The Effect of Daily Intake of Vitamin D-Fortified Yogurt Drink, With and Without Added Calcium, on Serum Adiponectin and Sirtuins 1 and 6 in Adult Subjects with Type 2 Diabetes

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Research

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Abstract

Background

There is evidence suggesting an effect of vitamin D on glycemic status through mediators including adiponectin and sirtuins.

Objective

To evaluate the effects of daily intake of vitamin D-fortified yogurt drink, either with or without added calcium, on serum adiponectin, sirtuins (SIRT)1 and 6.

Design

Briefly, 75 adults aged 30-60 yrs from both sexes with type 2 diabetes were randomly allocated to one of the three groups: (i) D-fortified-yogurt drink (DY; containing 1000 IU vitamin D and 300 mg calcium), (ii) Ca+D-fortified-yogurt drink (CDY; containing 1000 IU vitamin D and 500 mg calcium) and (iii) plain yogurt drink (PY; containing no detectable vitamin D and 300 mg calcium). All assessments were performed initially and after 12 weeks.

Results

A significant within-group increment in serum adiponectin concentrations was observed in both DY and CDY groups (+60.4± 8.6, +57.5± 6.4 µg/L, respectively; p<0.001 for both). The concentrations of SIRT1 and SIRT6 had a significant within-group increment only in the CDY group (p=0.003, p=0.001 respectively). Being in CDY group was more favorable predictor of improvement in SIRT6 concentrations. Changes of 25(OH)D was a significant predictor of changes of adiponectin. However, this association disappeared following adjustment for changes of SIRT1. In contrast, the association between changes of 25(OH)D and HbA1c remained significant even after adjustment for SIRT1.

Conclusion

Daily consumption of vitamin D-fortified yogurt drink for 12 weeks resulted in an increase in circulating concentrations of SIRT1 and SIRT6 in T2D subjects and D+Ca-fortified yogurt drink was more in favor of SIRT6 increment.

Introduction

Diabetes, the most prevalent metabolic disease globally, is accompanied by several devastating complications including cardiovascular disease (CVD), nephropathy, neuropathy, depressed immunity, impotence and infertility, stroke, retinopathy, cataracts, myocardial infarction and premature death [1]. Over 90 percent of diabetes cases are type 2 (T2D) which is predisposed by positive family history, central obesity and sedentary lifestyle. Therefore, T2D is partly preventable or at least can be remarkably
delayed by weight control, healthy diet and increased physical activity [2]. The importance of prevention of diabetes and its complications lies in the health care, social as well as economic burden of this disease [3–5].

While weight control and healthy lifestyle including healthy diet still consists the core of T2D treatment [6], attempts have been made to find an alternative therapy, including dietary and micronutrient supplements, to control blood glucose in the subjects with diabetes [7, 8]. Among the micronutrients, vitamin D has attracted more attention as it may be associated with both onset and treatment of T2D and consequently it has been, and continues to be, the subject of countless studies [9–14]. The other facet is the global high prevalence of vitamin D deficiency, its relation with health [15] and the necessity of reaching to vitamin D adequacy through appropriate interventions including supplementation and food fortification [16].

The ameliorating effects of raising vitamin D status of subjects with T2D on glycemic status as well as parathyroid hormone, some antioxidative and inflammatory biomarkers have been already documented [10, 13, 14, 17–19]. Though the regulating effect of vitamin D on pancreatic β-cell function and insulin signaling has been described [20], there is evidence suggesting an indirect effect of vitamin D in T2D through other mediators including adiponectin and sirtuins [13, 21].

Adiponectin is an adipokine secreted mainly by adipose tissue and also by muscle [22] involving in regulation of blood glucose and lipids [23]. Sirtuins, a family of highly conserved nicotinamide adenine dinucleotide (NAD⁺)-dependent enzymes that modify histones and some other proteins post-translationally, are related to aging and longevity [24]. However, a growing body of evidence indicates a role for sirtuins in insulin resistance, inflammation and oxidative stress in diabetes [25–30]. Even sirtuins have been proposed as a target in treatment of T2D [31]. Among seven mammalian sirtuins, sirtuin (SIRT)1 and SIRT6 have been reported to have more relevance to glucose homeostasis in T2D [32, 33]. This study was, therefore, undertaken to evaluate (i) if regular daily intake of vitamin D-fortified yogurt drink affects circulating concentrations of adiponectin, SIRT1 and SIRT6 and (ii) if consuming added calcium to D-fortified yogurt drink influences these effects.

Methods

Subjects and study design

We used serum samples kept at our biobank and data from a previously reported clinical trial registered at clinicaltrials.gov as NCT01229891[14]. Sample size was calculated using G power 3.19.2 [34], based on 80% of power and effect size of 0.4. It was determined that a minimum of 66 participants was required. We randomly selected seventy five participants (30 men and 45 women) from our databank. As described earlier [14], our subjects were initially recruited from the registered T2D patients at the Iranian Diabetes society. Eligible study participants were adults with confirmed T2D aged 30–60 years. Pregnant or lactating women as well as subjects with regular intake of nutritional supplements during the three
months prior to the study and those with any clinical diseases affecting vitamin D metabolism were excluded.

The interventions consisted of a 12-week, randomized, placebo-controlled, double-blind trial conducted during late fall and winter when dermal vitamin D synthesis is minimal [35]. The participants were randomly assigned to one of the three groups: (i) D-yogurt drink (DY), consumed as part of their usual diet 500 mL/day D-fortified yogurt drink containing 1000 IU vitamin D and 300 mg calcium (n = 25), (ii) Ca + D-yogurt drink (CDY), consumed 500 mL/day fortified yogurt drink containing 1000 IU vitamin D and 500 mg calcium (n = 25) and (iii) plain (unfortified) yogurt drink (PY) consumed 500 mL/day unfortified yogurt drink containing no detectable vitamin D and 300 mg calcium (n = 25). Yogurt drinks were in 250 mL bottles so participants had to drink two bottles a day, preferably one with lunch and one with dinner.

All procedures were approved by the Research Ethics Committee of National Nutrition and Food Technology Research Institute (NNFTRI). All subjects signed an informed written consent.

**Assessments**

All measurements including dietary, anthropometric and laboratory assessments were described in detail elsewhere [13, 14]. In the original project, dietary intake was evaluated using 24-h recall questionnaire for two days (including a weekend). Weight and height were measured using standard methods to the nearest of 0.1 kg and 0.1 cm, respectively. Body mass index was calculated by dividing weight (kg) by height$^2$ (m).

Serum samples were obtained from our biobank. Serum concentrations of 25-hydroxycalciferol (25(OH)D) were measured using high performance liquid chromatography (HPLC) [36] at the Laboratory of Nutrition Research that has been participating in the Vitamin D External Quality Assessment Scheme (DEQAS, www.DEQAS.org.uk) since 2012. Methods of analysis for glycated hemoglobin (Hb A1c), adiponectin and percent of total body fat mass (FM) have been described elsewhere [13, 14].

We measured circulating concentrations of SIRT1 and SIRT6 using enzyme immunoassay (EIA) method and commercial kits (both from ZellBio, Veltlinerweg, Ulm, Germany) and a microplate reader (Statfack 3200; Awareness Technology, Inc., Palm City, FL).

**Statistical analyses**

All data are presented as means ± standard deviation (SD) or 95% confidence interval (CI), unless stated. Group comparisons at baseline were done by analysis of variance (ANOVA) for continuous variables and chi square test for categorical variables. Multivariate linear regression analysis was conducted to reveal the estimated effect of interventions with vitamin D on outcomes. All statistical analyses were performed with Stata Statistical Software release 16 (Stata, College Station, TX, USA). A $p$ value of $< 0.05$ was considered significant.
Results

Data were collected from 75 participants (mean age: 50.7 ± 6.1 years) of whom 45 were women (60.0%). Three groups were similar in terms of distribution of age (p = 0.496) and gender (p = 0.513).

There were no significant within- or between-group differences in dietary intakes (Table 1). Distribution of the studied variables did not show any significant between-group difference at the baseline (Table 2). Serum 25(OH)D concentration significantly increased from baseline in both vitamin D-supplemented groups. A significant within-group increment in serum adiponectin concentrations was observed in both DY and CDY groups (+ 60.4 ± 8.6, + 57.5 ± 6.4, respectively; p < 0.001 for both). However, the between-group difference was not statistically significant. When compared with baseline values, we found a significant decrease in BMI, FM and HbA1c in both vitamin D-supplemented groups. Interestingly, the concentrations of SIRT1 and SIRT6 had a significant within-group increment compared to baseline, only in the CDY group (p = 0.003, p = 0.001 respectively), but not in DY or PY groups (Table 2).
Table 1
Comparison of mean daily intake of energy, macronutrients, calcium and vitamin D* between three groups

| Variables   | PY              | DY              | CDY             | p value¹ |
|------------|-----------------|-----------------|-----------------|----------|
| **Energy (kcal)** |                 |                 |                 | 0.832    |
| Before     | 1684.3 ± 710.2  | 1659.9 ± 460.0  | 1584.3 ± 483.8  |          |
| After      | 1631.4 ± 639.7  | 1662.5 ± 449.9  | 1507.6 ± 357.7  |          |
| p value²   | 0.642           | 0.978           | 0.223           |          |
| **Protein (g)** |                 |                 |                 | 0.372    |
| Before     | 69.7 ± 30.9     | 58.6 ± 19.4     | 58.3 ± 23.8     |          |
| After      | 60.9 ± 20.6     | 63.6 ± 31.4     | 57.8 ± 21.1     |          |
| p value²   | 0.109           | 0.321           | 0.872           |          |
| **Carbohydrate (g)** |              |                 |                 | 0.653    |
| Before     | 237.5 ± 106.4   | 253.7 ± 84.2    | 225.6 ± 79.8    |          |
| After      | 211.1 ± 74.9    | 247.0 ± 91.6    | 211.0 ± 65.0    |          |
| p value²   | 0.117           | 0.719           | 0.201           |          |
| **Fat (g)** |                 |                 |                 | 0.781    |
| Before     | 53.0 ± 26.6     | 48.6 ± 14.1     | 53.3 ± 26.2     |          |
| After      | 59.9 ± 42.9     | 55.9 ± 19.3     | 50.4 ± 18.1     |          |
| p value²   | 0.380           | 0.134           | 0.593           |          |
| **Fiber (g)** |                |                 |                 | 0.558    |
| Before     | 18.9 ± 13.0     | 18.3 ± 7.1      | 16.4 ± 6.1      |          |
| After      | 18.8 ± 8.1      | 19.8 ± 8.9      | 15.3 ± 6.4      |          |
| p value²   | 0.965           | 0.521           | 0.394           |          |

* The added amounts of calcium and vitamin D to the yogurt drinks are not considered here.

1 donated significance of between-group comparisons at baseline (one-way ANOVA)

2 donated significance of within-group comparisons (Paired sample t.test)

Abbreviations: CDY, calcium+D−fortified yogurt drink; DY, D−fortified yogurt drink; PY, plain (unfortified) yogurt drink
| Variables          | PY       | DY       | CDY       | p value¹ |
|--------------------|----------|----------|-----------|----------|
| **Calcium (mg)**   |          |          |           | 0.807    |
| Before             | 687.9 ± 280.4 | 714.4 ± 291.1 | 679.2 ± 262.3 |          |
| After              | 687.6 ± 283.2 | 734.3 ± 311.7 | 670.8 ± 268.0 |          |
| p value²           | 0.996    | 0.779    | 0.875     |          |
| **Vitamin D (IU)** |          |          |           | 0.127    |
| Before             | 34.6 ± 45.2 | 28.5 ± 25.4 | 22.1 ± 29.6 |          |
| After              | 19.5 ± 8.0 | 21.7 ± 10.2 | 15.0 ± 5.6 |          |
| p value²           | 0.129    | 0.203    | 0.289     |          |

* The added amounts of calcium and vitamin D to the yogurt drinks are not considered here.

1 donated significance of between-group comparisons at baseline (one-way ANOVA)

2 donated significance of within-group comparisons (Paired sample t-test)

Abbreviations: CDY, calcium+D-fortified yogurt drink; DY, D-fortified yogurt drink; PY, plain (unfortified) yogurt drink
| Variables       | PY     | DY     | CDY    | p value¹ | p value² |
|-----------------|--------|--------|--------|----------|----------|
| **BMI (kg/m²)** |        |        |        |          |          |
| Before          | 29.2 ± 4.5 | 28.5 ± 3.9 | 28.1 ± 4.8 | 0.698    |          |
| After           | 29.6 ± 4.8 | 27.5 ± 3.9 | 27.7 ± 4.9 |          | 0.263    |
| **FM (%)**      |        |        |        |          |          |
| Before          | 34.8 ± 8.2 | 32.7 ± 10.1 | 35.3 ± 10.6 |          | 0.068    |
| After           | 36.1 ± 7.2 | 31.0 ± 9.8 | 34.0 ± 10.0 |          | 0.007    |
| **25(OH)D (nmol/L)** |        |        |        |          |          |
| Before          | 35.1 ± 22.7 | 44.3 ± 18.5 | 38.1 ± 23.8 |          | 0.343    |
| After           | 32.3 ± 25.1 | 75.7 ± 21.5 | 68.9 ± 23.9 |          | < 0.001  |
| **HbA1c, %**    |        |        |        |          |          |
| Before          | 7.6 ± 1.5 | 7.5 ± 1.8 | 8.0 ± 1.8 | 0.656    | 0.001    |
| After           | 8.6 ± 1.4 | 6.7 ± 2.0 | 7.1 ± 1.4 |          | 0.027    |
| **Adiponectin (µg/L)** |        |        |        |          |          |
| Before          | 99.7 ± 58.8 | 103.0 ± 59.1 | 105.2 ± 39.5 |          | 0.935    |
| After           | 119.4 ± 57.5 | 163.4 ± 85.2 | 162.8 ± 57.1 |          | < 0.001  |

1 Between-group comparisons at baseline (one-way ANOVA), 2 Within-group comparisons (Paired sample t test)

Abbreviations: BMI, body mass index; CDY, calcium+D–fortified yogurt drink; DY, D–fortified yogurt drink; FM, total body fat mass; HbA1c, hemoglobin A1c; 25(OH)D, 25–hydroxy vitamin D, PY, plain (unfortified) yogurt drink; SIRT, sirtuin.
| Variables   | PY (ng/mL) | DY (ng/mL) | CDY (ng/mL) | p value<sup>1</sup> |
|-------------|------------|------------|-------------|----------------------|
| **SIRT1**   |            |            |             |                      |
| Before      | 3.73 ± 1.1 | 3.89 ± 1.0 | 3.59 ± 0.9  | 0.595                |
| After       | 3.49 ± 1.1 | 4.14 ± 0.9 | 4.31 ± 0.7  |                      |
| p value<sup>2</sup> | 0.471 | 0.388 | 0.003 |          |
| **SIRT6**   |            |            |             | 0.189                |
| Before      | 1.56 ± 0.5 | 1.41 ± 0.4 | 1.32 ± 0.4  |                      |
| After       | 1.45 ± 0.5 | 1.47 ± 0.5 | 1.81 ± 0.5  |                      |
| p value<sup>2</sup> | 0.465 | 0.643 | 0.001 |          |

<sup>1</sup> Between-group comparisons at baseline (one-way ANOVA), 2 Within-group comparisons (Paired sample t test)

Abbreviations: BMI, body mass index; CDY, calcium+D-fortified yogurt drink; DY, D-fortified yogurt drink; FM, total body fat mass; HbA1c, hemoglobin A1c; 25(OH)D, 25-hydroxy vitamin D, PY, plain (unfortified) yogurt drink; SIRT, sirtuin.

Table 3 shows the results of the regression models adjusted for baseline values indicating that daily intake of D-fortified yogurt drink either with (B = 0.93, p < 0.001) or without added calcium (B = 0.52, p = 0.002) could increase SIRT1 concentrations compared with PY. Moreover, being in each of the vitamin D-supplemented groups, compared with PY, had a significant effect on serum concentrations of 25(OH)D and adiponectin, as well as on BMI and FM after 12 weeks intervention period. Likewise, being in CDY group, as compared to PY group, had a significant effect on serum concentrations of SIRT6. The pairwise comparisons after adjustment for multiple comparison (Tukey's) indicated that among subjects in both D-fortified groups, being in CDY group was more favorable predictor of improvement in SIRT6 concentrations as compared with the DY (p = 0.007) and PY (p < 0.001) (Table 3).
| Variables | B      | St. error | p value | 95% CI      | $r^2$ |
|-----------|--------|-----------|---------|-------------|------|
| **BMI**   |        |           |         |             |      |
| PY        | -      | -         | -       | -           | 0.93 |
| DY$^{1,3}$| -1.5   | 0.3       | < 0.001 | -2.2 - 0.8  |      |
| CDY$^{1,2}$| -0.8  | 0.3       | 0.02    | -1.5 - 0.1  |      |
| **FM**    |        |           |         |             | 0.91 |
| PY        | -      | -         | -       | -           |      |
| DY$^1$    | -3.2   | 0.7       | < 0.001 | -4.8 - 1.6  |      |
| CDY$^1$   | -2.4   | 0.7       | 0.002   | -4.0 - 0.9  |      |
| **25(OH)D** |       |           |         |             | 0.68 |
| PY        | -      | -         | -       | -           |      |
| DY$^1$    | 36.5   | 4.9       | < 0.001 | 26.6 - 46.3 |      |
| CDY$^1$   | 34.3   | 4.8       | < 0.001 | 24.6 - 44.1 |      |
| **HbA1c** |        |           |         |             | 0.48 |
| PY        | -      | -         | -       | -           |      |
| DY$^1$    | -1.8   | 0.3       | < 0.001 | -2.6 - 1.1  |      |
| CDY$^1$   | -1.7   | 0.3       | < 0.001 | -2.5 - 1.0  |      |
| **Adiponectin** |    |          |         |             | 0.7  |
| PY        | -      | -         | -       | -           |      |
| DY$^1$    | 40.6   | 10.9      | < 0.001 | 18.8 - 62.4 |      |
| CDY$^1$   | 37.6   | 10.9      | 0.001   | 15.8 - 59.4 |      |
| **SIRT1** |        |           |         |             | 0.71 |
| PY        | -      | -         | -       | -           |      |
| DY$^{1,3}$| 0.52   | 0.16      | 0.002   | 0.2 - 0.84  |      |
| CDY$^{1,2}$| 0.93  | 0.16      | < 0.001 | 0.61 - 1.25 |      |
| **SIRT6** |        |           |         |             | 0.37 |
| PY        | -      | -         | -       | -           |      |
| DY$^3$    | 0.12   | 0.12      | 0.344   | -0.13 - 0.37|      |
| CDY$^{1,2}$| 0.51  | 0.12      | < 0.001 | 0.26 - 0.77 |      |

Model 1: adjusted for value of variables before interventions

Abbreviations: BMI, body mass index; CDY, calcium+D−fortified yogurt drink; DY, D−fortified yogurt drink; FM, total body fat mass; HbA1c, hemoglobin A1c; 25(OH)D, 25−hydroxy vitamin D, PY, plain (unfortified) yogurt drink; SIRT, sirtuin.
Table 4 displays the results of the regression models incorporating changes of 25(OH)D, serum SIRT1 and SIRT6 individually with changes of HbA1c as outcome and also the regression models incorporating these variables with changes of serum adiponectin and changes of FM as outcomes. The models showed that changes of serum 25(OH)D was a significant predictor of changes of serum adiponectin, HbA1c and FM. However, the association between changes of circulating 25(OH)D and adiponectin disappeared when it was adjusted for changes of serum SIRT1. In contrast, the association between changes of circulating 25(OH)D and HbA1c remained significant even after adjustment for SIRT1. There were no associations between changes in serum SIRT6 and changes in HbA1c and changes in serum adiponectin.
| Dependent variables | Predictors          | Models     | B    | St. error | p value | 95% CI         | adjusted r² |
|---------------------|---------------------|------------|------|-----------|---------|----------------|-------------|
| Changes of FM       | Changes of 25(OH)D  | Unadjusted | -0.03| 0.01      | 0.018   | -0.05-0.005    | 0.10        |
|                     |                     | Model¹     | -0.03| 0.51      | 0.008   | -0.06-0.01     |             |
|                     |                     | Model²     | -0.03| 0.01      | 0.008   | -0.06-0.01     |             |
| Changes of SIRT1    | Unadjusted          |           | -0.44| 0.51      | 0.391   | -1.4-0.57      | 0.10        |
|                     |                     | Model¹     | 0.03 | 0.51      | 0.943   | -0.99-1.07     |             |
| Changes of SIRT6    | Unadjusted          |           | -0.62| 0.70      | 0.381   | -2.02-0.785    | 0.10        |
|                     |                     | Model²     | 0.01 | 0.71      | 0.979   | -1.4-1.44      |             |
| Changes of HbA1c    | Changes of 25(OH)D  | Unadjusted | -0.02| 0.006     | < 0.001 | -0.03-0.01     | 0.23        |
|                     |                     | Model¹     | -0.01| 0.006     | 0.061   | -0.02-0.001    |             |
|                     |                     | Model²     | -0.02| 0.007     | 0.011   | -0.03-0.004    |             |
| Changes of SIRT1    | Unadjusted          |           | -0.94| 0.26      | 0.001   | -1.4-0.42      | 0.05        |
|                     |                     | Model¹     | -0.05| 0.22      | 0.795   | -0.51-0.39     |             |
| Changes of SIRT6    | Unadjusted          |           | -0.84| 0.37      | 0.029   | -1.60-0.08     | 0.17        |
|                     |                     | Model²     | -0.49| 0.38      | 0.204   | -1.25-0.273    |             |

1 Variables in model 1: changes of serum 25(OH)D, changes of serum SIRT1

2 Variables in model 2: changes of serum 25(OH)D, changes of serum SIRT6

Abbreviations: FM, total body fat mass; HbA1c, hemoglobin A1c; 25(OH)D, 25-hydroxy vitamin D, SIRT, sirtuin.
| Dependent variables | Predictors | Models | B  | St. error | p value | 95% CI       | adjusted r^2 |
|---------------------|------------|--------|----|-----------|---------|--------------|--------------|
| Changes of adiponectin | Changes of 25(OH)D | Unadjusted | 0.38 | 0.15 | 0.017 | 0.06–0.69 | 0.12 |
|                     |            | Model^1 | 0.114 | 0.19 | 0.549 | -0.26-0.49 | 0.06 |
|                     |            | Model^2 | 0.23  | 0.19 | 0.250 | -0.16-0.61 | 0.06 |
| Changes of 25(OH)D   |            |        |      |           |         |              |              |
| Changes of SIRT1     |            | Unadjusted | 17.3 | 6.4  | 0.009 | 4.47–30.1 | 0.12 |
|                     |            | Model^1 | 15.6 | 6.8  | 0.026 | 8.91–29.2 | 0.12 |
| Changes of SIRT6     |            | Unadjusted | 9.2  | 9.2  | 0.321 | -9.2-27.7 | 0.06 |
|                     |            | Model^2 | 5.1  | 9.7  | 0.604 | -14.3-24.5 | 0.06 |

1 Variables in model 1: changes of serum 25(OH)D, changes of serum SIRT1

2 Variables in model 2: changes of serum 25(OH)D, changes of serum SIRT6

Abbreviations: FM, total body fat mass; HbA1c, hemoglobin A1c; 25(OH)D, 25−hydroxy vitamin D, SIRT, sirtuin.

In univariate regression, changes of circulating 25(OH)D was a significant determinant of FM changes (B= -0.03, 95%CI= -0.05–0.005, p = 0.018). Likewise, in multiple regression analysis, serum 25(OH)D remained significant predictor of FM even after adjustment for changes of serum concentrations of SIRT1 (B= -0.03, 95%CI= -0.06–0.01, p = 0.008) and SIRT6 (B= -0.03, 95%CI= -0.06–0.01, p = 0.008).

**Discussion**

Our findings showed that improvement of vitamin D status via daily intake of D-fortified yogurt drink resulted in a significant increase in serum concentrations of sirtuins 1 and 6. These findings are in accord with several experimental studies. For example, in rats fed on low vitamin D diet, secretion of SIRT1 was decreased [37] whereas in mice fed on high fat diet, vitamin D₃ supplementation resulted in up-regulation of SIRT1 [38]. Current evidence shows that association of vitamin D and sirtuins may be through both direct and indirect pathways. Direct association of vitamin D with SIRT1, through vitamin D receptor (VDR), has been shown in experimental models [39–41]. On the other hand, vitamin D-induced up-regulation of SIRT1 together with pAMPK and GLUT-4 in adipose tissue suggests a role for these insulin-independent signaling molecules in glycemic control through vitamin D [38].
Sirtuin 6 contributes to glucose homeostasis by enhancing insulin secretion and inhibiting gluconeogenesis as well as lipogenesis [33]. In macrophages, SIRT6 suppresses obesity-induced inflammation and insulin resistance [28]. In fat-specific SIRT6 knockout mice fed on a high-fat diet, there was an augmented tendency to obesity, inflammation and insulin resistance. An under-expression of SIRT6 and related reduced adipose triacylglycerol lipase activity was observed in obese subjects [42]. Nevertheless, various studies have documented that both inhibition and enhancing SIRT6 may improve glucose tolerance in T2D. In murine model of T2D, inhibition of SIRT6 for ten days resulted in over-expression of muscular GLUT-1 and GLUT-4, enhanced glycolysis, decreased serum insulin as well as blood lipid concentrations and improved oral glucose tolerance [43]. On the other hand, experimental studies provided strong evidence for SIRT6 in pancreatic β-cells function [44, 45]. Notwithstanding, our findings provide clinical evidence for vitamin D-induced increased SIRT6, which was accompanied by a formerly reported significant improvement of glycemic status in T2D subjects [14].

We found consuming yogurt drink fortified with both vitamin D and calcium is more favorable to increase SIRT6. It has been shown that mitochondrial matrix calcium has a regulatory effect on sirtuin expression [46]. The effect of calcium intake on different aspects of diabetes including body weight and insulin resistance has been vastly studied [47–49] but still is controversial [50]. It is noteworthy that the mean calcium intake in our participants was about 700 mg/d, considerably less than the recommended intake for this age group. It is therefore likely that supplementing calcium intake in CDY group might, at least in part, contribute to its SIRT6 enhancing effect.

Disappearance of the association of changes of serum 25(OH)D and adiponectin concentrations following adjustment for changes of serum SIRT1 indicates a SIRT1-mediated effect of vitamin D on adiponectin secretion. Thus, vitamin D up-regulates SIRT1, as demonstrated in both animal model [51] and randomized clinical trial [21] and then SIRT1 in turn regulates adiponectin secretion. This finding is in accord with the report of regulation of adiponectin secretion by SIRT1 and endoplasmic reticulum oxidoreductase Ero1-Lα [52]. It is also documented that SIRT1 can potentiate 1,25-dihydroxycalciferol, the active form of vitamin D, via enzymatic deacetylation of VDR [53].

In the current study, there was a significant decrease in BMI in both vitamin D-supplemented groups despite no significant change in energy intake during 12 weeks intervention period. Though the enhancing effect of dairy calcium intake on weight loss in subjects with diabetes has already been reported [54], we found no significant change in PY group. Along the same line, a prospective study in Australia demonstrated an association of higher circulating 25(OH)D concentrations, but not dietary calcium intake, with lower risk of diabetes in adults [55].

In this study, reduction of BMI in both vitamin D supplemented groups was independent of changes of serum SIRT1 and SIRT6 concentrations. In oppose to this finding, it has been shown that vitamin D may have a fat-storing inhibitory effect on adipocytes which is mediated by NAD and SIRT1[56]. It is, therefore, likely that the effect of vitamin D on body weight may be mediated through both SIRT1-dependent and SIRT1-independent pathways. In a study, adipocyte and muscle cell culture media were treated by adding
sera obtained from the overweight/obese subjects fed a low or high dairy diet for four weeks. The results demonstrated activation of SIRT1 and SIRT1-independent pathways in media treated with high-dairy dieters’ sera resulting in enhanced mitochondrial biogenesis [57]. The regulatory action of SIRT1 on energy metabolism has been reported earlier [58].

The limitations of present study must be acknowledged. Firstly, the short term effects observed in this study does not necessarily reflect any possible long-term effects. Secondly, the other sirtuins with possible effect on pancreatic β-cell function, notably SIRT3 [59, 60], were not examined, either.

Conclusions

On the whole, daily consumption of vitamin D-fortified yogurt drink for 12 weeks resulted in an increase in circulating concentrations of SIRT1 and SIRT6 in T2D subjects and D + Ca-fortified, as compared with only D-fortified, yogurt drink was more in favor of SIRT6 increment. It is likely that the improving effect of vitamin D on adiponectin is SIRT1-dependent whereas its effect on HbA1c is SIRT1-independent. These findings shed some light on the mechanism of action of vitamin D on different aspects of diabetes including body weight and glycemic status.

Abbreviations

ANOVA: analysis of variance; CDY: calcium+vitamin D-fortified yogurt drink; CI: confidence interval; CVD: cardiovascular disease; DY: D-fortified yogurt drink; FM: total body fat mass; GLUT: glucose transporter; HbA1c: hemoglobin A1c; 25(OH)D: 25-hydroxycholecalciferol; NAD: nicotinamide adenine dinucleotide; NNFTRI: National Nutrition and Food Technology Research Institute; pAMPK: adenosine monophosphate-activated protein kinase; PY: plain (unfortified) yogurt drink; SD: standard deviation; SIRT: sirtuin; T2D: type 2 diabetes.

Declarations

B. Acknowledgement

All laboratory bench works were performed at the Laboratory of Nutrition Research, NNFTRI. We wish to thank all the subjects who assisted us by their sincere participation in this project. This study received no fund.

C. Ethical and consent to participate

All procedures were approved by the Research Ethics Committee of National Nutrition and Food Technology Research Institute (ethics code: IR.SBMU.NNFTRI.REC.1398.024). All subjects signed an informed written consent.

D. Authors' contributions
TN and BN designed the study and performed all laboratory works. Statistical analyses were done by BN who also prepared the preliminary manuscript. TN finalized the manuscript with the intellectual aid of BH.

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F. Availability of data and materials

All data generated are included in this published article.

G. Consent for publication

Not applicable.

H. Competing interests

BN, BH and TRN declare no conflicts of interest.

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