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Evaluation level of vitamin D for the population of Basrah government

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1. Abstract

The present study was carried out to evaluate vitamin D levels in local samples from different regions in Basrah governorate by using ELISA tests. Serum were collected from (90) samples, of different ages and both genders . Nine clinically healthy local samples were considered as contro Results show that suspected testers showed different clinical manifestation belong to vitamin D deficiency and significant decrease ($p<0.05$) were encountered in D (16.03±1.5) ng/ml compared with control (28.6±7.2) ng/ml. It had been concluded the residents of Basra Governorate are exposed to a small percentage of the beneficial and necessary sunlight for a certain period, in addition to suffering from health problems that prevented the benefit from this necessary solar energy. or reduction of nutritional supplements containing important vitamins and minerals.

2- Introduction

Vitamin D get special consideration in human by several worker in the world. Vitamin D, a fat-soluble prohormone, is synthesized in response to sunlight. Vitamin D requires two metabolic conversions, 25-hydroxylation in the liver and 1alpha-hydroxylation in the kidney, to become active hormone.(Zehnder et ,al.1999). The active form, 1alpha,25-(OH)2D, binds to the vitamin D receptor (VDR) to modulate gene transcription and regulate mineral ion homeostasis. Vitamin D plays several roles in the body, influencing bone health as well as serum calcium and phosphate levels. Furthermore, vitamin D may modify immune function, cell proliferation, differentiation and apoptosis. (Carmeliet and Verlinden2008) Vitamin D deficiency has been associated with numerous health outcomes, including risk of rickets in children or osteomalacia in adults, increased risk of fractures, falls, cancer, autoimmune disease, infectious disease, type 1 and type 2 diabetes, hypertension and heart disease, and other diseases such as multiple sclerosis. Here, vitamin D physiology and metabolism, its genomic action and association of polymorphisms in vitamin D pathway genes .(Haddad,1995). In Basrah , Iraq little information

had been provided on vitamin D deficiency therefore the study were aimed to evaluation and measurement of vitamin D in Basrah of the south of Iraq .

3. History:

Although rickets, scurvy, beri-beri and other such diseases were known for

centuries, the cause of them remained elusive until the twentieth century. On the basis of the dogma put forth by the influential German chemists in the nineteenth century led by von Liebig (von Liebig,1957). Another discovery of a substance that prevented scurvy among sailors was made by Hoist and Frohlich in1907 .They found that scurvy experienced by seamen could be prevented or cured by citrus fruits or a substance found therein. Yet, the idea of essential micronutrients of an organic type had yet to be conceived. The idea of vitamins was first suggested by (Funk,1911) who envisioned that a ‘vital amine' present in foods was required for health and survival. Unknown to Funk and without evidence, this would prove to be a term that would describe the accessory food factors later to be discovered. Although the idea of vitamin D became very clear and it was found in a non-saponifiable fraction, the actual identification of the vitamin structure was not to take place (Askew et al.1931) were able to isolate vitamin D2 from an irradiation mixture of ergosterol. Vitamin D1 had proved to be an artefact of an adduct between vitamin D2 and lumisterol by(Windaus and Linser,1928). Thus, vitamin D2 proved to be the first vitamin D to be isolated and identified

In 1935, 7-dehydrocholesterol was isolated by (Windaus et al.1935) and vitamin D3 was identified in 1937 by the(Windaus and Bock,1937) Vitamin D3 is the natural form of vitamin D formed in the skin as a result of UV irradiation of 7-dehydrocholesterol. This then raised the question of whether vitamin D is a true vitamin or whether it is normally produced in the skin and is not found in natural foods. Although it was surmised that vitamin D3 arises in skin via the irradiation of 7-dehydrocholesterol, this was not proven until 1978 when(Esvelt et al, 1978)actually isolated and identified vitamin D3 by mass spectrometry. Before this,(Holick et al,1977). provided evidence that previtamin D3 is formed in the skin on UV irradiation. The actual chemistry of the irradiation process was defined by the work of Velluz et al.34 and also by the contributions of (Havinga1973).

4. Structure:

Vitamin D is not technically a vitamin, since it is not an essential dietary factor. It is rather a prohormone produced photochemically in the skin from 7-dehydrocholesterol. Vitamin D and its metabolites may be categorized as either cholecalciferols or ergocalciferols. Cholecalciferol (vitamin D-3) is the parent compound of the naturally occurring family and is produced in the skin from 7-dehydrocholesterol on exposure to the ultraviolet B portion of sunlight. Vitamin D-2 (ergocalciferol), the parent compound of the other family, is manufactured by irradiation of ergosterol produced by yeasts and its potency is less than one-third of vitamin D-3's potency. The steps in the vitamin D endocrine system include the following:

1) the photoconversion of 7-dehydrocholesterol to vitamin D-3 in the skin or dietary intake of vitamin D-3.

2) metabolism of vitamin D-3 by the liver to 25-hydroxyvitamin-D-3 [25(OH)D-3], the major form of vitamin D circulating in the blood compartment .

3) conversion of 25(OH)D-3 by the kidney (functioning as an endocrine gland) to the hormone 1,25-dihydroxyvitamin D-3 [1,25(OH)(2)D-3.

4) systemic transport of the dihydroxylated metabolite 1,25(OH)(2)D-3 to distal target organs.

5) binding of 1,25(OH)(2)D-3 to a nuclear receptor (VDR) at target organs, followed by generation of appropriate biological responses. The activation of vitamin D to its hormonal form is mediated by cytochrome P450 enzymes. Six cytochrome P450 (CYP) isoforms have been shown to hydroxylate vitamin D.

Four of these, CYP27A1, CYP2R1, CYP3A4 and CYP2J3, are candidates for the enzyme vitamin D 25-hydroxylase that is involved in the first step of activation. The highly regulated, renal enzyme 25-hydroxyvitamin D-1 alpha-hydroxylase contains the component CYP27B1, which completes the activation pathway to the hormonal form 1,25(OH)(2)D-3. A five-step inactivation pathway from 1,25(OH)(2)D-3 to calcitroic acid is attributed to a single multifunctional CYP, CYP24A1, which is transcriptionally induced in vitamin D target cells by the action of 1,25(OH)(2)D-3. An additional key component in the operation of the vitamin D endocrine system is the plasma vitamin D binding protein (DBP), which carries vitamin D-3 and its metabolites to their metabolism and target organs. DBP is a specific, high-affinity transport protein. It is synthesized by the liver and circulates in great excess, with fewer than 5% of the binding sites normally occupied. 1,25(OH)(2)D-3, acts as a ligand for a nuclear transcription factor; vitamin D receptor - VDR, which like all other nuclear receptors, regulates gene transcription and cell function. The widespread presence of VDR, and the key activating (1 alpha-hydroxylase, CYP27B1) and inactivating (24-hydroxylase, CYP24A1) enzymes in most mammalian cells means that the cells in these tissues have the potential to produce biological responses, depending on the availability of appropriate amounts of vitamin D-3. Thanks to this widespread presence of elements of vitamin D endocrine system, its biological features are being recognized outside bone tissue, i.e. calcium and phosphate metabolism(Bouillon et,al.2008).

5. Sources:

Vitamin D (calciferol) comprises a group of fat soluble seco-sterols found naturally only in a few foods, such as fish-liver oils, fatty fish, mushrooms, egg yolks, and liver. The two major physiologically relevant forms of vitamin D are D2 (ergocalciferol) and D3 (cholecalciferol). Vitamin D3 is photosynthesized in the skin of vertebrates by the action of solar ultraviolet (UV) B radiation on 7-dehydrocholesterol (Fieser 1959). Vitamin D2 is produced by UV irradiation of ergosterol, which occurs in molds, yeast, and higher-order plants. Under conditions of regular sun exposure, dietary vitamin D intake is of minor importance. However, latitude, season, aging, sunscreen use, and skin pigmentation influence the production of vitamin D3 by the skin (Institute of Medicine 1997). Most of the dietary intake of vitamin D comes from fortified milk products and other fortified foods such as breakfast cereals and orange juice (Institute of Medicine

1997). Both vitamin D2 and D3 are used in nonprescription vitamin D supplements, but vitamin D2 is the form available **by prescription in the United States (Holick 2007)**

6. Vitamin D Function:

Active vitamin D functions as a hormone, and its main biologic function in people is to maintain serum calcium and phosphorus concentrations within the normal range by enhancing the efficiency of the small intestine to absorb these minerals from the diet (DeLuca 1988; Reichel 1989). When dietary calcium intake is inadequate to satisfy the body's calcium requirement, 1,25(OH)2 D, along with PTH, mobilizes calcium stores from the bone. In the kidney, 1,25(OH)2 D increases calcium reabsorption by the distal renal tubules. Apart from these traditional calcium-related actions, 1,25(OH)2 D and its synthetic analogs are increasingly recognized for their potent antiproliferative, prodifferentiative, and immunomodulatory activities (Nagpal 2005)

Intestinal Absorption:

The primary site of vitamin A and carotene absorption is the proximal jejunum (Donald, et. al. 2001) Normal pancreatic, liver and bilary function and adequate fat intake are required for absorption of vitamin A and its precursors. Dietary retinol esters are hydrolyzed within the intestinal lumen and absorbed directly in the intestines .(Ross, and Gardner,1994). The ingested B-carotene are oxidatively cleaved by B-carotene dioxygenase, by cleavage utilizing molecular oxygen and requires bile salt . The retinaldehyde is reduced by a specific reductase utilizing to formed retinol in the intestinal mucosa . A small fraction of the retinal generated from the B-carotene is oxidized to retinoic acid in the intestine. (Krinsky, et. al,1990).

Tissue Metabolism:

Vitamin D3 produced in the epidermis must be further metabolized to be active. The first step, 25-hydroxylation, takes place primarily in the liver, although other tissues have this enzymatic activity as well. As will be discussed below, there are several 25-hydroxylases. 25OHD is the major circulating form of vitamin D. However, in order for vitamin D metabolites to achieve maximum biologic activity they must be further hydroxylated in the 1α position by the enzyme CYP27B1; 1,25(OH)2D is the most potent metabolite of vitamin D and accounts for most of its biologic actions. The 1α hydroxylation occurs primarily in the kidney, although as for the 25-hydroxylase, other tissues have this enzyme. Vitamin D and its metabolites, 25OHD and 1,25(OH)2D, can also be hydroxylated in the 24 position. In the absence of 25-hydroxylation this may serve to activate the metabolite or analog as 1,25(OH)2D and 1,24(OH)2D have similar biologic potency. However, 24-hydroxylation of metabolites with an existing 25OH group reduces their activity and leads to further catabolism. The details of these reactions are described below. (Whistler,et,al.1645)

Metabolism of vitamin D

Once vitamin D enters the circulation, it is bound by an α2-globulin known as vitamin D-binding protein(Holick and Chen,2008). Vitamin D is not active as such but undergoes a series of metabolic transformations first in the liver and then in the kidney to form the active metabolite, 1,25-dihydroxyvitamin D.1, 2 In the liver, an enzyme, vitamin D-25-hydroxylase, converts vitamin D to 25-hydroxyvitamin D (Fig. 3).(Kumar R,1982).This enzyme is located in both the microsomal and the mitochondrial fractions of the hepatocyte(Fig. 4). (Kumar R,1982).

Causes of vitamin D deficiency :

A deficiency in vitamin D can result from inadequate exposure to sunlight, inefficient production in the skin, not enough vitamin D in your diet, and health conditions that can affect it including, gastrointestinal disorders, renal diseases, and liver

Darker skin

Melanin is what gives skin its color. Light-skinned people have less melanin than those with darker skin. Melanin is able to absorb UV-B radiation from the sun and reduce the skin's capacity to produce vitamin D3 by 95%-99%. Dark-skinned individuals have natural sun protection and require at least three to five times longer exposures to make the same amount of vitamin D as a person with a white skin tone. African-Americans have a population mean serum 25(OH)D level of 16 ng/mL, whereas white Americans have a level of 26 ng/mL. (Rochel,et,al.2000).

Weight

Being overweight or obese may put you at risk for a vitamin D deficiency. A recent review of 23 studies showed that obese subjects had 35% higher rates of vitamin D deficiency compared with normal-weight subjects and 24% higher rates compared with overweight subjects. While diet and decreased sun exposure may have some impact on this, there appears to be an increased need that cannot be met without a supplement.(Leo and Chen2000). One study tested the blood levels of vitamin D after sun exposure in both obese and non-obese subjects. Both saw an initial rise in vitamin D levels after similar exposures, but 24 hours later, there was 57% less vitamin D in the blood of the obese subjects. Both groups had a similar capacity of the skin to produce the vitamin. The difference was seen in the release of vitamin D from the skin into the circulation.(Kwok,et,al.1994).

Limited exposure to the sun

You may look out your window and see the sun shining and think that you are safe from this deficiency, but that is not always the case. Even in sunny climates, there is an increased prevalence of vitamin D deficiency(Pollyet,al.2000). We have all heard about the dangers of skin cancer and the need for sunscreen to protect us from this disease. This knowledge and the preventive actions we take have significantly decreased our vitamin D levels. Sunscreen protects so well against UV-B rays that

an SPF of 30 decreases vitamin D synthesis in the skin by more than 95%. On top of this, we tend to spend more time indoors. One study found that it took Caucasians exposure of more than 30% of their body every day in the summer to make the optimal amounts of vitamin D. Most adults work indoors and wear more clothing during the workweek, which leaves only about 10%-15% of their body exposed to UV for short periods, so they cannot meet their vitamin D needs through the sun alone (Glass and Rosenfeld2000). Even if you do have some exposure to the sun, the total amount of vitamin D you can produce is affected by the season, time of day, ozone amount, latitude, and the number of clouds in the sky.(Chowdhury,et,al.2014)

The important thing about using the sun for vitamin D production is to know that less is more. You are better off with short regular exposures to the sun rather than prolonged exposure for many reasons. The process is not as simple as the sun hitting your skin and vitamin D appearing in your blood. What actually happens is that vitamin D3 is first transformed by a process known as hydroxylation in the liver to 25-hydroxyvitamin D3, often written as (25(OH)D3), and then again in the kidney to its active form, 1,25-dihydroxyvitamin D3, written as (1,25(OH)2D3). The level that is checked in your blood is 25-hydroxyvitamin D, often written as 25(OH)D, which includes vitamin D2 and D3. By staying in the sun, you limit this process and can actually get less vitamin D. You also have a lower risk of burning and damaging your skin with short exposures. (Betty,2011).

Pathophysiology:

Decreased exposure of the skin to sunlight is a common cause of vitamin D deficiency. People with a darker skin pigment with increased amounts of melanin may have decreased production of vitamin D. Melanin absorbs ultraviolet B radiation from the sun and reduces vitamin D production. Sunscreen can also reduce vitamin D production. Medications may speed up the metabolism of vitamin D, causing a deficiency (Chiang et,al.2016)

Liver diseases: The liver is required to transform vitamin D into 25-hydroxyvitamin D. This is an inactive metabolite of vitamin D but is a necessary precursor (building block) to create the active form of vitamin D(Cherniack et,al.2008)

Kidney disease: The kidneys are responsible for converting 25-hydroxyvitamin D to 1,25-hydroxyvitamin D. This is the active form of vitamin D in the body. Kidney disease reduces 1,25-hydroxyvitamin D formation, leading to a deficient effects of vitamin D(Winzenberg and Jones 2013).

Intestinal conditions that result in malabsorption of nutrients may also contribute to vitamin D deficiency by decreasing the amount of vitamin D absorbed via diet. (Holick 2008). In addition, a vitamin D deficiency may lead to decreased absorption of calcium by the intestines, resulting in increased production of osteoclasts that may break down a person's

bone matrix(Wang et,al.2016). In states of hypocalcemia, calcium will leave the bones and may give rise to secondary hyperparathyroidism, which is a response by the body to increase serum calcium levels. The body does this by increasing uptake of calcium by the kidneys and continuing to take calcium away from the bones. If prolonged, this may lead to osteoporosis in adults and rickets in children(Winzenberg and Jones 2013)

Clinical Findings:

Vitamin D deficiency may only be detected on blood tests, but is the cause of some bone diseases and is associated with other conditions:[1]

• Rickets, a childhood disease characterized by impeded growth and deformity of the long bones.[8] The earliest sign of vitamin D deficiency is craniotabes, abnormal softening or thinning of the skull(Zella et, al.2010).

• Osteomalacia, a bone-thinning disorder that occurs exclusively in adults and is characterized by proximal muscle weakness and bone fragility. Women with vitamin D deficiency who have been through multiple pregnancies are at elevated risk of Osteomalacia(Omdahl et, al.2001).

• Osteoporosis, a condition characterized by reduced bone mineral density and increased bone fragility.

• Increased risk of fracture.

• Muscle aches, weakness, and twitching (fasciculations), due to reduced blood calcium (hypocalcemia).

• Periodontitis, local inflammatory bone loss that can result in tooth loss.

• Pre-eclampsia: There has been an association of vitamin D deficiency and women who develop pre-eclampsia in pregnancy. The exact relationship of these conditions is not well understood(Carlberg and Polly,1998)

Maternal vitamin D deficiency may affect the baby, causing overt bone disease from before birth and impairment of bone quality after birth(Shaffer and Gewirth,2002)

• Respiratory infections and COVID-19: Vitamin D deficiency may increase the risk of severe acute respiratory infections and COPD. Emerging studies have suggested a link between vitamin D deficiency and COVID-19 symptoms. A review has shown that vitamin D deficiency is not associated with a higher chance of having COVID-19, but is associated with a greater severity of the disease, including 80% increases in the rates of hospitalization and mortality.(Jolliffe, et,al.2020)

Laboratory Finding :

Two forms of vitamin D can be measured in the blood, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D. The 25-hydroxyvitamin D is the major form found in the blood and is the relatively inactive precursor to the active hormone, 1,25-dihydroxyvitamin D. Because of its long half-life and higher concentration, 25-hydroxyvitamin D is commonly measured to assess

and monitor vitamin D status in individuals. (Nguyen and Chernoff 2012)

25-hydroxyvitamin D

A low blood level of 25-hydroxyvitamin D may mean that a person is not getting enough exposure to sunlight or enough dietary vitamin D to meet his or her body's demand or that there is a problem with its absorption from the intestines. Occasionally, drugs used to treat seizures, particularly phenytoin (Dilantin), can interfere with the production of 25-hydroxyvitamin D in the liver.

(Tangpricha and Khazai2012).

There is some evidence that vitamin D deficiency may increase the risk of some cancers, immune diseases, and cardiovascular disease.

A high level of 25-hydroxyvitamin D usually reflects excess supplementation from vitamin pills or other nutritional supplements.

1,25-dihydroxyvitamin DA low level of 1,25-dihydroxyvitamin D can be seen in kidney disease and is one of the earliest changes to occur in persons with early kidney failure.A high level of 1,25-dihydroxyvitamin D may occur when there is excess parathryoid hormone or when there are diseases, such as sarcoidosis or some lymphomas, that can make 1,25-dihydroxyvitamin D outside of the kidneys.(Rennert,2011).

High levels of vitamin D and calcium can lead to the calcification and damage to organs, particularly the kidneys and blood vessels. If magnesium levels are low, they can cause a low calcium level that is resistant to vitamin D and parathyroid hormone regulation. It may be necessary to supplement both magnesium and calcium to regain normal function(Glass and Rosenfeld2000).

Materials and methods

- ✓ Area of the study

This study was conducted in Basrah govornorate / Iraq , which was divided into five regions included center of Basra, Al-Qurna, AL-zubair, Shatealarab and Abu-Alkassib.

- ✓ Sample

- ✓ Ninty different ages and both sexes which were used in this study. Eighty one show signs of vitamin D deficiency, moreover nine , clinically healthy local were served as controls Clinical examination

Routine clinical examination were performed to all suspected human with special examination reported in the questionnaire attached (Accessory 1).

- ✓ Collection of samples

Ten milliliter of blood were drained from each 90. Those samples were put in a sterile labeled tubes without anti-coagulant, left for overnight or centrifuged at 1500 r.p.m. within 5 minutes to separation .

Then subsequently the serum transferred to Ependorf tubes, then was freezed at -20$_o$C for further serum analysis of vitamin A and beta carotene using tow Enzyme Linked Immune Sorbent Assay(ELISA) tests interact

- ✓ Statistical Analysis

The data were expressed as mean ± standard error (mean ±SE).Least significant different test (LSD) was used to test the difference between the disease group and control by using statistical program for social science SPSS, $p<0.05$ was considered significant

Our Human Vitamin D3,VD3 ELISA kit is to assay VD3 levels in Human serum, plasma, culture media or any biological fluid.

- ✓ Serum preparation

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at

2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again

- ✓ Procedure

1.Dilution of Standards

Ten wells are set for standards in a Microelisa stripplate. In Well 1 and Well 2, 100µl Standard solution and 50µl Standard Dilution buffer are added and mixed well. In Well 3 and Well 4, 100µl solution from Well 1 and Well 2 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50µl solution is discarded from Well 3 and Well 4. In Well 5 and Well 6, 50µl solution from Well 3 and Well 4 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. In Well 7 and Well 8, 50µl solution from Well 5 and Well 6 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. In Well 9 and Well 10, 50µl solution from Well 7and Well 8 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50µl solution is discarded from Well 9 and Well 10. After dilution, the total 5 volume in all the wells are 50µl and the concentrations are 30ng/ml,20ng/ml, 10ng/ml, 5ng/ml and 2.5ng/ml, respectively.

2.In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.

3. Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.

4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T

5 . wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.

6. Add 50 µl HRP-Conjugate reagent to each well except the blank control well.

7.Incubation as described in Step 3.

8. Washing as described in Step 5.

9. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37 ℃ for 15 minutes. Please avoid light during coloring.

10. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.

11 . Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after 30ng

Treatment:

•For children 1-18 years of age who are vitamin D deficient, we suggest treatment with 2,000 IU/d of vitamin D3 for at least six weeks or with 50,000 IU once a week for at least six weeks to achieve a blood level of 25(OH)D above 30 ng/mL, followed by maintenance therapy of 600-1,000 IU/day.

•We suggest that all adults who are vitamin D deficient be treated with 50,000 IU of vitamin D3 once a week for eight weeks or its equivalent of 6,000 IU of vitamin D3 daily to achieve a blood level of 25(OH)D above 30 ng/mL, followed by maintenance therapy of 1,500-2,000 IU/day.

•In obese patients, patients with malabsorption syndromes, and patients on medications affecting vitamin D metabolism, we suggest a higher dose (two to three times higher; at least 6,000-10,000 IU/day) of vitamin D to treat vitamin D deficiency to maintain a 25(OH)D level above 30 ng/mL, followed by maintenance therapy of 3,000-6,000IU/day. One study found that for every 33 lbs. of body weight the serum 25(OH)D level was 4 ng/mL lower at the end of one year of monitoring. This could lead to a significant change in the amount required to supplement based on your body weight and starting serum level.

•African-Americans: The population means serum 25(OH)D level is lower in African-Americans than whites, but supplementation is as effective in this population.(Bischoff-Ferrari et al2016).

Control and prevention :-

- Daily or weekly or monthly dose
- Single-dose therapy

- Vitamin D doses and meals
- Special cases(Aziz2016).

Accessory (1)

N	Location	Name	Date	Sex	Age	Clinical sings	Types of management

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