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Effects of vitamin D supplements on influenza A illness during the 2009 H1N1 pandemic: a randomized controlled trial

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In a prior randomized trial, we found that the incidence of influenza A was less in the vitamin D3 group than among those on placebo, but the total incidence of either influenza A or B did not differ between groups. In this trial, the incidence of influenza A or B was less in the vitamin D3 group than in the placebo group only during the first half of the study. To elucidate whether vitamin D3 has preventive actions against influenza A, we conducted another trial during the 2009 pandemic of the H1N1 subtype of influenza A. Students (n = 247) of a Japanese high school were randomly assigned to receive vitamin D3 supplements (n = 148; 2000 IU per day) or a placebo (n = 99) in a double-blind study for 2 months. The primary outcome was incidence of influenza A diagnosed by a rapid influenza diagnostic test by medical doctors. Influenza A was equally likely in the vitamin D3 group (20/148: 13.5%) compared with the placebo group (12/99: 12.1%). By *post hoc* analysis, influenza A occurred significantly less in the vitamin D3 group (2/148: 1.4%) compared with the placebo group (8/99: 8.1%) (risk ratio, 0.17; 95% confidence interval, 0.04 to 0.77; P = 0.009) in the first month. However, during the second month, the vitamin D3 group experienced more events and effectively caught up with the placebo group. Vitamin D3 supplementation did not lower the overall incidence of influenza A during the 2009 H1N1 pandemic. A *post hoc* analysis suggests that the initial benefit during the first month of treatment was lost during the second month.

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1. Introduction

A novel influenza A (H1N1) virus of swine-origin emerged in April 2009.[1] This influenza expanded from North America to Europe, Asia, and the southern hemisphere, which prompted the World Health Organization to declare it a "pandemic of influenza: phase 6." In an analysis of hospitalized patients, most subjects infected by the 2009 pandemic influenza (H1N1) virus recovered without complications, but certain patients, particularly patients with chronic diseases, obese adults, young children, and pregnant/postpartum women, had severe and prolonged infections.[2] In the southern hemisphere (*i.e.*, Australia and New Zealand), the pandemic influenza accelerated markedly in June, reaching a peak within 4 to 6 weeks, and then declined after mid-July,[3,4] which was earlier than the traditional peak of seasonal influenza. Accordingly, the peak in the northern hemisphere, including Japan, was predicted to occur from October to November, which is earlier than the traditional peak of seasonal influenza.

During the preceding influenza season, from 2008 to 2009, we showed that vitamin D supplementation significantly reduced the incidence of influenza A in school children by means of a randomized, double-blind, placebo-controlled trial (RCT).[5] However, we also noted a non-significant excess of influenza B in the vitamin D3 group (39/167: 23.3%) compared to the placebo group (28/167: 16.8%).[5] It was difficult to explain why vitamin D supplementation was effective only in preventing influenza A but not in influenza B. In Japan, influenza A is usually prevalent in the early period of the influenza season (*i.e.*, January), whereas the incidence of influenza B increases later in the influenza season (*i.e.*, March). In a *post hoc* analysis of this trial, we found that the incidence of influenza A or B was less in the vitamin D3 group than in the placebo group only during the first half of the study. Moreover, another randomized trial from Finland showed that vitamin D supplementation similarly prevented the incidence of acute respiratory tract infections during the first 6 weeks of use.[6] Therefore, we hypothesized that vitamin D supplementation may have delayed the susceptible period to influenza, whether type A or type B. To test this hypothesis, we conducted another RCT comparing vitamin D3 supplements with placebo in high school students during the 2009 influenza A (H1N1) pandemic.

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2. Results and discussion

2.1. Participants

2.1.1. Characteristics. A total of 895 students were asked to participate in this study at their own discretion during September–October 2009 *via* a letter and an assembly at the school; 105 did not meet the inclusion criteria (69 had already been infected by influenza A after May 2009, 35 had underlying diseases such as bone fracture or asthma and had taken medicine, and 1 had serious allergies). Among the remaining 790 students, 543 chose not to participate (no reason was specified), and 247 volunteered to participate; 148 were randomly assigned to take vitamin D3 and 99 to take placebo for 2 months starting October 18 and ending December 17, 2009 (Fig. 1). The two randomly assigned groups did not differ according to the sex or year in the school (Table 1).

2.1.2. Follow-up. All 247 students were followed until the end of the study without any loss to follow-up. Six students from the vitamin D group and four from the placebo group declined to participate shortly after randomization; although reasons were not specified; it was not due to the presence of clear adverse events. Adherence to vitamin D supplements or placebo was evaluated on the basis of the daily logs; 99% of students reported taking the supplement daily, as directed, and 1% forgot to take the study supplement once per week on average. Consistent with an intention-to-treat analysis, data from all 247 participants were included in the analysis.

2.2. Primary outcome

2.2.1. RIDT-positive influenza A in total. Rapid influenza diagnostic test (RIDT)-positive influenza A occurred in 32 students (13.0%) over the 2 month period. No students were infected by influenza B or infected more than once with RIDT-positive influenza A during the study period.

2.2.2. RIDT-positive influenza A in the vitamin D3 group and in the placebo group. Overall, RIDT-positive influenza A occurred in 20/148 (13.5%) students in the vitamin D3 group compared with 12/99 (12.1%) in the placebo group, with no significant difference between groups (risk ratio (RR), 1.11, 95% confidence interval (CI), 0.57–2.18, $P = 0.75$) (Table 2).

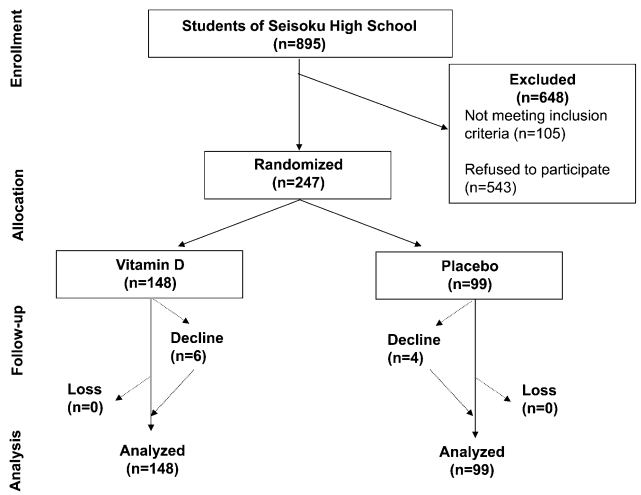

Fig. 1 Participant flow.

Table 1 School year by a randomly assigned group

	Vitamin D3	Placebo	Total
Male – *n*, (%)	98 (67)	64 (65)	163
School year – *n*, (%)			
Freshman	55 (37)	36 (36)	91
Sophomore	45 (30)	32 (32)	77
Senior	48 (33)	31 (32)	78
Total	148	99	246

2.2.3. RIDT-positive influenza A during the first month. When the comparison was limited to the first month, RIDT-positive influenza A occurred significantly less in the vitamin D3 group (2/148: 1.4%) compared with the placebo group (8/99: 8.1%) (RR 0.17; 95% CI, 0.04–0.77, $P = 0.009$) (Table 2).

2.2.4. Discussion in the primary outcome. In Tokyo, prevalence of influenza pandemic peaked during a week from October 29 to November 4, which was included in the first month of this study period: October 18th to December 17th. We found that vitamin D3 supplementation did not decrease the overall incidence of RIDT-positive influenza A. However, the RCT also suggests that short-term (*i.e.*, 1 month) use of vitamin D3 dietary supplementation may temporarily decrease the incidence of influenza A during an influenza pandemic. In a previous article, both low and high levels of cord blood 25-hydroxyvitamin D (25OHD) were associated with increased aeroallergen sensitization.[7] Therefore, we hypothesized that effects of vitamin D supplementation were U-shaped: the risk of influenza increases in both lower and higher 25OHD levels, but the risk decreases in optimal 25OHD levels, there is a possibility that vitamin D reduced the incidence of infection in the first month whereas vitamin D increased that in the second month, resulting in "caught up" with those in the placebo group (Fig. 2), thereby yielding no net difference between groups. In our prior RCT using 1200 IU of vitamin D3, we found that the incidence of influenza A was lower in the vitamin D3 group than in the placebo group.[5] However, in a *post hoc* analysis of this earlier RCT, influenza A or B was observed in 11 children in the vitamin D3 group (1200 IU per day: 60% dose of this study) *vs.* 26 children in the placebo group during the first 1.5–2.0 months of the 3.5 to 4 month trial ($P = 0.009$). This result differs from that over the course of the entire trial, where incident influenza A or B was observed in 57 (33.5%) of the vitamin D3 group *vs.* 59 (34.1%) of the placebo group ($P = 0.91$). Thus, both the current RCT and our prior RCT show a similar trend – namely, that vitamin D3 may be preventive to influenza in an initial phase, but that the incidence of influenza in the vitamin D3 group then seems to catch up with that in the placebo group during subsequent weeks. Although it would be easy to dismiss one such result as due to chance, we now have found this pattern in two similar Japanese RCTs. Outside Japan, Laaksi *et al.* conducted a RCT of vitamin D3 (400 IU) *versus* placebo among 164 military conscripts with a primary outcome of acute respiratory

Table 2 Risk ratio of rapid influenza diagnostic test (RIDT)-positive influenza and influenza-like illness by the randomly assigned group

	Total 2 month period (Oct 18th to Dec 17th)				The first month (Oct 18th to Nov 17th)			
	Vitamin D	Placebo			Vitamin D	Placebo		
	Case/total (%)	Case/total (%)	RR (95% CI)	*P*-value	Case/total (%)	Case/total (%)	RR (95% CI)	*P*-value
RIDT-positive influenza	20/148 (13.5)	12/99 (12.1)	1.11 (0.57, 2.18)	0.75	2/148 (1.4)	8/99 (8.1)	0.17 (0.04, 0.77)	0.009
Influenza-like illness[a]	32/148 (21.6)	17/99 (17.2)	1.26 (0.72, 2.14)	0.39	11/148 (7.4)	11/99 (11.1)	0.67 (0.30, 1.48)	0.32

[a] Influenza-like illness includes both RIDT-positive influenza and RIDT-negative but clinically relevant to influenza-like illness.

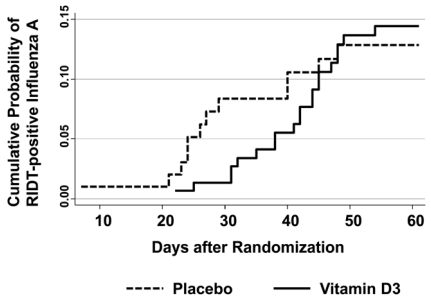

Fig. 2 Probability of the rapid influenza diagnostic test (RIDT)-positive influenza according to a randomly assigned group.

tract infection.[6] The authors reported that there was an effect during the first 6 weeks of the study, with a mean (SD) of 0.7 (0.2) days of absence in the intervention group and 1.4 (2.6) days of absence in the placebo group, but that there tended to be no difference between the two groups after the first 6 weeks. These results are similar to our two Japanese RCTs.

2.3. Secondary outcome

2.3.1. Influenza-like illness. Influenza-like illness, including both RIDT-positive and -negative results, occurred in 49 students (19.8%). The incidence of influenza-like illness did not differ between groups over the 2 month study period, with 32/148 (21.6%) of participants affected in the vitamin D3 group and 17/99 (17.2%) of participants affected in the placebo group (RR 1.26, 95% CI, 0.74–2.14, $P = 0.39$) (Table 2). Even when the study period was limited to the first month of intervention, the incidence of influenza-like illness was not significantly different between the vitamin D3 group (11/148: 7.4%) and the placebo group (11/99: 11.1%) (RR 0.67, 95% CI, 0.30–1.48, $P = 0.32$).

2.3.2. School absence. Frequencies and duration of school absence did not differ between groups (Table 3).

2.3.3. Influenza-related symptoms. Frequency of subjective symptoms reported by students on daily logs (*e.g.*, fever including influenza-like illness, runny nose, cough, sore throat, and arthralgia) did not differ between groups (Table 3).

2.3.4. Discussion in secondary outcomes. In contrast to the primary outcome (RIDT-positive influenza A), the incidence of influenza-like illness, school absence, and influenza-related symptoms was not significantly different between the two groups (Fig. 3). In our prior RCT, RIDT-negative influenza-like illness, nonspecific febrile disease, pneumonia, admissions to hospital, and days absent from school also were not different between the vitamin D3 and placebo groups. However there are still insufficient numbers of participants in this and previous studies to conclude that these secondary outcomes did not differ between the two groups. Since the completion of these Japanese RCTs in 2009, several RCTs of vitamin D supplementation to prevent acute respiratory infection have been published around the world, and they have yielded conflicting results. For children's pneumonia in Kabul, a single high-dose oral vitamin D3 supplementation to young children along with antibiotic treatment for pneumonia reduced the occurrence of repeat episodes of pneumonia,[8] whereas quarterly bolus doses of oral vitamin D3 supplementation were not an effective intervention to reduce the incidence of pneumonia in infants.[9] Among adults whose mean baseline 25OHD levels were approximately 30 ng mL^{-1}, there was no difference in the incidence or severity of upper respiratory infection between the vitamin D and placebo groups.[10,11] In contrast, among Mongolian children whose median 25OHD levels were 7 ng mL^{-1}, vitamin D supplementation significantly reduced the risk of

Table 3 School absence and influenza-related symptoms, by the randomly assigned group

	Vitamin D3	Placebo	*P*-value[g]
Absent students: number[a] (%)	68 (46)	38 (38)	0.24
Absent days: mean ± SD	1.7 ± 2.5	1.1 ± 1.9	0.14
Fever: number[b] (%)	52 (35)	34 (34)	0.85
Runny nose: number[c] (%)	23 (16)	17 (17)	0.73
Coughing: number[d] (%)	25 (17)	21 (21)	0.39
Sore throat: number[e] (%)	19 (13)	16 (16)	0.46
Arthralgia: number[f] (%)	13 (9)	10 (10)	0.73

[a] Number of students absent at least one day, as reported by the school. [b] Number of students who had 'fever more than 37.0' in the log file at least one day. [c] Number of students who checked 'runny nose' in the log file at least one day. [d] Number of students who checked 'cough' in the log file at least one day. [e] Number of students who checked 'sore throat' in the log file at least one day. [f] Number of students who checked 'Arthralgia' in the log file at least one day. [g] Chi-square test was used for all comparisons except absent days, where the Mann–Whitney test was used.

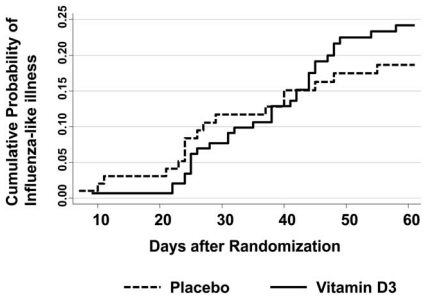

Fig. 3 Probability of influenza-like illness according to a randomly assigned group.

acute respiratory infection in winter.[12] These divergent results probably reflect the heterogeneity of outcomes but also of study populations and interventions, with large differences in subjects' ages, baseline vitamin D status, vitamin D dosing regimens, study duration, and season. The role of differences in genetic background is unknown, but also merits investigation.

2.4. Safety

The supplements were well-tolerated, without any reported adverse events.

2.5. Study limitations

The study has several limitations. First, since we could not predict the differential effects of vitamin D supplementation between the early and late phase prior to starting the trial and since we did not want to reduce the participant rate by painful blood sampling, we did not measure serum levels of 25OHD, which probably explains why vitamin D supplements reduce a risk of acute respiratory infection in one population,[12] but have no effect in another.[11] Without knowing the vitamin D status of participants during the course of the current study, we are reluctant to speculate on the mechanism of how vitamin D3 supplementation may delay susceptibility to RIDT-positive influenza A. Second, when we separated the first half from the whole study period, the event numbers were small. The similarity of results in two previous RCTs,[5,6] and this RCT does, however, support a true biological finding. Third, this trial was performed at a single Japanese high school during the 2009 H1N1 pandemic and not in a more diverse population during a more typical influenza season, a study design that reduces generalizability. It may be that particular individuals (*e.g.*, adolescents and young adults) and highly pathogenic subtypes of influenza A (*e.g.*, H1N1) are less likely to benefit from interventions such as vitamin D3 supplementation. Fourth, the investigators did not perform RITD directly or consult medical records, which are shortcomings of this study. Fifth, since we made a protocol of this trial in early phase of the pandemic and we could not predict the incidence of pandemic influenza A H1N1 among high school students between middle of October and middle of December, the incidence of RIDT-positive influenza A was 13% which was far less than 25% we had expected. So, the sample size was too small to conclude that vitamin D supplementation may not lower overall incidence of influenza A during the 2009 H1N1 pandemic. Sixth, we did not measure individual UVB/sun exposure per day and diet as parts of the questionnaire.

3. Experimental

3.1. Study design

3.1.1. Randomized, double-blind, placebo-controlled, parallel-group trial. We conducted a randomized, double-blind, placebo-controlled, parallel-group trial at Seisoku High School in Minato-ku (35° N), Tokyo, Japan, over a 2 month period, from October 18, 2009, to December 17, 2009, during the high season of pandemic influenza A H1N1 in Japan. The study protocol was reviewed and approved by the institutional review board of Seisoku High School. The entire process of study design and protocol, data monitoring, and analyses was performed at the Division of Molecular Epidemiology, Jikei University School of Medicine; there was no industry support or involvement in the study. The safety review board consisted of two physicians from Jikei University Hospital who are not co-authors of this study. Both vitamin D3 and placebo were purchased from Zenyaku Co., Ltd. (Otsuka, Bunkyo-ku, Tokyo, Japan) as a dietary supplement. This trial was registered at UMIN Clinical Trials Registry as UMIN000002532.

3.1.2. Study population, eligibility, and consent. Because the first outbreak of the influenza pandemic among high school students occurred during May and June in Japan, we planned to target only high school students in this study. The background, aims, methods, and possible risks/benefits of this study were explained to 895 Seisoku High School students aged 15 to 18 years and their parents, first by a letter and then *via* talks and communication by the first author (M.U.) at the school. Participants were asked to start taking the study supplements from October 18 and to continue taking them until December 17. Exclusion criteria were: (1) students who had already been infected with an influenza-like illness after May 2009; (2) those who had a history of urinary tract stones or diseases of calcium/bone metabolism; (3) those who had a bone fracture; (4) those who were already taking vitamin D supplements or activated vitamin D; (5) those who had asthma, as asthma may be an exacerbating factor in the pandemic influenza; and (6) those who had serious allergies, in order to avoid severe reactions to ingredients in the study supplement. Parents and students were asked to provide written informed consent. Study participation was completely voluntary.

3.1.3. Randomization, blinding, and intervention. We (M.U. and H.M) used a central computerized procedure to randomly assign students in permutated blocks of five to receive either vitamin D3 or placebo in a 3 : 2 ratio. Parents were provided with one numbered bottle containing 450 capsules. One capsule contained 400 IU of vitamin D3 or placebo; active and placebo capsules were identical in appearance. Participants were asked to take five capsules daily (total 2000 IU vitamin D3 or placebo); all five capsules could be taken at the same time or divided into two daily doses. The tolerable upper intake is currently set at 2000 IU per day by the Japanese Ministry of

Health, Labour and Welfare. According to the estimated dose–response curve reported by Gallagher *et al.*, by taking 2000 IU as a daily dose, 25OHD levels after the plateau phase would be 37 ng mL^{-1}.[13] Therefore, we chose 2000 IU as a daily dose in this study. Sesame oil, gelatin derived from swine and glycerin were used as the formulation for placebo as well as the active supplement. Blinding of the study was achieved by bottle numbering. Staff at the data monitoring center had no contact with participants. Thus, participants (high school students), care providers (parents), and medical doctors who assessed outcomes were blinded to the supplements.

3.2. Follow-up procedures and ascertainment of outcomes

3.2.1. Primary outcome. The students were asked to visit a doctor's clinic if they developed a fever (defined as body temperature higher than 37.0 °C) during the pandemic phase. As a school rule in Japan, students or parents are required to inform homeroom teachers of a doctor's diagnosis. Then, the homeroom teacher was asked to send a fax to the data monitoring center providing a detailed description from the students/parents as told to them and/or a certificate provided by the doctor regarding the diagnosis of or recovery from influenza A. Diagnosis was made by means of a RIDT and not polymerase chain reaction at the primary care setting in Japan. Participants were also asked to complete a daily log during the study period to: (1) reconfirm the diagnosis of influenza by a medical doctor, (2) assess adherence with the study supplement, and (3) assess other subjective symptoms, such as fever, runny nose, cough, sore throat, and arthralgia. For case identification, the study number was used and private information such as names and addresses of participants was not disclosed to the data monitoring center.

Because it takes several days for serum levels of 25OHD to start increasing, and a few days to allow for the incubation period of influenza, outcomes were only included in the analysis if they occurred after October 25 (7 days after starting study supplements). The primary outcome was the occurrence of influenza A, diagnosed by medical doctors with RIDT using nasopharyngeal swabs (*i.e.*, RIDT-positive influenza A). The sensitivity of the RIDT used in Japan for 2009 pandemic influenza A (H1N1) virus infection confirmed by polymerase chain reaction is approximately 77%.[14]

3.2.2. Secondary outcome. Secondary outcomes were: (1) doctor-diagnosed influenza-like illness, including not only RIDT-positive but also RIDT-negative influenza cases suspected by doctors due to clinical signs (*e.g.*, fever, headache, arthralgia, runny nose, and coughing) and close contact with patients with influenza; and (2) school absence and the reason for absence. Homeroom teachers were asked to send a fax to the data monitoring center to report any case of adverse events, including urinary tract stones or other serious signs/symptoms, as described to them by the students/parents.

3.3. Statistical analysis

3.3.1. Sample size calculation. We estimated that the primary outcome (RIDT-positive influenza A) would occur in 25% of students in the placebo group. A 3 : 2 divided sample of 260 was calculated as being sufficient for the detection of a 60% reduction in outcome, with a type I error (two-sided) of 5% and a power of 85%, on the assumption of no loss to follow-up. To detect the same 60% risk reduction with 85% power using a 1 : 1 ratio, the calculated sample size was 254. Because this number was almost the same if we used a 3 : 2 ratio, we chose the latter ratio because we assumed that the number of participants would increase in a study using a 3 : 2 ratio rather than a 1 : 1 ratio. Interim analyses were not used as the study period was only 2 months.

3.3.2. Efficacy analysis. Efficacy was assessed using an intention-to-treat analysis, which includes all students in the study, regardless of whether they were taking supplement after randomization. The incidence of both primary and secondary outcomes in the two groups was compared using a RR and 95% CI. All reported P values are two-sided and $P < 0.05$ was considered statistically significant. No adjustments were made for multiple comparisons. All analyses were performed using Stata 12.1 (StataCorp LP, College Station, TX).

4. Conclusions

We found that vitamin D3 supplementation did not decrease the overall incidence of RIDT-positive influenza A or influenza-like illness. However, the RCT also suggests that short-term (*i.e.*, 1 month) use of vitamin D3 dietary supplementation may temporarily decrease the incidence of influenza A during an influenza pandemic; the similarity of this finding with that of two other published RCTs[5,6] suggests that this finding is a result of more than chance. Future studies, with larger populations and serial 25OHD testing, are needed to further explore this novel finding and determine the optimal dose and duration of vitamin D supplementation to potentially decrease the risk of influenza and other acute respiratory infections.

Conflicts of interest

The authors declare no conflicts of interest.

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