ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/237479612

Effect of iron on serum 25-hydroxyvitamin D and 24,25- dihydroxyvitamin D

Article

CITATIONS
7

READS
4

3 authors, including:

Gershon Tenenbaum
Florida State University
68 PUBLICATIONS **1,035** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Effects of a creativity supportive environment on children's creativity and adaptation behaviors. View project

Effects of an Improvisation Intervention on Elite Figure Skaters Performance, Self Esteem, Creativity and Mindfulness Skills View project

All content following this page was uploaded by Gershon Tenenbaum on 17 December 2014.

The user has requested enhancement of the downloaded file.

Effect of iron on serum 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D concentrations[1,2]

Dov Heldenberg, Gershon Tenenbaum, and Yosef Weisman

ABSTRACT In 13 of 17 infants (aged 10.5 ± 4.3; $\bar{x}$ ± SD mo) with iron-deficiency anemia, the serum 24,25-dihydroxyvitamin D concentration was below the normal range and in 9 of these 13 the serum 25-hydroxyvitamin D concentration was below the normal range despite the fact that these infants received 10 μg vitamin D/d from the age of 1 mo. The infants were treated with intramuscular iron dextran (Imferon). The iron-dextran treatment increased the hemoglobin and serum iron concentrations as well as 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D concentrations. It is known that iron deficiency impairs fat and vitamin A intestinal absorption. Therefore, it is suggested that absorption of vitamin D may also be impaired. This may contribute to the development of vitamin D deficiency. Iron supplementation may have improved the absorption of vitamin D in the small intestine and hence increased the vitamin D concentration in the plasma. *Am J Clin Nutr* 1992;56: 533–6.

KEY WORDS Infancy, iron-deficiency anemia, vitamin D deficiency, iron dextran

Introduction

Severe iron-deficiency anemia may cause malabsorption syndrome. Our assumption was that mild iron-deficiency anemia may impair fat absorption including vitamin D, which is fat soluble, and hence decrease vitamin D concentrations in the plasma. If this assumption is correct, treatment with iron will correct the anemia and will also improve the absorption of vitamin D and increase its concentration in the plasma.

Dietary iron-deficiency anemia is very common in Israel (1). Because of the abundant sunlight and regular vitamin D supplementation in infants from 1 mo of age on, vitamin D deficiency is expected to be a rare phenomenon. Previously we diagnosed a few cases of rickets in infants with iron-deficiency anemia, despite regular vitamin D supplementation. Therefore, we examined the prevalence of iron-deficiency anemia and vitamin D deficiency in randomly selected patients with iron deficiency.

Subjects and methods

The study was performed from May through September, 1990, a period with a substantial amount of sunlight in Israel. Twenty-five infants aged 6–24 mo (10.51 ± 4.30; $\bar{x}$ ± SD) with anemia were referred to the outpatient clinic of the Hillel-Yaffe Hospital. The hospital provides medical services to a population of 220000 Jewish and Arab residents. The ratio of Jews to Arabs is 3:2, respectively. In the patients' group the ratio of Jews to Arabs was 1:7, respectively. The patients were dark skinned and came from a low socioeconomic class. The maximal duration of breast-feeding was 3 mo, mainly because the mothers were obliged to return to work.

When breast-feeding was discontinued, cow milk was introduced. Later in the second year the mothers reported that "the infants were eating the usual family diet". Every infant in the study group received prophylactically 10 μg vitamin D orally daily as Vitamidyne drops (Fischer Corp, Tel Aviv, Israel), from 1 to 12 mo of age; each drop contained 5 μg cholecalciferol. This is a regular treatment in Israel given to infants, despite the abundance of sunshine. The infants in this study were exposed to much sunshine because they were raised in a rural environment and spent much time outdoors with their parents. Every infant underwent a physical examination in which weight and height were recorded. A roentgenogram of the wrist was performed on every infant. The criteria for iron-deficiency anemia were as follows: hemoglobin (Hb) < 1.69 mmol/L, mean corpuscular volume (MCV) < 75 fL, red cell distribution width (RDW) > 15%, transferrin saturation < 15%, and a normal hemoglobin electrophoresis. Criteria for vitamin D deficiency were as follows: 25-hydroxyvitamin D [25(OH)D] < 24.7 nmol/L, and 24,25-dihydroxyvitamin D [24,25$(OH)_2$D] < 205 nmol/L (2, 3). This study was approved by the human subjects committee of our institution.

Blood was drawn for Hb, hematocrit (Hct), MCV, RDW, serum iron, total-iron-binding capacity, albumin, alkaline phosphatase, calcium, phosphorus, blood urea nitrogen (BUN), creatinine, 25(OH)D, and 24,25$(OH)_2$D. The concentration of 25(OH)D and 24,25$(OH)_2$D were measured in lipid extracts of serum by competitive radioimmunoassay (4). Hemoglobinopathy was found in five infants; they were excluded from the study. Twenty infants fulfilled the criteria of iron-deficiency anemia and therefore were included in the study. Iron dextran

[1] From the Department of Pediatrics, Hillel-Yaffe Memorial Hospital, Hadera, Israel; the Center for Research and Sport Medicine Sciences, Wingate Institute, Netanya, Israel; and the Bone Disease Unit, Ichilov Hospital, Sackler Faculty of Medicine, Tel-Aviv University, Israel.

[2] Address reprint requests to D Heldenberg, Department of Pediatrics, Hillel-Yaffe Memorial Hospital, Hadera, Israel.

Received July 10, 1991.

Accepted for publication February 14, 1992.

The American Journal of Clinical Nutrition

Downloaded from ajcn.nutrition.org by guest on June 8, 2013

TABLE 1
Hematological and vitamin D values in infants with iron-deficiency anemia pre- and posttreatment with iron*

Group	Hemoglobin		25(OH)D	
	Pre	Post	Pre	Post
	mmol/L		*mmol/L*	
Low 25(OH)D and low $24,25(OH)_2D$ (n = 9)	1.23 ± 0.14[a]	1.70 ± 0.04	17.80 ± 4.24[a]	63.90 ± 32.80
Normal 25(OH)D and low $24,25(OH)_2D$ (n = 4)	1.41 ± 0.03[b]	1.85 ± 0.07	44.95 ± 15.51[b]	55.87 ± 16.15
Normal 25(OH)D and normal $24,25(OH)_2D$ (n = 4)	1.44 ± 0.04[b]	1.76 ± 0.04	45.62 ± 16.84[b]	71.13 ± 14.42
P	0.008	0.24	0.001	0.73

* $\bar{x}$ ± SD. Means not sharing a common superscript letter are significantly different based on Tukey's test at the P values shown.

(Imferon; Fisions, Leicestershire, UK) was given intramuscularly to all infants. The total intramuscular dose of iron dextran was calculated according to Dallmar (5). The parenteral medication was used because of poor compliance of the mothers with oral medication. At 8 wk posttreatment, 17 infants reattended and further investigations were carried out, including repeated blood tests. The pre- and posttreatment results were compared. Retrospectively, the infants were divided for further analysis into three groups by their concentration of vitamin D metabolites at the outset of the study: *1*) low 25(OH)D and low $24,25(OH)_2D$ (n = 9); *2*) low $24,25(OH)_2D$ and normal 25(OH)D (n = 4); and *3*) normal concentrations of both vitamin D metabolites (n = 4).

A repeated-measures (RM) analysis of variance (ANOVA) was applied to the pre- and posttreatment data for all the dependent measures (6).

A control group consisted of 10 healthy Arab infants of similar age, with an Hb > 169 mmol/L, who were hospitalized for elective minor surgery. These infants received an iron-fortified formula immediately after discontinuation of breast-feeding. In addition, they received a supplement of 10 μg vitamin D orally daily, from the age of 1 mo.

Results

The infants' physical examinations were unremarkable. They were at the 25th-50th percentiles for their age, height, and weight. Roentgenographic findings revealed no rachitic changes. Parasitic ova were not found. The mean (± SD) values for BUN and creatinine were 3.2 ± 0.6 mmol and 3.78 ± 0.72 μmol/L, respectively (normal values for all infants with no exceptions).

At the first stage all the hematological and vitamin D measures were subjected to a descriptive statistics broken down by three groups (**Tables 1 and 2**). At the second stage a one-way ANOVA was applied to the data, with vitamin D status as the independent measure. The results (Tables 1 and 2) indicated that before iron supplementation, the group with low 25(OH)D and low $24,25(OH)_2D$ had on the average lower Hb, 25(OH)D, $24,25(OH)_2D$, MCV, and transferrin saturation values than the other two groups

TABLE 2
Values for calcium, phosphorus, and alkaline phosphatase in infants with iron-deficiency anemia pre- and posttreatment with iron*

Group	Calcium		Phosphorus		Alkaline phosphatase		$24,25(OH)_2D$		Mean corpuscular volume		Red cell distribution width		Percent saturation	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
	mmol/L		*mmol/L*		*U/L*		*mmol/L*		*fL*		%		%	
Low 25(OH)D and low $24,25(OH)_2D$ (n = 9)	2.38 ± 0.12	2.39 ± 0.10	1.87 ± 0.40	1.97 ± 0.20	4377.8 ± 1325.5	4022.2 ± 791.8	0.69 ± 0.82[a]	4.60 ± 1.59[b]	58.44 ± 5.36[a]	76.22 ± 1.39	18.20 ± 0.83	14.62 ± 0.48	6.89 ± 2.82[a]	17.97 ± 2.29
Normal 25(OH)D and low $24,25(OH)_2D$ (n = 4)	2.29 ± 0.21	2.41 ± 0.10	2.01 ± 0.39	1.68 ± 0.21	3650.0 ± 1226.1	3000.0 ± 697.6	1.62 ± 0.15[b]	2.62 ± 1.02[a]	66.25 ± 3.10[b]	76.50 ± 1.73	18.27 ± 0.76	14.65 ± 0.57	11.40 ± 1.52[b]	17.40 ± 0.41
Normal 25(OH)D and normal $24,25(OH)_2D$ (n = 4)	2.29 ± 0.16	2.43 ± 0.12	1.84 ± 0.20	1.77 ± 0.17	3675.0 ± 1187.1	3500.0 ± 778.9	3.26 ± 0.56[c]	5.37 ± 0.35[b]	63.50 ± 2.52[ab]	76.75 ± 1.26	17.57 ± 0.43	14.22 ± 0.22	10.72 ± 1.22[b]	16.55 ± 0.44
P	0.94	0.77	0.76	0.07	0.53	0.11	0.0001	0.02	0.03	0.83	0.34	0.33	0.008	0.42

* $\bar{x}$ ± SD. Means not sharing a common superscript letter are significantly different based on Tukey's test at the P values shown.

The American Journal of Clinical Nutrition

Downloaded from ajcn.nutrition.org by guest on June 8, 2013

of infants. After iron supplementation these differences disappeared.

Before applying a more advanced statistical procedure the matrix data were transformed into logarithmic values to avoid the requirement of normal distribution, because of an insufficient number of infants within each of the groups. An RM ANOVA applied to Hb data showed a significant interaction effect ($F_{[2,14]} = 5.21$; $P \leq 0.02$) between the group and the time elapsed before and after supplementation with iron to the infants. In addition, the pretreatment and posttreatment effects were also significant ($F_{[1,14]} = 168$; $P \leq 0.00001$). All groups significantly increased Hb from the outset to the end stage of the study. However, the significant differences at the outset of the study disappeared at the end, where the differences among the three groups were insignificant.

A similar analysis (RM ANOVA) was applied to the 25(OH)D data. As shown in Table 1, significant differences were obtained at the outset of the study. However, all groups increased significantly ($F_{[1,14]} = 16.91$; $P \leq 0.001$) from pre- to posttreatment periods. However, the largest average increase was obtained by infants with low 25(OH)D and low $24,25(OH)_2D$ concentrations. At the end, no significant differences among the groups were found. This trend was indicated by a significant tendency for time × group effect ($F_{[2,14]} = 2.08$; $P \leq 0.10$).

RM ANOVA was applied to the $24,25(OH)_2D$ data. The trend obtained for 25(OH)D was also obtained for $24,25(OH)_2D$. At the outset of the study the group with normal 25(OH)D and normal $24,25(OH)_2D$ averaged higher values than the other two groups. At the end, all groups significantly increased their $24,25(OH)_2D$ values ($F_{[1,14]} = 26.72$; $P \leq 0.0001$). The substantial increase in the group with normal 25(OH)D and normal $24,25(OH)_2D$ was indicated also by a significant time × group interaction ($F_{[2,14]} = 4, 54$; $P \leq 0.03$). It should be added that at the end of the study, although all mean values were within the normal range, infants with normal 25(OH)D and low $24,25(OH)_2D$ concentrations were still significantly ($P < 0.05$) lower than the others in $24,25(OH)_2D$ after iron supplementation (*see* Table 1).

Finally, differences in MCV and transferrin saturation, which were low at the outset of the study in the group with low 25(OH)D and low $24,25(OH)_2D$, disappeared after iron supplementation. The results obtained in the control group were as follows: Hb, 1.81 ± 0.05 mmol/L; transferrin saturation, 25.07 ± 5.5.0%; 25(OH)D, 64.78 ± 19.86 nmol/L; and $24,25(OH)_2D$, 4.37 ± 1.52 nmol/L. The concentrations of calcium, phosphorus, and alkaline phosphatase were similar in all groups with no significant differences between pre- and posttreatment of iron dextran (Table 2).

Discussion

In the present study in > 50% of infants with iron-deficiency anemia, a decreased concentration of 25(OH)D and $24,25(OH)_2D$ was reported, despite the fact that the infants received daily supplementation with vitamin D. Treatment with iron dextran increased concentrations of serum iron and vitamin D metabolites. The increase in serum vitamin D metabolites occurred even though no change was made in the diet or vitamin D supplementation. This observation raises the question of whether there is a causal relationship between iron deficiency and serum vitamin D metabolite concentrations. Vitamin D produced in the skin or absorbed from the gut is carried to the liver. There vitamin D is hydroxylated to 25(OH)D, the major vitamin D metabolite in the serum. 25(OH)D is subsequently hydroxylated to either 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] or $24,25(OH)_2D$ in the kidney. There is emerging evidence that $24,25(OH)_2D$ is involved in the development of embryonic cartilage and in endochondral bone formation (7). Rickets or osteomalacia due to vitamin D deficiency is usually detected when the plasma concentration of 25(OH)D is < 24.7 mmol/L (2, 3). When vitamin D deficiency is in its earliest stage and calcium homeostasis is put under strain, the kidney reacts by producing less $24,25(OH)_2D$. Some investigators therefore believe that a reduction in the ratio of serum $24,25(OH)_2D$ to its precursor, 25(OH)D, could be an even more sensitive index of vitamin D deficiency than that of serum 25(OH)D concentration (8, 9). We can also find a decreased concentration of $24,25(OH)_2D$ and normal 25(OH)D in moderate renal insufficiency. Some of the 25(OH)D formed in the liver is secreted into the bile, mainly in the form of glucuronides and may be reabsorbed in the enterohepatic circulation (10). The significance and magnitude of this enterohepatic circulation is controversial. Even if it is small, the continual loss of 25(OH)D as a result of impaired reabsorption from the gut could lead to vitamin D deficiency. Gastrointestinal abnormalities of function associated with iron deficiency include impaired intestinal absorption of fat, vitamin A, and xylose (11). Iron deficiency in some way inhibited the metabolism of vitamin D to 25(OH)D. It may also be that iron deficiency impairs vitamin D absorption and 25(OH)D reabsorption in the gut, and this may contribute to the state of vitamin D deficiency found in our patients.

Iron-deficiency anemia is very common in Israel (1). Winter found that 45% of the infants developed an Hb < 1.69 mmol/L and 15% of the infants had an Hb < 1.54 mmol/L (1). In contrast to the high prevalence of iron deficiency, it was expected that the exposure to sunlight in Israel, together with a daily supplementation of 10 μg vitamin D, would prevent the infants from developing vitamin D deficiency (12). However, surprisingly, vitamin D deficiency was found in 13 of the 17 infants. Grindulis et al (13) found a combined deficiency of iron and vitamin D in Asian toddlers. Both deficiencies were because of inadequate nutrition and were associated with evidence of underprivileged environment, crowded living conditions, low social class, and poor maternal education. They also noticed that children with low vitamin D concentrations had lower Hb and serum iron concentrations. Grindulis et al (13) failed to demonstrate any relationship between iron and vitamin D concentrations. In the present study a combined deficiency of iron and vitamin D was found despite the fact that the patients received a daily supplementation of vitamin D and were exposed to sunlight. It is possible that the observations that were made are explained by the fact that the infants in our study were 2 mo older and were more likely to be outdoors and be exposed to sunlight. The treatment of their anemia may also have made the children feel better, and therefore, be more active outdoors, thereby increasing the likelihood of making more vitamin D in their skin. However, note that the infants in our study live in a very poor Arab village, and most of their time from early infancy is spent outdoors. Therefore, it is not likely that there would be a significant change in their exposure to sunlight during the 2 mo. In contrast with the findings of Grindulis et al, a relationship between iron and vitamin D concentration was found. Elevation of serum iron

The American Journal of Clinical Nutrition

Downloaded from ajcn.nutrition.org by guest on June 8, 2013

was associated with elevation in vitamin D concentration. The increase in the concentration of vitamin D occurred even though no change was made in the diet. We assume that the increase in vitamin D concentration was affected by the iron, which significantly increased the absorption of vitamin D from the intestine.

Other possible effects of iron therapy on serum 25(OH)D concentrations may be alterations in vitamin D binding protein or inhibition of the conversion of 25(OH)D to $1,25(OH)_2D$.

Rickets in tropical or subtropical countries with an abundance of sunlight is surprising (14). One study has suggested that Asians and dark-skinned infants may tend to have a poorer vitamin D status than whites (15). Another study showed that melanin reduces the amount of vitamin D produced in response to ultraviolet (UV) radiation exposure. Asians do not impair vitamin D synthesis from dehydrocholesterol in the skin, but they require longer exposure to UV radiation to have vitamin D production similar to Caucasians (16). If this explanation is accepted, some doubts still arise as to the very high prevalence of rickets in those countries. It is suggested that iron-deficiency anemia, which is common in those countries, contributes to the high prevalence of rickets because the amount of vitamin D synthesized in the skin is insufficient and does not satisfy the infants' vitamin D requirements. Furthermore, the absorption of vitamin D in the small intestine is limited, as the result of iron deficiency, resulting in the development of rickets.

During the first several months of rickets' development, normal concentrations of calcium, phosphorus, and alkaline phosphatase may occur whereas concentrations of vitamin D may be lower than normal.

In summary, 17 patients with both iron and vitamin D deficiencies were investigated. The results do not represent the status of iron and vitamin D in the whole Arab population in Israel. Vitamin D supplements alone given to infants with iron-deficiency anemia may not necessarily prevent vitamin D deficiency. The maximal absorption and efficiency of vitamin D may only occur if the infants' diet is fortified with iron. Therefore, it is recommended to supply to infants a formula containing both iron and vitamin D.

References

1. Winter S. Letter to the editor. Prevalence of anemia among Israeli infants. Harefuah 1988;114:525 (in Hebrew).
2. Kanis JA. Vitamin D metabolism and its clinical application. J Bone Joint Surg (Br) 1982;64:542–60.
3. Clemens TL, O'Riordan LH. Vitamin D. In: Becker L, ed. Principles and practice of endocrinology and metabolism. Philadelphia: JB Lippincott, 1990;422–38.
4. Weisman Y, Reiter E, Rott A. Measurement of 24,25 dihydroxyvitamin D in sera of neonates and children. J Pediatr 1977;91: 904–8.
5. Dallman PR. Iron deficiency and related nutritional anemias. In: Nathan DG, Oski FA, eds. Hematology of infancy and child-hood. Philadelphia: WB Saunders, 1981;298–343.
6. Winer BJ. Statistical principles in experimental design. New York: McGraw-Hill, 1971.
7. Somjen D, Binderman I, Weisman Y. The effects of 24R, 25-dihydroxy cholecalciferol and of 1 alpha, 25 dihydrochole-calciferol on ornithine decarboxylase activity and on DNA synthesis in the epiphysis and diaphysis of rat bone and in the duodenum. Biochem J 1983;214:293–8.
8. Lawoyin S, Zerwekh JE, Glass K, Pak CY. Ability of 25-hydroxyvitamin D_3 therapy to augment serum 1,25- and 24,25-dihydroxy vitamin D in postmenopausal osteoporosis. J Clin Endocrinol Metab 1980;50:593–6.
9. Weisman Y, Schen RJ, Eisenberg Z, Edelstein S, Harell A. Inadequate status and impaired metabolism of vitamin D in the elderly. Isr J Med Sci 1981;17:19–21.
10. Arnaud SB, Goldsmith RS, Lambert PW, Go VL. 25-hydroxyvitamin D_3: evidence of an enterohepatic circulation in man. Proc Soc Exp Biol Med 1975;149:570–2.
11. Naiman JL, Oski FA, Diamond LK, Vawter GF, Shwachman H. The gastrointestinal effects of iron deficiency anemia. Pediatrics 1964;33:83–99.
12. Nehama H, Weintroub S, Eisenberg Z, Birger E, Milbauer B, Weisman Y. Seasonal variation in paired 24,25 dihydroxy-vitamin D concentration in Israel. Isr J Med Sci 1987;23:274–7.
13. Grindulis H, Scott PH, Belton NR, Wharton BA. Combined deficiency of iron and vitamin D in Asian toddlers. Arch Dis Childh 1986;61:843–8.
14. Belton RB. Rickets—not only the "English Disease". Acta Paediatr Scand [Suppl] 1986;323:68–75.
15. Clemens TL, Adams JS, Henderson SL, Hollick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D. Lancet 1982;1:74–6.
16. Lo CW, Paris PW, Hollick MF. Serum vitamin D in response to ultraviolet irradiation in Asians. In: Norman AW, Schaefer K, Grigoleit HG, Herrath DV, eds. Vitamin D, chemical, biochemical and clinical update. Berlin: De Gruyter, 1985;709–10.

The American Journal of Clinical Nutrition

Downloaded from ajcn.nutrition.org by guest on June 8, 2013

View publication stats