed were 25–54 years of age at entry and had baseline and follow-up measures for the assessments listed below. The sample was restricted based on hs-CRP values to include measures < 10 mg/L to avoid the influence of persons with acute inflammation. One year measures were considered at 365 ± 185 days and two year at 730 ± 185 days. Participants who reported a diagnosis of diabetes or reported taking medications for diabetes were excluded from the study.

Participants were interviewed and assessed by health care professionals at each program visit to collect demographic information, lifestyle information (including fruit/vegetable and fish consumption, exercise, tobacco and alcohol use) medical history, medication use (including diabetic medications), biometric measurements were obtained (including waist circumference and BMI) and non-fasting blood work was collected.

Biochemical assessments included HbA1c, 25(OH)D, vitamin B12, and hs-CRP. Measurements were made at Calgary Laboratory Services (Calgary, Alberta) from the start of our sample period until April 2013, after which time samples were sent to Doctor's Data (St. Charles, IL). During the transition between labs, samples were sent to both labs simultaneously, correlations were: HbA1c r = 0.96 (n = 242, p < .001); hs-CRP r = 0.99 (n = 241, p < .001) by the same method. Samples for 25(OH)D measurement were compared between labs, where CLS used the Liaison (chemiluminescent reaction) and Doctor's Data employed a liquid chromatography, tandem mass spectrometry (LC/MS-MS) method, giving a correlation of r = 0.801 (n = 3015, p < .001), a typical correlation between 25(OH)D assay methods.

Categorical glycemic status was defined according to HbA1c values: normoglycemic ≤ 5.8%, prediabetic 5.8–6.4%, and diabetic ≥ 6.4% [27,28]. Prediabetic participants were considered at risk for developing diabetes.

Statistical analyses

All statistical analyses were performed using Stata v10 (StatCorp LP, TX) and included descriptive statistics and t-tests for significance. Ordinary least squares (multiple) regression modeling and Pearson correlation analyses were performed for continuous measures of biomarker changes, and Probit regression techniques for changes in categorical diabetic statuses. Marginal effects from a Probit model are interpreted the same way as coefficients from most regressions.

Multiple regression analysis was used to determine differences

Table 2
Changes in Metabolic Variables and Diabetic Status Over One and Two Years.

Variable	Vital 1						Vital 2					
	N	Base-line	One Year	N	Base-line	Two Years	N	Base-line	One Year	N	Base-line	Two Years
HbA1C (%)	223	5.51 ± 0.39	5.65 ± 0.4[a]	101	5.55 ± 0.43	5.74 ± 0.66[a]	790	5.64 ± 0.35	5.56 ± 0.38[a]	269	5.68 ± 0.51	5.51 ± 0.53[a]
25(OH)D (nmol/L)	223	79 ± 35	111 ± 49[a]	101	77 ± 33	123 ± 57[a]	790	87 ± 45	119 ± 52[a]	269	92 ± 48	117 ± 51[a]
Hs-CRP (mg/L)	223	2.08 ± 2.0	1.91 ± 1.89	101	2.14 ± 2.00	1.61 ± 1.71[a]	790	1.60 ± 1.78	1.52 ± 1.73	269	1.53 ± 1.70	1.46 ± 1.66[a]
Normoglycemic to Prediabetes or Diabetes	185	–	0.16 (0.37)	81	–	0.23 (0.43)	549	–	0.078 (0.27)	184	–	0.02 (0.15)
Prediabetic or Diabetic to Normoglycemic	39	–	0.08 (0.27)	20	–	0.15 (0.37)	245	–	0.44 (0.5)	85	–	0.48 (0.5)

Values are mean ± standard deviation or mean (standard error).
Please note: Baselines differ for each year of follow-up due to discontinuation in the program.
[a] Indicates significant difference from baseline.

Table 3
Estimation for Changes in HbA1C and hs-CRP after one year and two years in the Pure North program (Ordinary Least Squares).

	Δ HbA1C (%)			Δ hs-CRP (mg/L)		
	(1)	(2)	(3)	(4)	(5)	(6)
Variables	One Year	One year	Two Years	One Year	One year	Two Years
Vital1	0.185[a]	0.123[b]	0.345[a]	0.134	-0.061	-0.050
	(0.018)	(0.055)	(0.050)	(0.114)	(0.161)	(0.179)
25(OH)D[¥] (nmol/L)	−0.001[a]	−0.002	−0.001	−0.004[a]	−0.003[b]	−0.003[b]
	(0.001)	(0.001)	(0.001)	(0.001)	(0.001)	(0.001)
25(OH)D^2	5.14e-06[a]	6.25e-06	2.28e-06	–	–	–
	(1.66e-06)	(5.00e-06)	(4.02e-06)	–	–	–
Age[¥]	0.002	−0.002	0.006[b]	0.011[b]	0.019[b]	0.009
	(0.001)	(0.003)	(0.003)	(0.005)	(0.008)	(0.009)
Male	0.034[b]	−0.012	0.017	−0.023	−0.068	−0.120
	(0.015)	(0.047)	(0.044)	(0.094)	(0.141)	(0.158)
Centered Value of Biomarker	−0.167[a]	0.050	−0.233[a]	−0.500[a]	−0.494[a]	−0.580[a]
	(0.022)	(0.049)	(0.047)	(0.026)	(0.039)	(0.044)
Constant	−0.071[a]	0.072	−0.195[a]	0.010	−0.150	−0.011
	(0.022)	(0.075)	(0.062)	(0.118)	(0.185)	(0.190)
Observations	1013	370	370	1013	370	370
R-squared	0.173	0.026	0.191	0.276	0.322	0.341

Please note: Baselines differ for each year of follow-up due to discontinuation in the program.
Constant terms in all models refer to the mean value of the dependent variable for the Vital 2 cohort.
[a] (Standard error); Indicates p < .01.
[b] (Standard error); Indicates p < .05.
[¥] Baseline value. The dependent variable is change in the biomarker. In columns (2), (3), (5) and (6), the constant term is for 25(OH)D of 50 nmol/L at follow-up, for a female with mean age and mean value of the biomarker at baseline for the entire sample.
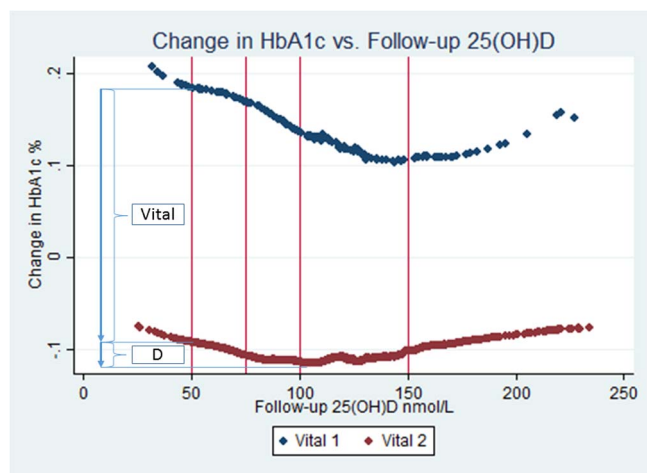
Fig. 2. The effect of vitamin D3 (Vital 1) in comparison to vitamin D3 with other supplements (Vital 2) on change in glycosylated hemoglobin. LOWESS plot of ΔHbA1c versus 25(OH)D level at follow-up for the Vital 1 and Vital 2.

between Vital 1 and Vital 2 interventions and what could be attributed to vitamin D supplementation (Vital 1) and the full program including vitamin D (Vital 2). The dependent variable in all models was the change in the biomarker over one year regressed on a constant and on an indicator variable for Vital 1 (Fig. 2). Further models controlled for age, sex, use of PGX® and changes in BMI, waist circumference, fruit and vegetable consumption, exercise, tobacco and alcohol use, and the baseline value of the biomarker (to account for any tendencies in the data toward mean reversion). Models are based on the observed quadratic function of serum 25(OH)D concentrations. To confirm this functional form implication of including baseline bio-marker values in a model of biomarker changes we regressed the value of the biomarker at one year on the baseline value and its baseline value squared along with other control variables to confirm that we get the same coefficient estimates for the Vital 1 indicator variable and the 25(OH)D controls. We also used the strategy of limiting the sample to participants with baseline hs-CRP greater than two, greater than three and greater than five. This approach confirmed the results in the models including

baseline hs-CRP where < 10 limit was used.

Probit models are equivalent to Logistic regression but are based on the standard normal distribution rather than the Logistic for modeling the probability for the outcome of interest. Probit models are useful for estimating absolute risk and reported marginal effects were interpreted as the change in the probability of the outcome of interest occurring. Odds ratios in Logistic regression are measures of relative risk and are not clearly interpretable when explanatory variables are continuous rather than dichotomous.

Changes in biomarker levels were used in modeling rather than absolute values to account for any fixed, but unobserved, characteristics of participants or potential confounding influences. Consequently participants who had both a baseline measure within 30 days of beginning the Pure North program and a one-year follow-up measure of all variables were included in the statistical model. BMI, waist circumference, fruit and vegetable consumption, exercise, alcohol and tobacco use were not reported consistently so models incorporating these covariates had smaller sample sizes.

Statistical modeling equations and explanation

A simple statistical model for explaining changes in HbA1C over a year was used:

ΔHbA1 c= f(vitD,multivitamin) + ε

The model suggests that the change in HbA1c is a function of changes in 25(OH)D, changes in other micronutrients status and random variation in HbA1c. Given two cohorts, one of which only received vitamin D3 (Vital 1 (V1)) and one of which received vitamin D3 in combination with other supplements (Vital 2 (V2)).

ΔHbA1 c= f_{V2}(vitD,multivitamin) + f_{V1}(vitD) + ε

If we assume that fV2 and fV1 are linear functions, then we can re-write the equation as:

ΔHbA1 c= γ_{V2} + γ_{V1}V1 + β_{V2}(vitD) + β_{V1}(vitD∗V1) + ε

V1 is an indicator variable equal to one for an observation of a member of Vital 1 and 0 otherwise (Vital 2) and epsilon is the error term of the model. Comparison of γV2 and γV1 identified the effect of vitamin D3

in combination with other supplements (Vital 2) but not Vital 1 for a given 25(OH)D serum level. If those coefficients were equal then the parts of the Vital 2 program absent for the Vital 1 cohort would have no effect on HbA1c.

The regression model specifies that the value of the outcome of interest is equal to the mean value plus some random difference. In a regression model, the mean of the distribution varies with observables like a vitamin D level. The error term of the model is a random variable assumed to have a normal distribution with mean 0 and constant standard deviation sigma. The purpose of the estimation method is to choose values of the model's constant term and slope coefficient(s) that best describe the relationship between the explanatory variable of interest and the outcome variable (the line of best fit).

If vitamin D had a direct effect on HbA1c, then $\beta V1 = 0$ and $\beta V2 < 0$, whereas increased 25(OH)D measured broader program adherence only, then $\beta V1 + \beta V2 = 0$ and $\beta V2 < 0$.

Fig. 2 illustrates how these effects were identified and shows LOWESS smooth plots of DHbA1C against the follow-up value of 25(OH)D for the Vital 1 and Vital 2 cohorts. These non-parametric curves suggest that higher 25(OH)D is associated with larger reductions in HbA1C. It is also clear that the relationship is U- or J-shaped. The curve for Vital 2 indicates that the cohort on average had reductions in HbA1C over the year whereas Vital 1 participants had increases in HbA1C. The vertical distance between the two curves would be interpreted as the effect of the full program whereas the change along a given curve from 50 nmol/L to a point further to the right would be the change in HbA1C attributable to higher 25(OH)D.

Results

There were 236 participants, 25–54 years, in Vital 1 from which 13 were excluded due to a self-reported diagnosis of diabetes and taking diabetic medication. Of the 223 participants in Vital 1 at one year, 101 participants returned for follow-up at two years. There were 814 participants in Vital 2 but 40 were excluded because of diabetes. Of 790 in Vital 2, 269 had a follow-up at two years. In both cohorts, of those who did not return for follow-up, approximately half were lost to follow-up and the other half attended the clinic outside the specified time frame. Fig. 3 is flow diagram of participant inclusion in the dataset.

Because some participants were lost to follow-up, the cohorts for comparison at baseline differ between one year of follow-up and those that continue in the program for two years of follow-up (Table 1). At baseline Vital 2 participants at one year in the program (n = 790) and at two years (n = 269) had higher HbA1c values (by 0.13% for both baseline comparisons), higher 25(OH)D concentrations (8 nmol/L and 14 nmol/L higher for one and two-year cohorts, respectively), and had more females (11% and 18% for one and two-year cohorts, respectively). Age, BMI and hs-CRP did not differ between Vital 1 and Vital 2 at baseline for the cohort with one year of follow-up. For the two-year cohort, Vital 2 participants at baseline had a statistically significantly lower BMI (5.68 ± 0.51 vs. 5.64 ± 0.35 for Vital 2 and 1, respectively) and lower baseline hs-CRP levels (1.53 ± 1.7 vs. 1.60 ± 1.78 for Vital 2 and 1, respectively).

To confirm our presumption, that the Vital 1 cohort had not been taking the multivitamin, we compared baseline and one-year values of serum vitamin B12 (from the multivitamin) and 25(OH)D (vitamin D_3 taken separately as drops). Fig. 1 indicates that serum 25(OH)D concentrations coincide with increases in B12 in the Vital 2 cohort, whereas vitamin B12 concentrations do not increase in Vital 1 even at higher 25(OH)D levels. There was no correlation between changes in serum vitamin B12 and 25(OH)D for Vital 1 whereas higher 25(OH)D concentrations were significantly correlated with increases in vitamin B12 in the Vital 2 cohort, suggesting that both groups were taking vitamin D supplementation but only Vital 2 was compliant with the multivitamin. This supports the natural experiment.

BMI did not change significantly at one year in either group, nor did

BMI change differ between groups at one year (−0.16 ± 1.6 for Vital 1 and −0.11 ± 2.5 for Vital 2). There was no change in BMI over two years in either cohort. At one and two years, HbA1c values were significantly lower in comparison to baseline in Vital 2 whereas in Vital 1 values increased (p < .001) (Table 2). Serum 25(OH)D concentrations were significantly higher in both groups at one and two years versus baseline, with mean increases that did not differ between groups (32 ± 48 nmol/L for Vital 1 and 32 ± 55 nmol/L for Vital 2) (Table 2).

Glycemic status is presented in Table 2. Despite having lower baseline HbA1c than the Vital 2 cohort, the proportion of Vital 1 participants progressing from normoglycemic to prediabetic or diabetic, and prediabetic to diabetic was double that for Vital 2. Conversely, improvement in diabetic status at one year (returning to normoglycemic) was 44% for Vital 2 and 8% for Vital 1 at one year (Table 2). The probability of a normoglycemic participant progressing to prediabetes/ diabetes after one year is 8% higher for Vital 1 than for Vital 2 (Table 2).

Probit Model (1) confirmed that HbA1c values increased at one year in Vital 1 participants and decreased in Vital 2 participants (Table 3). Higher 25(OH)D was associated with larger decreases in HbA1c for Vital 2 and lower increases for Vital 1. The effect of 25(OH)D levels on HbA1c change (inflection point) was greatest at a value of 125 nmol/L. All models were assessed for confounding variables that were theoretically important to the relationship under study.

Given the comparable magnitudes of the coefficient estimates for 25(OH)D controls for changes in HbA1c across the models, it is likely that the lower power of the smaller sample is the reason for this difference. Models (2) and (3) show that the changes in HbA1c at two years (0.345) were more than double the changes at one year (0.123), suggesting that the beneficial effects of the program continue to at least two years. The results do not differ when BMI, waist circumference, fruit and vegetable consumption, exercise, alcohol and tobacco use are included in the model.

Models for hs-CRP (Table 3) demonstrate a linear effect of 25(OH)D. Models (5) and (6) show decreases in hs-CRP at one year (−0.061) and two years (−0.050). In Vital 1, at 50 nmol/L of 25(OH)D there was an increase in hs-CRP over one year, but not in participants with a mean 25(OH)D serum concentration of 111 nmol/L. For Vital 2, there is decrease in hs-CRP values of 0.26 mg/L when mean 25(OH)D concentrations increase from 50 to 119 nmol/L. For hs-CRP, reductions were sustained to two years and almost entirely explained by 25(OH)D levels based on the similarities of the estimated coefficients. To investigate a "program" effect, changes in HbA1c and follow-up 25(OH)D levels were compared between Vital 1 and Vital 2 (Fig. 2). The portion of change statistically attributable to the other supplements was one-third to three-quarters of the total change whereas the effect of vitamin D varied between one quarter to two thirds of the total effect. These non-parametric curves suggest that higher 25(OH)D was associated with larger reductions in HbA1c. The vertical distance between the two curves was interpreted as the effect of the multivitamin and other supplements whereas the change along a given curve from 50 nmol/L to a point further to the right was interpreted as the change in HbA1c attributed to higher 25(OH)D.

Discussion

Our results suggest that a relatively straightforward preventative health program that provides nutritional supplementation may have the potential to reduce the risk of diabetes and improve glycemic status in individuals at risk. The combination of nutritional supplements taken by the Vital 2 participants was associated with a reduced risk of progressing from normoglycemia to prediabetes and an increased likelihood of a prediabetic participant improving to normoglycemia, changes that were sustained at two years after program entry.

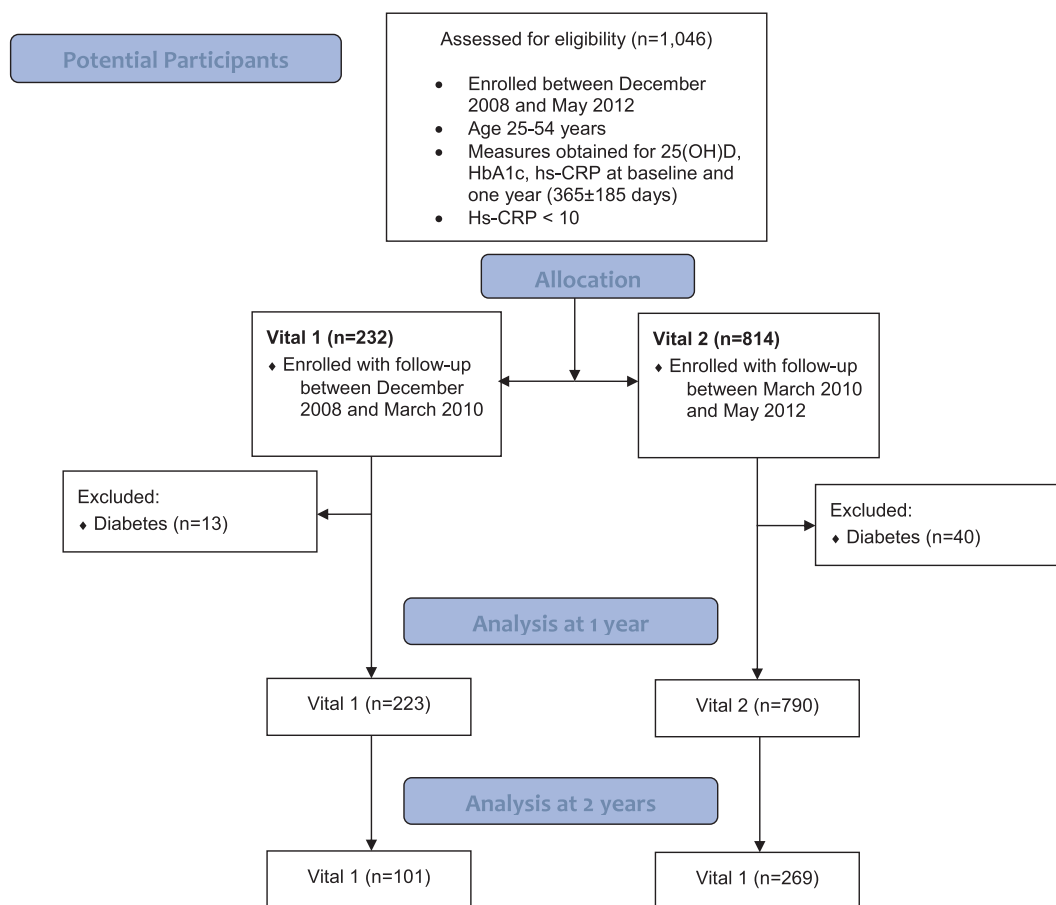Furthermore, the results in Table 3 suggest that while increasing

Fig. 3. Flowchart of participants included in the dataset.

25(OH)D concentrations through vitamin D$_3$ supplementation contributed to a reduced rate of increase in both HbA1c and hs-CRP, vitamin D in combination with other supplements was needed to decrease them. Further, the lack of change in BMI in both groups, the lack of effect of lifestyle measures (waist circumference, fruits and vegetables, etc.) on regression models and the reduction in Vital 2 but not Vital 1 suggest that the reductions in HbA1c and hs-CRP are due to the supplementation program.

With stable 25(OH)D concentrations at 50 nmol/L, the portion attributable to a change in 25(OH)D from 50 nmol/L to 119 nmol/L was one quarter to two thirds of the total effect, but neither HbA1c nor hs-CRP would have been reduced with vitamin D$_3$ supplementation alone. This may explain why the results of our study conflict with results of other studies examining the relationship between vitamin D$_3$ supplementation and diabetes risk. For example, a study of adults at risk for type 2 diabetes who received 2000 IU/d of vitamin D$_3$ did not find an effect on HbA1c [29]. Similarly, prediabetic patients with low 25(OH)D levels who were supplemented with 12,700 IU/d of vitamin D$_3$ did not demonstrate changes in HbA1c [30]. Based on our findings we posit that poor overall nutrition may be the reason for the discrepancy. The combination of achieving physiological levels of 25(OH)D with other nutrients (a multivitamin and omega-3 fatty acids) was required to observe reductions in HbA1c. Several lines of evidence suggest that vitamins D, C and E play roles in reducing oxidative stress and inflammation resulting in improved insulin sensitivity [31]. It is therefore plausible that overall nutritional support, and not just vitamin D alone, may be required to reverse the likely multifactorial mechanisms responsible for the progression to diabetes.

The relative risk of progression from prediabetes to diabetes in the general population has been estimated to be 12% compared to those with normoglycemia [32]. In contrast, regression from prediabetes to

normoglycemia occurred in approximately 8% of participants in the Diabetes Prevention Study [33]. At one year 8% of Vital 1 progressed to diabetes with 11% at two years, rates comparable to that expected in the general population. In contrast, Vital 2 had a significantly lower rate of progression than expected, with 3% progression from prediabetes to diabetes at one year and 1% at two years. Of note, 44% of Vital 2 participants regression from prediabetes to normoglycemia whereas only 8% of Vital 1 regressed. These results suggest that the Pure North program may be helpful in reducing the risk of, or even reversing, prediabetes and diabetes in a population.

Vital 2 reduced HbA1c from baseline, while Vital 1 (vitamin D$_3$ only) had slightly increased HbA1c levels. If we expect an increase in HbA1c levels, from 5.5% at baseline, a large majority of Vital 1 with normoglycemia would be in the prediabetic range at 2 years. In contrast, the mean baseline HbA1c was 5.65% for Vital 2 and if the same changes occurred in this cohort as were seen for Vital 1, they would have been in the prediabetic range on average after one year. Instead, Vital 2 was observed to have decreased HbA1c levels and 44% were normoglycemic at one year. This is in line with findings from the Diabetes Prevention Program, and intensive and expensive lifestyle intervention program, produced a 58% reduced risk of diabetes. Fig. 3 highlights the association of vitamin D with progression and regression rates in Vital 2. Higher levels of 25(OH)D were associated with better outcomes, with the effect being greatest at a value of 125 nmol/L.

Table 3 suggests that around 14% fewer normoglycemic persons would have progressed to prediabetes after one year. Applied to 1000 normoglycemic individuals this would result in ~ 140 persons who do not progress to diabetes. Given the mean increase in HbA1c for Vital 1 was 0.13 in one year, with mean baseline HbA1c of 5.7%, without the program these individuals would have been diabetic in about 5 years. Similarly, the effect sizes for returning from prediabetic to

normoglycemic, if the Vital 2 intervention were applied to 1000 persons with prediabetes, would see 510 persons returned to normoglycemic status.

The results of this study are of particular interest because of the scalable nature of this simple, relatively inexpensive, program. The supplements provided could be implemented at an annual cost of $350. If the results observed in this natural experiment could be replicated across a larger population, it could represent a highly cost effective option for reducing diabetes risk. From an economic perspective, Vital 1 demonstrates rates of transitions between glycemic statuses that are similar to those for the Canadian population [28]; Vital 1 rates remain steady whereas Vital 2 rates of prediabetes decline with a corresponding increase in normoglycemia. Accordingly, by comparing Vital 1 with Vital 2 we can estimate the cost implications possible for a co-hort of 1000 people. Assuming Vital 2 rates remain consistent over 5 years, an average health care utilization cost of $2053 [28], a 5% discount factor and a net lifetime cost from the mean age of Vital 2 (41–74 yr), the Net Present Value of costs for diabetes is $34,906 and for prediabetes is $27,262. For a cohort of 1000 people there would be 133 more normoglycemics, 130 fewer prediabetics ($3.6 million) and 10 fewer people with diabetes ($363,000). This results in a total avoidance of $4,000,000 in health care spending for 1000 individuals, or on average $4000 per person. At an annual cost of $350 for vitamin D_3 plus the other nutrients provided, the net present value is approximately $6000 per person, not including gains from improved quality of life or potential reductions in other chronic disease outcomes.

There are several limitations to the current retrospective study. To address selection bias we controlled for several variables (age, sex, BMI, etc.). There were slight differences between cohorts as outlined. Lifestyle counselling is a part of the Pure North program and may have been a confounder in the analyses, but it does not appear that weight loss was a contributing factor as there was no difference found in BMI between entry to the program and follow-up for either group. The lack of effect of waist circumference, BMI, fruit and vegetable intake, exercise, tobacco and alcohol on regression models use also suggests that lifestyle did not change significantly in either group.

There is also no way to elucidate which nutrients exactly were responsible for the effect on HbA1c and hs-CRP improvement. With modeling we were able to estimate the effect of vitamin D_3 to be between one quarter and one third with the other nutrients contributing the rest. However, we are unable to determine exactly which components of the multivitamin or omega-3 fatty acids were responsible. Biologically speaking, interactions between various nutrients make it plausible that more than one nutrient is responsible. Energy metabolism alone requires five different B vitamins, magnesium and manganese. Further, as in any population, participants may have been taking other nutrients to address their own deficiencies and symptoms. As such, the analysis presented assesses the success of a "real-life" prevention program that focused on optimizing nutritional status.

Although the design of this study as a retrospective, natural experiment limits the extent to which these results can be generalized to a larger population outside of the study group [30], the results suggest a real, positive effect of the Pure North intervention program on glycemic status in the participants. Diabetes is one of the fastest growing chronic diseases worldwide, and the role of nutritional supplementation in the prevention of this disease warrants further research. Well-designed clinical studies are required to ascertain if improving 25(OH)D levels in combination with multivitamin supplementation and/or omega-3 fatty acids could provide a safe, effective and economical means of reducing diabetes risk in the general population.

There are important implications of this study for the ongoing controversies over nutrient supplementation in healthy populations and the position on vitamin D adequacy. Our outcomes across two bio-markers, HbA1c and hs-CRP, are longitudinal outcomes in healthy subjects. We observed that the combination of vitamin D and multi-vitamin supplementation resulted in clinically important health improvements in our study's participants. We suggest that further examination of the role of vitamin D supplementation along with other nutrients through randomized controlled trials is required to further evaluate the potential of such nutritional intervention programs on diabetes risk.

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Author contributions

SMK, JCHE and RZL designed the study and had primary responsibility for final content, JCHE directed the statistical analysis, and SMK and RZL wrote the paper. All authors have read and approved the final manuscript

Conflicts of interest

RZL and JCE declare no conflict of interest. SMK is employed by the Pure North S'Energy Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcte.2017.11.002.

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