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Analysis of 25-Hydroxyvitamin D Status According to Age, Gender, and Seasonal Variation

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Background: The effects of age, gender, and seasonal variation on human levels of 25-hydroxyvitamin D (25(OH)D) are not well understood. In this study, we aimed to investigate 25(OH)D status according to these factors in a Korean population. *Methods:* A total of 303,943 serum 25(OH)D levels were measured using an electrochemiluminescence immunoassay between October 2011 and May 2014. Potential participants were ineligible for the study if they had significant renal, hepatic, or thyroid dysfunction, as well as any major ongoing disease that could influence serum 25(OH)D levels. *Results:* A total of 95,137 subjects (49,662 men and 45,475 women) were included in this study. The mean 25(OH)D levels were higher in men (42.4 nmol/l) than in women (32.9 nmol/l, $P < 0.001$). Among the men and women, 73.0% and 88.9%, respectively, had 25(OH)D levels <50 nmol/l, whereas only 3.8% of men and 1.4% of women had levels >75 nmol/l. The highest mean 25(OH)D value was noted in individuals aged ≥70 for both genders. The proportion of those with 25(OH)D levels <50 nmol/l appeared to be higher among younger subjects ($P < 0.001$). Lastly, there were significant differences between 25(OH)D levels in individuals during summer to fall and winter to spring in both genders, indicating seasonal periodicity ($P < 0.001$). *Conclusions:* Serum 25(OH)D status varied according to gender, age, and season. Therefore, analyses of vitamin D status require individualized gender, age, and seasonally adjusted thresholds. Clinicians should consider these factors when determining optimal serum 25(OH)D levels in clinical practice. J. Clin. Lab. Anal. **30**:905–911, 2016. © 2016 Wiley Periodicals, Inc.

Key words: age; gender; seasonal variation; 25-hydroxy vitamin D

INTRODUCTION

Vitamin D is thought to be an important nutrient for the growth and maintenance of bone. Importantly, the association of vitamin D levels with many different diseases, as well as with general well-being, has been demonstrated in a number of recent studies (1–4). Thus, the exact assessment of the epidemiology of populations with respect to vitamin D status is important in managing the different negative health effects and in establishing public health policy in order to avoid vitamin D deficiency (5). In recent studies, various aspects of vitamin D deficiency have been studied and the results support the importance of vitamin D management for health (6–9).

The elements necessary for the synthesis of vitamin D consist of exposure to sunlight and the ingestion of food and vitamin supplements (10). Vitamin D activity in the body results from two conversions of the parent compounds vitamin D_2 and vitamin D_3, which are hydroxylated to 25-hydroxyvitamin (25(OH)) D_2 and $25(OH)D_3$, respectively, in the liver. $25(OH)D_2$ and $25(OH)D_3$ are then hydroxylated to the biologically active hormone $1,25(OH)_2D$ in the kidney and to some extent in peripheral tissues. Furthermore, $1,25(OH)_2D$ may not be low even in the case of vitamin D deficiency because it is tightly regulated and changes little in response to vitamin D challenge. While 25(OH)D is an inactive precursor, it is the best measure of vitamin D nutritional status due to

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its long half-life and its ease of measurement at clinically relevant concentrations (11, 12).

There is actually little consensus with respect to the reference intervals used to determine optimal, sufficient, insufficient, deficient, and/or toxic levels of 25(OH)D (13). The Endocrine Society's 2011 clinical practice guidelines suggest 25(OH)D cutoffs for deficiency (<50 nmol/l), insufficiency (50–75 nmol/l) and sufficiency (>75 nmol/l) (14). On the other hand, the Institute of Medicine (IOM) defines four categories: risk of deficiency (<30 nmol/l), inadequacy (30–49 nmol/l), sufficiency (50–125 nmol/l), and above recommended levels (>125 nmol/l) (15). The 1997 Dietary Reference Intakes reported that concentrations less than 27.5 nmol/l are associated with rickets in children and osteomalacia in adults, and that concentrations <37.5 nmol/l are considered inadequate for bone health (16). In general, 25(OH)D levels <50 nmol/l are considered deficient while levels <25 nmol/l are strongly associated with medical problems (17–20).

The determinants of vitamin D status are multifactorial and include environmental, physiological, and personal characteristics (21, 22). In Korean individuals, vitamin D levels have largely been evaluated only in the context of other diseases (23–25). Although several studies have reported that vitamin D deficiency is relatively common in Korea, the reported proportion of people with vitamin D deficiency varies among studies (25–27). In this study, we analyzed the distribution of serum 25(OH)D levels in a large population of Korean individuals, and looked for potential associations with recent health care data as well as general variations according to age, gender, and season.

MATERIALS AND METHODS

Study Participants

The Kangbuk Samsung Health Study was a cohort study comprising Korean men and women who underwent a comprehensive annual or biennial examination at the Kangbuk Samsung Hospital Total Healthcare Center in Seoul and Suwon, South Korea (28, 29). The study population consisted of 303,942 men and women aged 19 or older who underwent a comprehensive health examination and for whom serum 25(OH)D levels were measured from October 2011 to May 2014. Data on demographic characteristics, visit date, visit times, medical history, medication use, and past medical history were also collected using standardized, self-administered questionnaires.

We excluded 208,805 participants for various reasons, chiefly abnormal laboratory findings for serum fasting glucose, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and thyroid stimulating hormone (TSH; $N = 57{,}882$); current use of vitamin D supplements ($N = 50{,}007$); a past history of malignancy ($N = 4{,}156$); and known liver disease ($N = 15{,}041$), kidney disease ($N = 6{,}911$), thyroid diseases ($N = 16{,}334$), and/or diabetes ($N = 4{,}465$). Because some individuals met more than one exclusion criterion or had several different measurements of 25(OH)D during the study period, a total of 95,137 individuals whose 25(OH)D levels were examined for the first single measurement and who were not excluded based on the criteria were deemed eligible for inclusion in our study. The requirement for informed consent was waived because we used nonidentified retrospective data that were routinely collected during the health screening process. This study was approved by the Institutional Review Board of Kangbuk Samsung Hospital (2014-01-120).

Measurements of Serum 25(OH)D Levels

Serum 25(OH)D levels were measured using an electrochemiluminescence immunoassay (ECLIA) using four Modular E170 instruments (Roche Diagnostics, Tokyo, Japan) with a Modular EEEE system. Two levels of Quality Control (QC) materials were used and the coefficient of variation (CV) of the low-level QC material was 3.21% to 5.65%, and that of the high-level QC material was 2.10% to 4.03% during the study period. The analytical measuring range (AMR) specified by the manufacturer was 7.5–175 nmol/l, and this range was validated in this study. The coefficient of determination (r) was 0.926 in the method comparison of the Modular E170 and LC-MS/MS. The clinical laboratory has been participating in annual surveys conducted by the Korean Association of Quality Assurance for Clinical Laboratories and the College of American Pathologists.

Blood specimens were sampled from the antecubital vein after a 10-h fast. First, samples were incubated with a pretreatment reagent (dithiothreitol and sodium hydroxide), which resulted in bound vitamin D (25-OH) being released from the vitamin D binding protein. The pretreated samples were then incubated with ruthenium-labeled vitamin D binding protein, which resulted in the formation of a complex between vitamin D (25-OH) and ruthenylated vitamin D binding protein. After the addition of streptavidin-coated microparticles and vitamin D (25-OH) labeled with biotin, unbound ruthenium-labeled vitamin D binding proteins were bound. Finally, a complex consisting of ruthenylated vitamin D binding protein and biotinylated vitamin D (25-OH) was formed and bound to a solid-phase column via the interaction with biotin and streptavidin. The maximum level of 25(OH)D that could be measured was 175.0 nmol/l, and thus levels over the maximum were recorded as 175.0 nmol/l. The minimum detection level was 7.5 nmol/l, and thus levels lower than 7.5 nmol/l were recorded as the minimum level.

TABLE 1. Mean 25(OH)D Levels According to Age and Gender

			Mean ± SD (nmol/l)			
Age	Men (*N*)	Women (*N*)	Men	Women	All	*P*-values
<30	4,304	7,572	36.2 ± 13.7	29.5 ± 12.5	31.9 ± 13.5	<0.001
30–39	28,117	26,112	41.2 ± 15.2	33.9 ± 14.0	37.7 ± 15.0	<0.001
40–49	13,492	9,139	44.9 ± 16.0	31.9 ± 13.7	39.7 ± 16.5	<0.001
50–59	2,608	1,742	46.4 ± 18.2	35.7 ± 18.2	42.2 ± 19.0	<0.001
60–69	955	758	49.7 ± 22.2	38.7 ± 21.0	44.7 ± 22.2	<0.001
≥70	186	152	52.4 ± 25.2	38.9 ± 21.5	46.4 ± 24.5	<0.001
Total	49,662	45,475	42.4 ± 16.0	32.9 ± 14.2	37.9 ± 15.7	<0.001

Statistical Analysis

To evaluate the distribution of serum 25(OH)D levels across the categories of age or seasons, we categorized the data into six groups consisting of age <30, 30–39, 40–49, 50–59, 60–69, and ≥70 and four groups for the season: spring (March to May), summer (June to August), fall (September to November), and winter (December to February). Because of a lack of uniform guidelines, we classified 25(OH)D levels in our study into four grades as follows: severe deficiency (<25 nmol/l) (17–19), deficiency (25–50 nmol/l) (14), insufficiency (50–75 nmol/l) (14), and sufficiency (>75 nmol/l) (14). The proportion of individuals falling into each of these categories was calculated for each age group and each seasonal group.

Continuous data for the two groups were compared using a Student's *t*-test or Mann–Whitney test. One-way ANOVA was used to compare the mean levels for each age group and season. After that, Tukey HSD was used for post hoc analysis to find pairs of groups that were statistically different from each other. With respect to the distribution of 25(OH)D levels within gender and age groups, we analyzed the data by cross tabulation using the chi-squared test. IBM SPSS version 18.0 (IBM, New York) was used to perform calculations and test for statistical significance

RESULTS

The mean ± SD level of 25(OH)D among those aged 19 to 84 was 37.9 ± 15.7 nmol/l (Table 1). The lowest mean level was 29.5 ± 12.5 nmol/l in woman aged <30 and the highest was 52.4 ± 25.2 nmol/l in men aged ≥70. For both genders, the levels of 25(OH)D evaluated by age group were highest in seniors (≥70 years) and lowest in younger individuals (< 30 years). Overall, 25(OH)D levels were significantly higher among men than women for all age groups.

An estimated 20.8% (19,783/95,137) of the study subjects (11.0% of men and 31.5% of women) had a 25(OH)D level <25 nmol/l, which indicated that they were severely deficient in 25(OH)D (Table 2). Approximately one-third of the study subjects (33.9%, 4,024/11,876) aged <30 were severely deficient with levels <25 nmol/l (20.7% of men and 41.4% of women). Just over 2.7% (2,533/95,137) of study subjects (3.8% of men and 1.4% of women) had levels >75 nmol/l, which is the level considered sufficient for bone health according to the Endocrine Society's 2011 clinical practice guidelines recommendations, which are currently under review (14). Moreover, only 27.1% of men and 11.0% of women had an 25(OH)D level >50 nmol/l, which has been proposed as the level associated with optimal health (18, 20). Overall, in almost all age groups, men

TABLE 2. Distribution of 25(OH)D Levels According to Age and Gender

	Men, N (%)				Women, N (%)				
Age	<25 nmol/l	25–50 nmol/l	50–75 nmol/l	>75 nmol/l	<25 nmol/l	25–50 nmol/l	50–75 nmol/l	>75 nmol/l	Total
<30	892 (20.7)	2,786 (64.7)	563 (13.1)	62 (1.4)	3,132 (41.4)	3,968 (52.4)	415 (5.5)	57 (0.8)	11,876
30–39	3,247 (11.5)	17,938 (63.8)	6,079 (21.6)	853 (3.0)	7,266 (27.8)	15,630 (59.9)	2,876 (11.0)	340 (1.3)	54,229
40–49	979 (7.3)	8,061 (59.7)	3,819 (28.3)	634 (4.7)	3,150 (34.5)	5,143 (56.3)	741 (8.1)	105 (1.1)	22,631
50–59	212 (8.1)	1,454 (55.8)	760 (29.1)	182 (7.0)	534 (34.5)	923 (53.0)	214 (12.3)	71 (4.1)	4,350
60–69	99 (10.4)	451 (47.2)	279(29.2)	126 (13.2)	209 (27.6)	389 (51.3)	106 (14.0)	54 (7.1)	1,713
≥70	19 (10.2)	83 (44.6)	47 (25.3)	37 (19.9)	44 (28.9)	69 (45.4)	27 (17.8)	12 (7.9)	338
Total	5,448 (11.0)	30,773 (62.0)	11,547 (23.3)	1,894 (3.8)	14,335 (31.5)	26,122 (57.4)	4,379 (9.6)	639 (1.4)	95,137

TABLE 3. Mean 25(OH)D Levels According to Season of Blood Collection, Age, and Gender

	Season of blood collection (Mean ± SD (nmol/l))							
	Men				Women			
Age group	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter
<30	32.7 ± 12.0	39.7 ± 14.5	41.2 ± 15.0	31.9 ± 12.2	27.0 ± 11.5	30.7 ± 13.0	31.0 ± 12.2	27.5 ± 12.2
30–39	35.2 ± 12.5	44.9 ± 15.7	44.7 ± 15.2	34.4 ± 12.7	29.5 ± 12.0	37.7 ± 14.7	36.2 ± 13.7	29.0 ± 12.2
40–49	39.4 ± 14.2	47.4 ± 16.0	47.4 ± 16.0	38.4 ± 14.5	27.5 ± 11.5	34.2 ± 14.0	33.7 ± 13.7	27.5 ± 13.0
50–59	41.9 ± 16.7	50.7 ± 18.2	50.4 ± 18.0	42.7 ± 18.2	33.4 ± 18.2	41.4 ± 18.7	38.9 ± 18.5	32.7 ± 16.2
60–69	47.4 ± 21.7	60.2 ± 23.7	54.9 ± 21.5	43.4 ± 19.5	37.4 ± 21.7	43.4 ± 20.7	41.9 ± 20.7	35.2 ± 18.7
≥70	48.9 ± 22.5	53.7 ± 23.5	60.9 ± 23.7	52.7 ± 30.0	33.4 ± 18.2	43.4 ± 22.2	48.7 ± 23.2	39.2 ± 22.5
Total	36.4 ± 13.7	45.4 ± 16.0	45.9 ± 15.7	36.9 ± 15.2	29.0 ± 12.7	35.4 ± 14.7	35.2 ± 14.0	29.2 ± 13.2
(*n*)	13,989	14,761	16,609	4,303	11,499	14,392	14,028	5,556

were more likely than women to have adequate concentrations. Men and women aged ≥60 were significantly more likely than younger subjects to have adequate concentrations of 25(OH)D.

Mean 25(OH)D levels varied significantly according to the season in which the blood sample was obtained (Table 3). Likewise, we found a significant difference in the distributions for mean 25(OH)D levels according to age groups and gender within the seasonal groups ($P < 0.001$). Although the highest levels of 25(OH)D were recorded in the summer for women and in the fall for men, the levels tended to be higher among those whose blood was drawn in the season consisting of summer to fall rather than from winter to spring. Therefore, we compared all groups using a post hoc comparison with the Tukey HSD. There was no statistical difference between summer and fall 25(OH)D levels ($P = 0.73$ in women and $P = 0.15$ in men) and between spring and winter 25(OH)D levels ($P = 0.92$ in women and $P = 0.11$ in men). However, the seasonal variation for summer to fall and winter to spring was statistically significant for both genders ($P < 0.001$).

We next divided each gender group into participant visit months, consisting of 32 months altogether, and analyzed the data according to mean 25(OH)D levels (Fig. 1). The highest level in women was obtained in August 2012 (40.4 ± 14.5 nmol/l), while the lowest level was recorded in February 2012 (26.5 ± 12.0 nmol/l). The highest level in men was recorded in September 2011 (50.4 ± 16.0 nmol/l), while the lowest level was seen in February 2013 (32.4 ± 14.5 nmol/l). Consistent with the seasonal variations described above, a month-by-month analysis revealed monthly fluctuations in 25(OH)D levels, with the highest levels observed in the summer to fall months, followed by lower levels in the winter and spring.

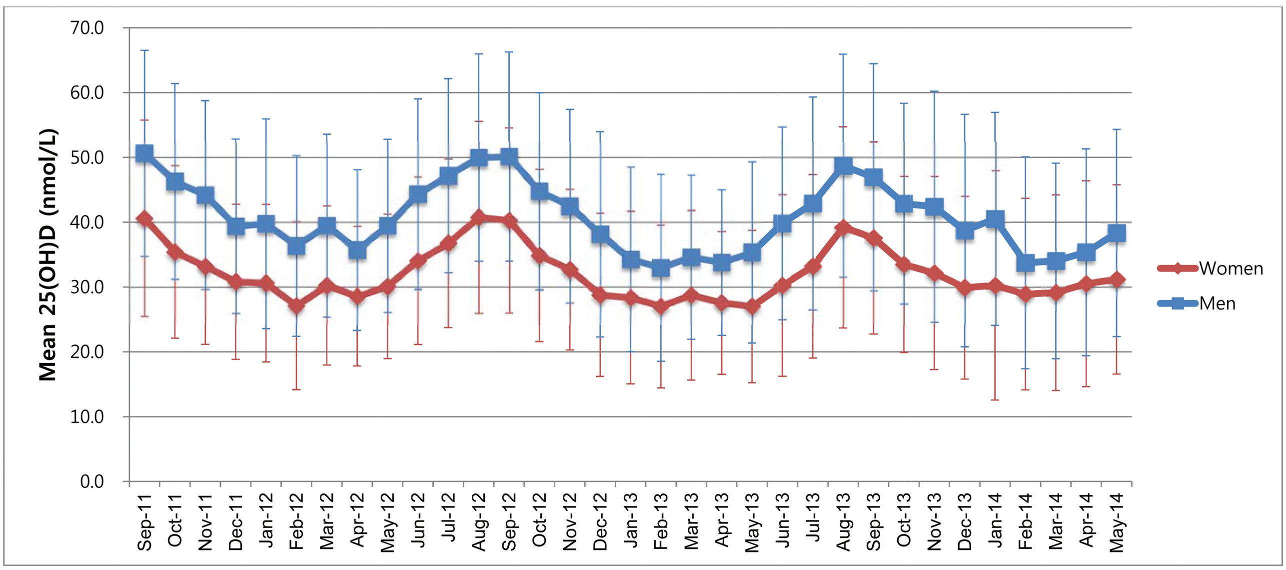

Fig. 1. Average 25(OH)D levels according to month and gender.

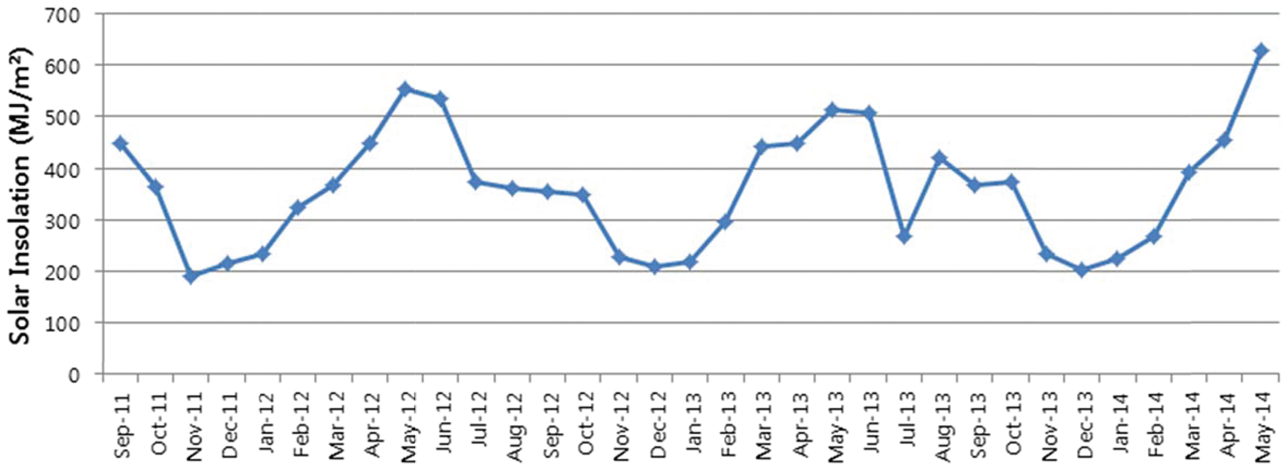

Fig. 2. Solar insolation according to month [24].

Interestingly, the 25(OH)D monthly graph was similar to that of a monthly solar insolation graph obtained from the Korea Metrological Administration (20) (Fig. 2).

DISCUSSION

The 2008 Korea National Health and Nutrition Examination Survey (KNHANES) analyzed 25(OH)D values from 3,047 males and 3,878 females in the Korean population. The KNHANES study reported that 47.3% of males and 64.5% of females had insufficient vitamin D levels, with the most prevalent level of insufficiency was noted in the 20- to 29-year-old age group, while that of the 60- to 69-year-old age group showed the least prevalent level of insufficiency. Additionally, a mean vitamin D level of 52.9 $\pm$ 18.7 nmol/l in males and 45.4 $\pm$ 17.7 nmol/l in females was reported; these levels were higher than our results. This study also reported that the predictors for vitamin D insufficiency included the spring and winter seasons, living in an urban area, and having an indoor occupation (9). In the United States, when the vitamin D statuses of 715,769 samples were measured at two academic medical centers, the average serum 25(OH)D levels were higher in males than in females for all age groups, and seasonal periodicity was noted based on higher 25(OH)D levels in the summer than in the winter (8). In a recent study analyzing 11,150 Italian outpatients, seasonal periodicity was also verified and the results were similar with past studies and with ours (30).

These studies described above were significantly different from the present study with respect to recruitment of the study population. Specifically, most big-data studies do not exclude ill patients who have diseases or factors that may influence vitamin D levels. On the other hand, in our study, patients who had a general illness, diagnosed or undiagnosed, or who were taking vitamin D supplements were screened and excluded from the study. In considering this exclusion step, we reasoned that it would be meaningful to determine if low levels of vitamin D levels are frequently observed in a healthy population, which would be different from the results of previous studies undertaken in hospitals. Another characteristic of our study was the habitation and occupation of our examinees. Many of the people in the study were city dwellers or employees at companies, which partially explained the age distribution of the study population. Specifically, the largest group of individuals in our study consisted of those 30–39 years of age (57% of both genders), while those who were more than 60 years old comprised only 2% of the study population.

We compared our results for monthly 25(OH)D levels with that of solar insolation data obtained from the Korea Meteorological Administration (Figs. 1 and 2). Based on the similar patterns between the two graphs, it appeared that seasonal 25(OH)D level fluctuation was associated with solar insolation.

The distribution of age in the study population was considered a limitation. Each age group consisted of a sufficient population size to make comparisons of the 25(OH)D levels meaningful; however, the mean 25(OH)D level of the total study population may have been influenced by the age distribution, making it lower than the true value. Another limitation of this study was that not all potential factors that may contribute to variations in 25(OH)D levels were examined, including skin pigmentation, amount or frequency of food ingestion, and variations in regions or occupations. Furthermore, these results were not obtained from repeated measurements of participants through follow-up studies.

The state and severity of vitamin D deficiency in this study was more pronounced than reported in other studies (8, 9). However, optimal concentrations of 25(OH)D

have not been established, with some researchers proposing that >75 nmol/l is desirable for overall health and disease prevention (31–33). Conversely, 25(OH)D levels <75 nmol/l are associated with a greater risk of breast cancer, colorectal cancer, and adenomas (34, 35). However, because the values obtained in this study were obtained from an otherwise normal healthy population, we are unable to propose vitamin D treatment in the vitamin D deficient population, as only 5.2% subjects met the criteria of >75 nmol/l 25(OH)D. Thus, the established reference levels from previous studies of vitamin D insufficiency and deficiency do not appear applicable to the Korean population.

In this study, we reported the distribution of the levels of 25(OH)D in a Korean population using recent data from health-care centers, and analyzed the levels according to age, gender, and seasonal variation. Establishment of specific reference ranges among genders, ages, or seasons may be useful for evaluating and managing vitamin D deficiency. In the future, studies evaluating the proper reference range of vitamin D levels in the Korean population should be performed at multiple institutes and in different regions, as well as among people with different lifestyles.

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