Serum 25-hydroxy cholecalciferol in infants and preschool children in the Western region of Saudi Arabia

Etiological factors

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ABSTRACT

Objective: Low vitamin D status has been frequently reported among Saudi subjects of all ages. No attempt has been made to relate this status to dietary intake or to diseases leading to malabsorption, for example diarrhea. This study was performed to investigate the various factors leading to low vitamin D status, and their relative importance in infants and preschool children.

Methods: Nine hundred and thirty-five healthy subjects aged 4-72 months were selected randomly form the Jeddah area of the Kingdom of Saudi Arabia. Medical history, time and frequency of exposure to sunlight and dietary intake were recorded. Blood samples were obtained form 739 subjects for the determination of 25-hydroxy cholecalciferol. The Subjects were divided into 5 age groups. The mean ± standard deviation and other statistical parameters for serum 25-hydroxy cholecalciferol were calculated. Mean ± standard deviations of exposure time and vitamin D intake were calculated, and subjects divided according to the adequacy of their intake.

Results: Age had no effect on the mean serum 25-hydroxy cholecalciferol ($p=0.63$). Mean dietary intake of the vitamin increased initially ($p<0.05$), decreased in the next group ($p<0.005$), then remained constant. There was significant correlation between serum level and dietary intake of the vitamin. No exposure to sunlight was noted in the youngest group. Low serum levels were associated mainly with repeated diarrheal attacks. This was the same in the next group. The mean exposure time in the 3rd group increased significantly ($p<0.001$). Low serum levels were found in subjects with low exposure time plus either or both of low dietary intake and repeated attacks of diarrhea. Increased mean exposure time ($p<0.0005$), and decreased incidence of repeated diarrheal attacks were found in the next age group with low serum levels noted in subjects with low exposure time and low dietary intake. In the oldest age group, mean exposure time increased further ($p<0.0005$), and low levels were found in subjects with low exposure time (mainly girls).

Conclusions: Diet was the major source of the vitamin in subjects <12 months of age, and hence low levels were associated with frequent diarrheal attacks. A decrease in dietary intake, and more dependence on endogenous vitamin synthesis was apparent in older children, leading to low vitamin status in ones with low dietary intake and inadequate exposure to sunlight.

Keywords: Serum 25-hydroxy cholecalciferol, diet, sunlight, diarrhea, infants, pre-school children.

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Two sources provide the body with its need of vitamin D namely, diet and endogenous synthesis from 7-dehydro cholesterol by the action of ultraviolet radiation on the skin.[1] In both cases, it has to be activated before it can perform its biological function. The first activation step occurs in the liver,

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where the vitamin is hydroxylated to its 25-hydroxy form (25-OHD) or 25-hydroxy cholecalciferol.[2] The 2nd activation step is another hydroxylation reaction in the kidney, giving rise to 1,25 $(OH)_2$ D or 1,25 dihydroxy cholecalciferol.[3,4] However, since 25-OHD is the storage form of the vitamin, and has the highest concentration in circulation, and the longest half-life,[5] it has long been recognized as the best measure of vitamin D status.[6-7] A large study on the vitamin D status of Saudis was conducted and covered the effect of age, sex, living accommodation[8] regional and environmental location,[9] prevalence of low levels,[10] and seasonal variation.[11] The age of the studied population ranged from <6 years to 90 years. No significant correlation was detected between 25-OHD and age, but female adolescents and preschool children had the lowest levels in comparison with other groups. The highest 25-OHD levels were found in the inhabitants of the western province whether male, female, children or adult. The frequency of low 25-OHD (5-10 ng/ml) was found to be high for children (22%),[10] and even more so for young children <6 years of age (23% for males and 24% for females). However, this frequency was for the entire child population from different parts of the country. There was no information regarding the frequency of low vitamin D status in the different regions, most probably due to small numbers in the different age groups (only 90 for children <6 years from different parts of the country). Furthermore, no attempt was made to relate vitamin D status to nutritional habits, dietary intake, and related diseases leading to malabsorption, such as diarrhea. Such a study would help to explain the other factors contributing to Vitamin D deficiency, in addition to limited exposure to sunlight. Therefore, our study was planned to cover these aspects, recruiting subjects who are most likely to suffer from effects of vitamin D deficiency on growth namely, <6 years of age, recording their dietary intake, and feeding patterns, and compare this to levels of 25-OH vitamin D in their bloods, in order to come up with clear recommendations regarding groups most likely to be at risk of vitamin D deficiency, and measures taken to correct the situation such as dietary modifications and additional supplements.

Methods. The subjects were healthy infants, and preschool children aged 4-72 months, with no internal disorders (including diseases of liver, kidney, or gastrointestinal tract, hormonal disturbances or genetic disorders) selected randomly from nurseries, kinder gardens, welfare centers, and vaccination clinics. Principles of random selection were used in choosing subjects.[12] Care was taken to cover all districts in the Jeddah area of the Kingdom of Saudi Arabia, and all the socioeconomic classes. Chosen subjects were included in the study only if their parents gave free informed consent, and were of Saudi origin or Arabic speaking, with at least 5-years residence in the country. Nine hundred and thirty-five subjects were seen, however blood samples were obtained from 739 subjects only, due to either refusal of the mother, or difficulty in drawing blood from very uncooperative subjects. This should not bias results as the exclusion was not selective but random, and large numbers remained in each age group to give a good representative sample of the studied population. The age of the subjects to the nearest month was calculated from their birth records. Medical history of past illness with special emphasis on frequency of diarrheal attacks (number of times during the last 6 months) and their duration (in days) was taken for each subject. Subjects who suffered from 4 or more attacks of diarrhea; each lasting more than a day; during the previous 6 months were classified as having frequent attacks of diarrhea. The subjects' diets were assessed by means of diet history and food frequency questionnaires, as well as a 24-hour recall method administered by personal interview with the mother. The 24-hour recalls were repeated 3 days later, and an average of the 2 recalls was taken. The other collected information was used to validate the 24-hour recalls. Vitamin D intake per day was calculated for each subject using the nutrient values given in various food composition bulletins,[13-16] as well as information on packets of ready-made foods. To evaluate the adequacy of vitamin D in the diet, the calculated intake of the vitamin was compared to the World Health Organization WHO/FAO standard for recommended daily intake (RDA=400 IU or 10 μg/day).[17] Subjects with an intake ≥ 77% of RDA will have <50% chance of inadequacy, and is more likely to have adequate intake, while those with an intake of <77%-54% of RDA will have ≥ 50% chance of inadequacy, and those with an intake of <54% of RDA will almost certainly have inadequate vitamin intake.[18] Duration and frequency of exposure to sunlight was also assessed for all subjects by questionnaire administered during the interview on number of exposure times/weeks and length of each period in minutes. Venous blood samples were collected into plain tubes, and serum was separated and stored at –70ºC until analysis. 25-hydroxy cholecalciferol was estimated in sera using a kit (INCSTAR corporation, Minnesota, USA). The procedure involved a rapid extraction of 25-OHD and other hydroxylated metabolites from serum with acetonitrile. The treated samples were then assayed using a competitive binding radioimmunassay (RIA) technique, in which 25-OHD in samples competed with 3H-labelled 25-OHD for binding to a specific antibody. Unbound 25-OHD was removed by incubation with dextran-coated charcoal. The radioactivity in the remaining antibody/25-OHD complex was measured by liquid scintillation counting on a rack Beta counter (LKB – Wallac 1211). The concentration in assayed samples was determined from a calibration curve constructed from

Table 1 - Serum 25-OHD concentration (ng/ml) for different age groups, and number of subjects with low levels.

Age (months)	**4 - <6 n = 110 (%)**	**6 - <12 n = 170 (%)**	**12 - <24 n = 166 (%)**	**24 - <36 n = 166**	**36 - 72 n = 221**
Mean	26.20	24.9	24.6	26.7	24.4
Mode	30.6	21.3	25.2	26.4	24.8
Median	24.6	22.9	23.7	25.3	23.1
SD	14.1	14.1	14.0	11.3	11.5
Minimum	2.8	2.6	2.90	3.1	4.1
Maximum	225.1	76.8	65.7	68.2	68.6
2.5th - 97.5th percentiles	3.9-69.2	4.1 - 67.3	3.8 - 57.8	4.9 - 49.3	6.3 - 51.2
n of subjects with 25-OHD 5 - 10 ng/ml	15 (14)	21 (12.5)	22 (13.5)	3 (4)	18 (8)
n of subjects with 25-OHD < 5ng/ml	3 (3)	5 (3)	5 (3)	1 (1)	1 (0.5)

SD - standard deviation; n - number of subjects in group

standards provided with the kit. Intra-assay coefficient of variation using a pool sera sample re-estimated 20 times in a single run (% coefficient of variation (CV)) was 6% while the inter-assay CV was 9% using the same pool sera estimated 15 times on separate days.

Statistical analysis. Results are presented as means ± standard deviation (SD), minimum and maximum, mode, median, 2.5 and 97.5 percentiles, and were calculated using SPSS – statistical package. Comparison between 2 means was carried out using unpaired students t-test. Comparison between more than 2 means was carried out using one-way analysis of variance. Significance was assigned at $p<0.05$. Relationship between dietary intake of the vitamin and its level in serum was assessed by Pearson product – moment coefficient of correlation(r). Significance of r was then tested by a t-test and assigned at $p<0.05$ also.

Results. The serum 25-OHD concentrations for the different age groups in the studied population are presented in Table 1. From the data, it is apparent that the value of 25-OHD is not normally distributed. However, all values lay between 2.6-76.8 ug/ml, except for one subject ($4^1/_2$ months of age) with a value of 225.1 ng/ml. The mother of this infant was over dosing herself with vitamins and mineral supplements all through pregnancy. No significant difference in the mean was noted between the different age groups ($p=0.63$). However, the

Table 2 - Dietary intake of vitamin D in different age groups.

Age (months)	**Mean + SD (ug)**	**Subjects with an intake of**		
		> 77% RDA n (%)	**> 77%- ≥54% RDA n (%)**	**<54% RDA n (%)**
4 - <6 (n = 154)	6.2 + 2.1	33 (21)	51 (33)	70 (45)
6 - <12 (n = 202)	6.9 + 1.8	57 (28)	91 (45)	54 (27)
12 - <24 (n = 219)	5.9 + 1.4	20 (9)	104 (47)	95 (43)
24 - <36 (n = 93)	5.7 + 1.2	8 (9)	43 (46)	42 (45)
36 -72 (n = 267)		25 (9)	109 (41)	133 (50)

RDA - Recommended daily allowance; n - number of subjects

Table 3 - Pattern of distribution of subjects between different frequencies of exposure to sunlight and mean exposure time/week ± SD in the different age groups.

Age of group	No exposure	7 times	5 times	2 times	Mean ± SD exposure time min/week
4 - < 6 months (n = 154)	154	-	-	-	zero
6 - < 12 months (n = 202)	51	21	78	52	16.0 ± 30.5
12 - < 24 months (n = 219)	-	83	102	33	150.0 ± 52.0
24 - 36 months (n = 93)	-	37	40	16	298.0 ± 52.0
37 - 72 months (n = 267)	15	252	-	-	405.2 ± 233

SD - standard deviation; n - number of subjects in group

percentage of subjects with low 25-OHD level (5-10 ng/ml), or insufficient levels (<5 ng/ml) showed some difference, being consistent for subjects up to 2 years of age (p=0.74), then dropping sharply and significantly (p<0.0001) after that in the group aged 24-<36 months. A significant increase in the percentage of subjects with low levels was noted in the next age group (36-72 months) (p<0.0001). This pattern could be related to dietary intakes (Table 2), and to exposure to sunlight as will be seen from later results. No exposure to sunlight was noted for any of the children <6 months of age (Table 3). One hundred and 12 subjects (73% of the infants in this group) were breast-fed, with 103 (67%) of them given supplements of milk, soft foods or both, while the remaining 9 infants depended solely on breast milk. Forty-two subjects (27%) were bottle fed with other supplements to all of them. The milk and soft foods given to infants in the group mostly contained added vitamins and minerals, raising the vitamin D intake in all infants receiving them. Only 23 subjects (including the 9 infants breast fed only) received no artificially added vitamin D in their diet. Their intake varied between 0.8 to 2.7 ug/day. However, since breast milk vitamin D content depends on the mother's nutritional status,[19] the calculated intake was only an average estimate using the mid point of the range for vitamin D content of human milk (namely 0.3 – 1.0 ug/L).[20]

Repeated attacks of diarrhea were noted in 19 subjects (Table 4) representing approximately 31% of artificially fed infants; being associated with poor hygiene, and 5% of breast fed infants, including the ones receiving additional soft foods. Significant correlation was found between dietary vitamin D intake, and serum 25-OHD concentrations (r=0.51, p<0.0001). Low serum levels (<10 ug/ml) were found in 14 subjects suffering from repeated attacks of diarrhea, even though their dietary intake was >77% RDA, and in 4 infants who were breast fed only whose dietary intake was <54% RDA. An increase in the mean intake of vitamin D was noted in the next age group (p<0.05). About 50% of the subjects (100 infants) were still breast fed either partially or completely, but all infants in the group received additional supplements of ready made or home made soft foods, which mostly contained added vitamin D. Only 29 infants received a diet with no

Table 4 - Pattern of subject distribution among different frequencies of diarrheal attacks in the different age groups.

Age of group	Frequencies of diarrheal attacks/6 months		
	0 - 1 time n (%)	2 - 3 times n (%)	> 4 times n (%)
4 - <6 (n = 154)	94 (61)	41 (27)	19 (12)
6 - <12 (n = 202)	64 (32)	73 (36)	65 (32)
12 - <24 (n = 219)	86 (39)	89 (41)	44 (20)
24 - <36 (n = 93)	42 (49.5)	42 (45)	5 (5)
36 -72 (n = 267)	263 (98.5)	4 (1.5)	-

n = number

added vitamin D, and their intake varied between 1.8-3.0 ug/day. Eggs and dairy products were the major source of the vitamin in these children. Exposure to sunlight in varying degrees (Table 3) was noted in about 75% of the infants (151 subject), but only 21 infants received daily exposure varying between 10 – 30 minutes (mean ± SD = 15 ± 6). Another 78 infants were exposed to sunlight 5 days a week with a mean ± SD of 18 ± 6 minutes/day, (range 10 – 30 minutes), and 52 infants received sunlight exposure only during the weekends but for longer periods (mean ± SD of 30 ± 10 minutes/day). With the increase in bottle-feeding in this age group the frequency of repeated diarrhea attacks increased to become approximately 32% in the group as a whole (65 infants). During these attacks milk products were stopped, leading to decreased intake of vitamin D, provided mainly by these products. There was significant correlation between serum 25-OHD concentrations and dietary intake of vitamin D in this age group also ($r = 0.42$, $P<0.0001$). However, low serum levels were found in 26 subjects, all having experienced repeated attacks of diarrhea but their dietary intake was > 63% of RDA and in 4 cases > 77% of RDA, not considered to have low dietary intake.

A decrease in the mean intake of vitamin D was found for the subjects aged 12-<24 months compared to the previous age group ($p<0.005$). This was probably due to part substitution of milk (mostly enriched with vitamin D). With other foods mostly home prepared with no added vitamins. Twelve subjects were still partially breast fed, but they were given additional milk in bottle (fortified with vitamin D), and soft foods. Their intake ranged between 2.6 – 4.2 ug/day. Only 20 subjects in the group received ≥77% of the RDA of the vitamin. The frequency of diarrheal attacks decreased in this age group to 20% (44 subjects), and were associated with poor hygiene and low socioeconomic status. Exposure to sunlight was noted to varying degrees in all subjects in this age group (Table 3). The mean weekly exposure time increased significantly compared to the mean of the previous age group ($p = 0.001$). Eighty-three subjects (39%) received daily exposure ranging between 20-40 minutes (mean ± SD of 25 ± 2), while 33 subjects (15%) received exposure on the 2 weekend days only for larger periods (mean ± SD of 60 ± 15 minutes/day), and 103 subjects (46%) received exposure during the 5 working days only for periods ranging between 20-40 minutes/day (mean ± SD of 28 ± 7). Children of the lower socioeconomic classes on the whole received more exposure to sunlight than others, while exposure to sunlight in the upper classes was more confined to weekends at the seaside. Again a significant correlation between dietary intake of vitamin D and serum 25-OHD concentrations could be found ($r = 0.33$, $p < 0.0001$). However, low serum levels were found in 27 subjects having low exposure to sunlight, (60-72 min/week) combined with either or both of repeated attacks of diarrhea or low dietary intake (namely < 54% of RDA), but no factor by itself was found to be the cause of low serum 25OHD.

A further, but insignificant decrease in the mean vitamin D intake was found in the next age group. However, with the stopping of breast-feeding completely, and the inclusion of vitamin D rich natural foods in the diet (for example liver, tuna fish, eggs), the lowest intake in the group was 3.2ug/day. The distribution of intake was similar to the distribution in the previous age group with 9% of the subjects receiving ≥77% RDA and the rest distributed almost equally between the 2 lower categories of intake (namely 77% - ≥54% RDA and <54% RDA). As in other groups significant correlation between dietary intake of the vitamin and serum levels was found ($r = 0.37$, $p<0.0005$). All subjects in the group were exposed to sunlight by varying degrees (Table 3), in a similar pattern to the previous groups but for longer periods of time, as many of them played more out of doors, and slept less during the day. This caused a further significant increase in the mean exposure time/week ($p <0.0005$). Thirty-seven subjects (40%) had daily exposure ranging between 40-120 minutes/day (mean ± SD of 63 ± 21), while 16 subjects (17%) received exposure on the 2 weekend days only for periods ranging between 60-180 minutes/day (mean ± SD of 126 ± 31), and the remaining 40 subjects (43%) received exposure during the 5 working days only for periods ranging between 30-50 minutes/day (mean ± SD of 37 ± 6). Again, children of the lower socioeconomic classes seemed to be more exposed to sunlight as they were allowed to play outside the house more often than other classes, while exposure to sunlight was limited to weekends for upper classes. Many working mothers took their children to daycare centers during working days, allowing exposure to sunlight during transport and playing time, but kept them indoor during the weekends. Except for 5 subjects, no repeated attacks of diarrhea were noted in this age group (Table 4). However, low levels were not associated with any single factor. Low dietary intake, combined with low exposure to sunlight was behind the low levels found in this group.

In the oldest age group, a further insignificant decrease was found in the mean intake and about 50% of the group was receiving very low intake <54% RDA. Exposure to sunlight increased considerably in most children reflected on a significant increased mean exposure time/week ($p<0.005$), however, a difference between the sexes appeared which was not noted in younger groups. All children going to kinder gardens (70% of subjects) received daily exposure ranging between 40-60 min/day during working days, and even more during the weekends (range 60-240 min/day). There was no difference between the 2 sexes in this subgroup of children. However, the children not going to school yet (all low socioeconomic class) showed a difference in sun exposure time between boys and girls. Boys were allowed to play out of the house all the time, allowing them an exposure time of more than 2 hours/day. On the other hand, girls were allowed out only to nearby shops, and only when company was available, giving them an exposure time of 20 minutes/day at the most. It was difficult to accurately estimate the exposure time, as it was irregular and varied according to circumstances. Fifteen girls were not allowed out at all, and hardly saw the sun except on family outings on weekends. These girls all had very low (<54%) RDA dietary intake), and low serum 25-OHD. The other subjects with low serum 25-OHD were 4 boys with very low dietary intake, who had suffered recently from measles keeping them in the house for periods up to 2 weeks. They also suffered from diarrhea during their recovery from measles. No repeated attacks of diarrhea were noted in this age group. Furthermore, as was the case for all other groups significant correlation between serum level of 25-OHD and dietary intake of the vitamin was found ($r = 0.36$, $p<0.0001$), however, many children with very low intake had high serum levels of 25-OHD.

Discussion. The percentage of subjects with low or insufficient levels in our studied population is lower than reported earlier for Saudi subjects <6 years of age.[10] Furthermore, our calculated means for 25-OHD in the various studied age groups are higher than the earlier reported mean for children <6 years of age in the kingdom as a whole.[8] This might be due to any or all of the following reasons: 1. Our study was conducted on subjects from the western region of the country, whose inhabitants were found to have the highest levels of 25-OHD compared to other regions.[9] 2. Earlier studies,[8-10] were conducted before vitamin D fortified milk was available in the market to such a wide scale, thus dietary intake must have been lower. 3. Our study did not include subjects with sickle cell disease which is reported to be associated with lower levels of serum 25-OHD.[21] 4. The iron status of our studied population might be different to that in the earlier study, as many of our subjects were ingesting iron fortified milk and infant products, which is reported to improve the absorption of vitamin D in the small intestine, and hence increase the vitamin D concentration in plasma.[22]

Due to social customs, dietary intake was the only source of the vitamin in infants <6 months of age. Therefore, significant correlation between intake and serum levels was found. In addition, there was a strong association between repeated diarrheal attacks leading to decreased absorption and low serum levels of the vitamin. Diet seemed also to be the major source of vitamin D in the group aged 6-<12 months, with only approximately 26% of the subjects receiving very low intake (<54% RDA). Therefore a significant correlation between dietary intake and serum levels of the vitamin in this age group was found. However, due to the interference of diarrhea with dietary intake, low serum levels were found in subjects who suffered from diarrhea ≥ 4 times during the previous 6 months and who had likely to be adequate dietary intake, while higher serum levels were seen in subjects with <54% RDA intake, thus indicating that the effect of exposure to sunlight cannot be ignored.

The mean dietary intake decreased significantly in the group age 12-<24 months and a higher percentage (approximately 43%) had <54%, RDA intake however, there was no such decrease in mean serum 25-OHD concentration even though this was expected as there was significant correlation between dietary intake and serum vitamin concentration. This indicates the importance of sunlight exposure in providing vitamin D in this age group. However, the effect of low dietary intake, and that of diarrhea in decreasing absorption; and hence serum vitamin concentration could not be ignored totally and could be of equal importance. In fact, a combination of the 3 factors not just one factor was found to be associated with low serum levels of the vitamin.

The decrease in percentage of subjects with low or insufficient serum 25-OHD in the group aged 24-<36 months in spite of decreased mean dietary intake and increased percentage of subjects receiving <54% RDA, indicates the increased dependence of the body on endogenous synthesis of the vitamin after exposure to sun light. As the subjects grow older they seem to become more dependent on sunlight to provide them with the vitamin, but diet might be important in certain groups not exposed to sunlight as in the young girls mentioned earlier. Social customs play a major role in the development of low vitamin D status, as it was found to be the cause of increased percentage of subjects with low vitamin status in the group aged 36-72 months, since many girls in the group were not exposed enough or even at all to sunlight.

Thus, it can be concluded from this study that even though artificial feeding with fortified milk should theoretically improve the vitamin D intake of infants <12 months of age to make it >77% of RDA, and thus likely to be adequate, the lack of hygiene leading to repeated attacks of diarrhea has interfered causing low serum vitamin level in many such subjects. Of course, social customs leading to low exposure to sunlight, as well as low vitamin intake in breast fed infants, have caused low serum levels in this group of subjects as well. The decrease in the intake of fortified milk in the group aged 12-<24 months was balanced by a decrease in the frequency of repeated attacks of diarrhea, and increased exposure to sunlight, thus, keeping the percentage of subjects with low or insufficient serum 25-OHD level similar to younger subjects. Increased exposure to sunlight as well as a decrease in the incidence of diarrhea, has led to a decrease in the percentage of subjects with low serum levels of the vitamin in the group aged 24-<36 months, in spite of decreased adequacy of the diet. Social customs keeping girls more at home has caused an increase in the percentage of the subjects with low levels in the oldest age group studied, and led to an obvious difference in vitamin D status between sexes even at this young age. However, no difference could be detected in children attending kinder gardens, proving that the difference is due to different length of time spent in the sun, rather than to dietary or other metabolic causes.

As it is not logical to expect a change in social customs overnight allowing more exposure to sunlight it might be more prudent to recommend vitamin D fortification of all infants' foods not already fortified, especially as working mothers depend on them greatly even if they were partially breast feeding. This should improve the dietary intake of a large percentage of the infants' population. As for breast fed infants it might be advisable to administer to them routinely vitamin D supplement during their 3rd vaccination visit as a preventative measure to protect them from low vitamin status, and hence rickets. More effort in the delivery of nutrition education and guidance programs to mothers emphasizing the importance of hygiene as a means of combating diarrhea, explaining the best methods for food preparation and preservation, and stressing on the importance of fortified milk in child nutrition, and the fact that it should not be discontinued as a way of encouraging children to eat other types of food, would certainly help in improving the vitamin D status of young children in this area of the world.

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