Milk fortified with the current adequate intake for vitamin D (5μg) increases serum 25-hydroxyvitamin D compared to control milk but is not sufficient to prevent a seasonal decline in young women

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Low vitamin D status in women of childbearing age may have implications for health. Vitamin D status of New Zealanders (NZ), based on low serum 25-hydroxyvitamin D (25OHD) is suboptimal. Vitamin D status may be improved with supplements and/or fortified foods. Recently an Adequate Intake (AI) for Australia and NZ was set at 5 μg/d vitamin D. We aimed to determine the effect of daily consumption of milk powder fortified with 5 μg vitamin D₃ on serum 25OHD concentration over 12 wks. 73 non-pregnant women (18 - 47 y) living in Dunedin, NZ (46°S) were randomised to receive either unfortified (control) or fortified (5 μg vitamin D₃) milk for 12 wks from January to April. Mean 25OHD was similar between groups at week 0 (control 74 vs 76 nmol/L) and fell significantly in both groups over the 12 weeks (control 53 nmol/L, fortified 65 nmol/L; p < 0.001). After 12 wks the fortified milk group had a serum 25OHD 19% (95% CI; 7, 32%) higher (10 nmol/L) than the control group after adjusting for baseline levels (p < 0.001). Daily consumption of fortified milk providing the current AI of 5 μg per day vitamin D₃ for 12 weeks resulted in higher 25OHD concentrations than control milk. This dose was not sufficient to prevent the seasonal decline in 25OHD. This study suggests an AI of 5 μg may be inadequate for New Zealanders to allow for seasonal changes in sunlight exposure, and is unlikely sufficient for other populations with low sunlight exposure.

Key Words: 25-hydroxyvitamin D, milk, vitamin D, New Zealand, women

INTRODUCTION
Vitamin D deficiency leads to rickets in children¹ and osteomalacia in adults.² Lesser forms of vitamin D deficiency, often termed insufficiency, may increase the risk of hyperparathyroidism, osteoporotic fracture and other negative health outcomes.³ The few natural food sources of vitamin D such as fatty fish are not regularly consumed by the population. Thus in the absence of vitamin D fortification or supplementation the major source of vitamin D is endogenous skin synthesis by UV light exposure. Anything that influences the amount of light reaching skin, such as season, latitude, clothing, and darker skin colour will influence vitamin D status. Recent surveys indicate high rates of vitamin D insufficiency in many countries including Australia and New Zealand, based on low circulating concentrations of 25 hydroxyvitamin D (25OHD).⁴⁵ For example, 80% of New Zealand adults in the 1997 National Nutrition Survey had a serum 25OHD less than 75 nmol/L with even higher rates of insufficiency in the winter months and among Maori and Pacific People.⁵

Due to inadequate UV light in the winter months and health concerns about UV light exposure, food fortification with vitamin D may be a practical strategy for improving vitamin D status.⁶ A Vitamin D intake recommendation, termed an Adequate Intake, for Australia and New Zealand were recently set at 200 IU/d (5 μg/d) for all people less than 50 y.⁷ While overseas studies suggest this level of intake may be too low to maintain circulating 25OHD concentrations at a level adequate for bone health,³ the effect of 5 μg vitamin D₃ on New Zealanders’ circulating 25OHD concentrations has not been determined.

Here we present results from a double blind randomised controlled trial investigating the effect of a fortified milk supplement containing 5 μg vitamin D₃ on serum 25OHD and intact parathyroid hormone (PTH) concentrations in NZ women of childbearing age.

MATERIALS AND METHODS
Subject Recruitment
Seventy-three women volunteers from Dunedin aged 18-45 years were recruited through advertisements in local newspapers and signs placed around the university.

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Intervention
This was a 12-week, double blind, randomised controlled trial beginning in January. Women were asked to attend an early morning clinic at the Department of Human Nutrition (week 0). The women were weighed, had their height measured and asked to complete a demographic and lifestyle questionnaire. Women were randomised to one of two treatment groups (fortified or control milk). They were provided with verbal and written instructions on how to prepare the milk powder and to complete a diary of their milk consumption. Participants were asked to return any unused milk powder on their return to clinic 12 weeks after baseline (April, week 12). Compliance was assessed by dividing the number of servings of milk consumed by the number of possible servings.

Milk
Fonterra Brands (Auckland, New Zealand) provided the fortified milk (ANMUM Materna™) and the control milk. The control milk powder was a mixture of whole milk and skim milk powders that were blended to match the fat level of the fortified milk. Participants were instructed to consume 75 g of milk powder daily as 37.5 g powder in 200 ml water twice daily (morning and evening). This amount (75 g) of fortified milk powder provided 200 IU (5 µg) of vitamin D₃. The control milk contained a negligible amount of vitamin D. The milks (75 g of powder) provided daily; 1330 KJ and 9 g of fat, 5 g of which was saturated fat.

Laboratory Assessment
Blood was drawn from subjects following an overnight fast at week 0 and week 12. Blood samples were processed for storage within 4 hours of collection; Blood was centrifuged (2500 × g, 7 min) and the serum was aliquoted to cryovials. All samples were stored at –70˚C. Serum 25OHD concentrations did not differ between control [74 (65, 85) nmol/L], and fortified [76 (66, 87) nmol/L] milk groups (Table 2). By Week 12 the 25OHD concentrations had declined to 53 (46, 62) nmol/L in the control milk group and 65 (57, 73) nmol/L in the fortified milk group (p < 0.001). There was a 19% (7, 32) (p <0.001) difference in 25OHD concentrations at 12 weeks after adjusting for baseline values, which translates to a mean difference of 10 (4, 20) nmol/L (p=0.001). At baseline 44% of women in the control group and 47% in the treatment group had a 25-hydroxyvitamin D concentration less than 75 nmol/L. By 12 weeks the prevalence of insufficiency significantly increased to 79% (p <0.001) in the control milk group and was 53% in the fortified milk group (p=0.727), with an odds ratio of 0.18 (p=0.011) for fortified versus control milks, adjusted for baseline values. Mean (95% CI) PTH concentrations did not differ between control [2.8 (2.6, 3.1) pmol/L] and fortified milk groups [2.9 (2.7, 3.1) pmol/L] and did not change over the course of the study.

DISCUSSION
Women consuming fortified milk had 10 nmol/L higher serum 25OHD concentrations than those consuming the placebo at 12 weeks, which translates to a mean treatment effect of ~2 ug/nmol/L 25OHD concentration. O’Donnell et al recently conducted a systematic review of the effects of fortified milk and 25OHD in women.

Table 1. Characteristics of study participants in each treatment group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo milk (n=36)</th>
<th>Fortified milk (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.8 (26.3, 31.3)</td>
<td>28.0 (25.5, 30.6)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.9 (61.2, 68.7)</td>
<td>67.4 (63.2, 71.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 (22.4, 25.0)</td>
<td>23.3 (22.9, 25.8)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>1 (8.3)</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>33 (91.7)</td>
<td>29 (78.4)</td>
</tr>
<tr>
<td>Asian</td>
<td>2 (5.6)</td>
<td>6 (16.2)</td>
</tr>
<tr>
<td>Indian</td>
<td>1 (2.8)</td>
<td>2 (5.4)</td>
</tr>
<tr>
<td>Compliance to treatment, %</td>
<td>96 (95, 98)</td>
<td>95 (92, 98)</td>
</tr>
</tbody>
</table>

Values are means (95% CI) unless otherwise stated.
of fortified foods (primarily dairy products) on 25OHD concentrations. Of 9 studies, which included a total of 889 subjects, all but one reported a significant beneficial effect on 25OHD concentrations. The individual treatment effects ranged from 14.5 to 34.5 nmol/L with 3.4 to 25 µg vitamin D per day, respectively. Our findings and those of O’Donnell et al indicate that the effect of vitamin D fortified foods on 25OHD concentrations is larger per ug of intake than that previously reported for vitamin D supplementation trials. For example, in a six-month vitamin D₃ supplementation trial, Aloia predicted that for every ug intake of vitamin D₃ there would be a 0.7 nmol/L rise in 25OHD somewhat lower than in our study. However, the lowest dose given was 50 ug/d and lower doses appear to have a greater effect on 25OHD change per ug than higher doses. Our findings suggest that vitamin D₃ added to milk is more bioavailable than a supplement, however no direct comparisons have been made to prove this.

Although 5 µg of vitamin D provided by the fortified milk resulted in higher 25OHD relative to control it was not sufficient to arrest the seasonal decline in serum 25OHD concentrations. While the prevalence of insufficiency did not change significantly in the treatment group, it would likely have increased had the study continued for longer than 12 weeks. The decline in serum 25OHD vitamin D concentrations between baseline and 12 weeks in women consuming the control milk was expected. A seasonal variation in 25OHD is well described in people living at higher latitudes. Circulating 25OHD concentrations are typically at their highest in early Autumn and then fall until early Spring. This study commenced in late January and finished in late April. This allowed us to determine 25OHD concentrations at their maximum for the year and to examine how the current AI of 5 µg/d might attenuate the seasonal decline in 25OHD. The 25OHD concentrations at baseline and at 12 weeks in the women receiving the control milk were similar to women 25 and 44 years of age surveyed in the 1997 National Nutrition Survey. In that survey mean 25OHD concentrations in this group were of 87 nmol/L in January and 59 nmol/L in April.

Parathyroid hormone concentrations are known to rise in the presence of low dietary calcium intake and/or low vitamin D status causes inadequate absorption of calcium from the diet, resulting in calcium being sourced from the skeleton to maintain circulating calcium. Seasonal fluctuations in parathyroid hormone concentration have been reported in New Zealanders between the months of February and October but there was no change in parathyroid hormone concentrations by 12 weeks (April) in either group of this study. It may be that a longer time period including winter months is necessary in order to detect changes in parathyroid hormone concentrations in New Zealand women. Further, the 1000 mg additional calcium provided by the milks may have attenuated a rise in parathyroid hormone concentration with decreasing 25OHD.

Our study had a number of limitations. We included only women of childbearing age only and our results cannot necessarily be extrapolated to other adults. Men had higher 25OHD concentrations than women in the 1997 New Zealand National Nutrition Survey. Further, 25OHD concentrations declined with age in New Zealand women suggesting that older women may require more vitamin D to maintain serum 25OHD concentrations. However, in a study of older people (n=92) living in an Australian residential care facility, 25OHD concentrations increased by greater than 3 nmol per µg vitamin D with supplementation. Our study was only 12 weeks in duration and we don’t know whether 25OHD concentrations had reached a plateau in the fortified group. Finally, there is uncertainty around the most appropriate cut-off for 25OHD to define vitamin D insufficiency. We chose 75 nmol/L, but cut-offs between 50 to greater than 100 nmol/L have been recommended.

In conclusion, daily consumption of fortified milk providing the current AI of 5 µg day vitamin D₃ for 12 weeks resulted in higher serum 25OHD concentrations than control milk. This dose, however, was not sufficient to prevent the seasonal decline in 25OHD. Our findings would seem to support the view of many experts that the current AI is insufficient to maintain optimal vitamin D status. If larger amounts of vitamin D are required to maintain optimal vitamin D- and our results suggest they are-

**Table 2. Serum 25-hydroxyvitamin D (25OHD), and intact parathyroid hormone (PTH) concentrations during the trial**

<table>
<thead>
<tr>
<th>25OHD nmol/L</th>
<th>Baseline</th>
<th>Week 12</th>
<th>% Difference</th>
<th>Insufficiency, 25OHD %&lt;75 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo milk</td>
<td>74 (65, 85)</td>
<td>53 (46, 62)</td>
<td>*</td>
<td>44 (27, 61)</td>
</tr>
<tr>
<td>Fortified milk</td>
<td>76 (66, 87)</td>
<td>65 (57, 73)</td>
<td>*</td>
<td>19 (7, 32)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intact PTH pmol/L</th>
<th>Placebo milk</th>
<th>Fortified milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo milk</td>
<td>2.8 (2.6, 3.1)</td>
<td>2.9 (2.7, 3.1)</td>
</tr>
<tr>
<td>Fortified milk</td>
<td>2.9 (2.7, 3.1)</td>
<td>2.9 (2.7, 3.2)</td>
</tr>
</tbody>
</table>

Values are geometric means (95% CIs).

*Significantly different from baseline, paired t-test; p < 0.05
**Significantly different from baseline, McNemar's chi square test for prevalence; p < 0.001
†Significantly different from placebo milk; p = 0.05
††Significantly different from placebo milk; p < 0.001
eration may need to be given to fortifying a greater range of foods with vitamin D in New Zealand or recommend that people take a vitamin D containing supplements.

**AUTHOR DISCLOSURES**

Fonterra Brands (Auckland, New Zealand) funded the study and provided the milk powders. TJG has consulted for Fonterra Brands Limited. There were no other conflicts of interest.

**REFERENCES**

Original Article

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維生素D (5μg) 強化的牛奶提高了血清25-羥維生素D水平但不足以預防年輕女性維生素D水平的季節性下降

維生素D不足會對育齡婦女的健康產生影響。由分析血清25-羥維生素D (25OHD)得知，紐西蘭人的維生素D營養狀態並不理想。攝取補充劑與/或者食品強化都可以提高維生素D水平。目前澳洲和紐西蘭的維生素D適宜攝入量(AI)訂為每日5μg。本研究檢測，每日食用添加5μg維生素D3的奶粉12周後，對血清25OHD水平的影響。居住在紐西蘭但尼丁(46°S)的73名未懷孕婦女(18-47歲)，隨機分配食用未強化奶粉(對照組)或者強化奶粉(5μg維生素D3)，從1月到4月連續12周。在第0周，兩組婦女的平均血清25OHD值相似 (74對照76nmol/L)。12周後，平均血清值均明顯下降 (對照組53nmol/L，強化奶粉組65nmol/L，p<0.001)。調整血清25OHD基礎值之後，食用強化奶粉12周的婦女血清25OHD值比對照組高19% (95% CI；7，32%)，即10nmol/L (p<0.001)。與對照組相比，連續12周每天食用添加5μg維生素D3的牛奶可以提高血清25OHD水平。但該劑量不足以預防25OHD的季節性下降。該研究顯示，對於紐西蘭人而言，5μg的適宜攝入量(AI)也許不足以彌補陽光照射的季節性差異。這個攝入量對於其他陽光照射較少地區的族群也很可能是不足的。

關鍵字：25-羥維生素D、牛奶、維生素D、紐西蘭、婦女