Exogenous and endogenous vitamin D are rapidly converted to 25-hydroxyvitamin D (25(OH)D) in the liver by vitamin D 25-hydroxylase.

Peter J. Tebben, M.D., with Endocrinology, Diabetes, Metabolism, and Nutrition and Pediatric Endocrinology and Metabolism at Mayo Clinic’s campus in Rochester, Minnesota, says: “Additional hydroxylation of 25(OH)D takes place in the kidney via 1-alpha hydroxylase (CYP27B1), producing the bioactive hormone 1,25-dihydroxyvitamin D (1,25(OH)₂D), which plays a critical role in calcium absorption through interaction with the vitamin D receptor (VDR). Production of 1,25(OH)₂D is tightly regulated through the concentrations of serum calcium, phosphorus and parathyroid hormone (PTH).

“The abundant precursor metabolite and storage form of vitamin D, 25(OH)D, can also be removed from circulation and converted to inactive 24,25-dihydroxyvitamin D (24,25(OH)₂D) — an enzymatic step catalyzed by 24-hydroxylase (CYP24A1) (Figure 1). The CYP24A1 gene, encoding the vitamin D 24-hydroxylase, is of major clinical and physiologic importance because it also serves to regulate the catabolism of 1,25(OH)₂D (Figure 2, page 2). Thus, excess 25(OH)D and 1,25(OH)₂D are catabolized by vitamin D 24-hydroxylase to the inert metabolites 24,25(OH)₂D and 1,24,25-trihydroxyvitamin D, respectively.”

Rajiv Kumar, M.D., with Nephrology and Hypertension at Mayo Clinic’s campus in Minnesota, explains: “CYP24A1 can be stimulated hormonally by 1,25(OH)₂D and by FGF23, whereas CYP27B1, encoding the vitamin D 1-alpha hydroxylase, is stimulated hormonally by PTH and downregulated by FGF23. Thus CYP24A1 and CYP27B1, together, provide for alternate and regulated fates of 25(OH)D and control the availability of the active metabolite, 1,25(OH)₂D, depending upon physiological needs. These two enzymes are therefore central to the homeostatic control of vitamin D metabolism, and as a result affect calcium metabolism in critical ways.”

Robert A. Wermers, M.D., with Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic’s campus in Minnesota, notes: “Loss-of-function mutations in the CYP24A1 gene can lead to inactive or no vitamin D 24-hydroxylase enzyme protein, resulting in increasing concentration of vitamin D metabolites and a clinical phenotype characterized by suppressed serum PTH, increased serum 1,25(OH)₂D, hypercalcemia, and hypercalciuria or nephrolithiasis or both. In a New England Journal of Medicine report in 2011, biallelic loss-of-function mutations (autosomal recessive) in CYP24A1 were reported in a population of children presenting with idiopathic infantile hypercalcemia (IIH).
Dr. Tebben adds: “Subsequent publications in the Journal of Clinical Endocrinology and Metabolism in 2012 and Osteoporosis International in 2016 showed that deficiency in 24-hydroxylase caused by pathogenic CYP24A1 gene mutations abrogates 24,25(OH)₂D synthesis, leading to hypercalcemia and predisposing patients to nephrolithiasis and nephrocalcinosis.”

Dr. Kumar notes: “As highlighted in Endocrine Reviews in 2016, although the prevalence of CYP24A1-associated hypercalcemia is rare — estimated to be in the order of 1 in 40,000 — the testing of 24,25(OH)₂D should be considered when other causes of hypercalcemia have been excluded. This is especially true for patients with non-PTH mediated hypercalcemia due to 1,25-dihydroxyvitamin D excess in association with a strong family history of nephrolithiasis or hypercalcemia or both. “The clinical manifestations of hypercalcemia depend largely on the age at diagnosis. Infants present with weight loss or failure to thrive, vomiting, dehydration, lethargy, and hypotonia, whereas adults with mutations in CYP24A1 most frequently present with renal manifestations such as nephrolithiasis or nephrocalcinosis or both, and may experience polyuria. The degree of hypercalcemia and symptoms can vary from mild and intermittent to severe but in general are less pronounced compared with those who manifest disease during infancy. As with other causes of vitamin D-mediated hypercalcemia, adults may develop neuropsychiatric symptoms such as lethargy, confusion and irritability with severe hypercalcemia.”

Ravinder J. Singh, Ph.D., with Laboratory Medicine and Pathology at Mayo Clinic’s campus in Minnesota, explains: “Formation of 24,25(OH)₂D is dependent in part on the concentration of 25(OH)D. In healthy individuals, the circulating concentration of 24,25(OH)₂D is 10 to 25 percent of the total 25(OH)D. Patients who are homozygous for CYP24A1 mutations with no enzyme activity develop undetectable levels of 24,25(OH)₂D.

“Importantly, testing and reporting the 24,25(OH)₂D result alone can mislead the interpretation of the test. Hence, the ratio of 25(OH)D-to-24,25(OH)₂D is necessary to estimate the calculation of vitamin D 24-hydroxylase enzyme activity, and this ratio is anticipated to be available as an orderable test in midyear 2017 (Mayo Test ID 2425D, 63416).

“The sample requirement is similar to a 25(OH)D measurement (Mayo Test ID 25HDN, 83670) where serum can be collected in a red top tube and shipped to the lab in refrigerated pack-
As highlighted in the prior issue of this newsletter, 2017 marks the 50th anniversary of the formal founding of the Division of Endocrinology at Mayo Clinic. *Endocrinology Update* will recognize this milestone in several ways over the year. The publication will highlight some of past division members who had major impacts on the development of the division. This issue honors Dr. Edward H. Rynearson (Figure 1), who was on staff at Mayo Clinic from 1932 to 1966.

Known by most as “Eddie” or “Ry,” Dr. Rynearson earned his Bachelor of Arts degree from Ohio Wesleyan University in 1922 and his M.D. degree from the University of Pittsburgh in 1926. In the summer of 1924 he was hitchhiking from Pittsburgh to Yellowstone National Park, where he worked as a ranger each summer. When he arrived in St. Paul, Minnesota, he decided to take a detour to Mayo Clinic to visit his lifelong friend Dr. Philip S. Hench (who would go on to win the Nobel Prize in physiology and medicine in 1950 with Drs. Edward C. Kendall and Tadeus Reichstein). Drs. Rynearson and Hench grew up in the same neighborhood in Pittsburgh, and their families were close friends.

Reflecting on this visit many years later, Dr. Rynearson wrote: “In all of my hitchhiking from Pittsburgh to Yellowstone, the stretch from St. Paul to Rochester was the most difficult. Let us remember that there was not an inch of paved road north of Rochester, and very little south of St. Paul.” On arriving in Rochester, Dr. Rynearson recalled: “I telephoned Phil, he told me where to meet him in the 1914 Building. … Within a few minutes I had the great pleasure and honor of being introduced to Dr. William J. Mayo, who had come to Phil’s section to see a patient.”

Dr. Rynearson went on to intern at Mercy Hospital in Pittsburgh and came to Mayo Clinic from homozygous or compound heterozygous CYP24A1 mutations (no 24-hydroxylase activity), 24,25(OH)2D levels should be close to undetectable despite the presence of adequate amounts of the substrate 25(OH)D.

“Nearly all patients described to date with biallelic CYP24A1 mutations or deletions have had a ratio of 25(OH)D-to-24,25(OH)2D greater than 80. The ratio results should be interpreted in the context of other biochemical findings including serum calcium, PTH and 1,25-dihydroxyvitamin D concentrations. If the ratio of 25(OH)D-to-24,25(OH)2D is less than 25, the result would be considered normal, but such findings can be observed in heterozygous carriers of CYP24A1 mutations who may have an attenuated clinical phenotype compared with individuals with biallelic mutations. A ratio of 25(OH)D-to-24,25-(OH)2D between 25 and 80 may be seen in patients with low serum vitamin D concentrations or in individuals with heterozygous CYP24A1 mutations. Confirmation with molecular testing is recommended. Germline mutation testing is available from Fulgent Diagnostics.”

**For more information**


on Oct. 1, 1927, as a fellow in medicine in the Mayo Graduate School of Medicine.

Dr. Rynearson:
• Made first assistant in medicine in 1930
• Was appointed to the Mayo Clinic staff in 1932
• Was appointed head of a section of medicine devoted to endocrinology and metabolism in 1947
• Chaired all three endocrine sections from 1954 to 1962 (Figure 2)

Dr. Rynearson wrote about the weekly metabolism seminars during those days: “Senior physicians sat in the front row and they would pass their comments back and forth between them, which were absolutely inaudible to anyone else in the room. I sat in the last row and, since I was quite deaf to start with, I would sit back there and bellow, ‘Louder please, those of us in the cheap seats can’t hear you!’”

Dr. Rynearson was a scholarly clinician. His areas of clinical interest included obesity, insulinoma, hypopituitarism, pituitary tumors, adrenal disorders, hyperparathyroidism, thyroid dysfunction, diabetes mellitus, and dermatologic disorders in patients with diabetes, gynecomastia and Cushing’s syndrome. He participated in the national debate on the futility of prolongation of life when a fatal outcome was inevitable and imminent. He published his thoughts on this topic in CA: A Cancer Journal for Clinicians in 1959.

Dr. Rynearson authored 46 peer-reviewed publications — literally covering the breadth of endocrinology. He took great pride in being a clinician and sharing the lessons he learned by caring for patients. Dr. Rynearson was concerned about the prevalence of health fads and the vitamin concoctions that were sold at exorbitant prices for “nutritional health” or as weight-loss aids. His last peer-reviewed publication, titled “Americans love hogwash,” was published in Nutrition Reviews in 1974. In an interview with the Rochester, Minnesota, Post-Bulletin, Dr. Rynearson was quoted as saying: “People want to be duped. They want super-health and there is no such thing.”

In 1950, Dr. Rynearson was elected president of the Endocrine Society. That was a time when the society was alternating the presidency between a clinician and a basic scientist in an effort to recognize clinical practice and basic science. However, soon after Dr. Rynearson’s presidency, this distinction was ignored. He was the last clinician of his era to become president of the Endocrine Society.

Dr. Rynearson was widely recognized as a colorful speaker with spontaneous wit and ready satire. Dr. Donald Scholz, who was on staff at Mayo Clinic from 1953 to 1991, recalls: “He was a good-hearted man. But what he did for our morning conferences, you had better be on time or don’t come. If you were late coming in and had not been seeing patients, don’t bother, brother. Turn around. Bye-bye. We will see you next week! That was spelled out real well.”

Dr. Scholz also recounted a story when Dr. Rynearson was on a trip to Augusta, Georgia. He purchased three prepackaged postcards for 25 cents and included 2-cent prepaid stamps. He only needed two postcards. So instead of discarding the extra postcard, he addressed it to “World’s Greatest Doctor, World’s Greatest Medical Clinic.” On returning to Rochester, he found the postcard in his Mayo Clinic mailbox. Along the same lines, he would sign many of his letters to friends and colleagues with the initials T.G.H., short for “The Great Healer” (Figure 3).

Dr. Rynearson retired from Mayo Clinic

Figure 2. Members of the three sections of Endocrinology in 1951. Front row left to right: Dr. Clifford F. Gastineau, Dr. Laurentius O. Underdahl, Dr. Edward H. Rynearson, Dr. William M. Balfour, Dr. Harold L. Mason. Middle row left to right: Dr. Alexander Albert, Dr. Charles M. Blackburn, Dr. Robert M. Salassa, Dr. F. Raymond Keating Jr., Dr. Samuel F. Haines, Dr. Austin C. Davis. Back row left to right: Dr. Marschelle H. Power, Dr. William W. Winchester, Dr. Llewelyn P. Howell, Dr. Randall G. Sprague, Dr. William M. McConahey Jr., Dr. Warren A. Bennett, Ph.D.

Figure 3. “The Great Healer” signature of Dr. Edward H. Rynearson.
Hypoparathyroidism is a rare endocrine disorder characterized by absent or inappropriately low concentrations of circulating parathyroid hormone (PTH), which leads to hypocalcemia, hyperphosphatemia and increased fractional excretion of calcium in the urine. Bart L. Clarke, M.D., with Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic’s campus in Rochester, Minnesota, says: “It is estimated that 60,000 to 115,000 patients in the U.S. have this disorder. Roughly 75 percent of cases of hypoparathyroidism are due to anterior neck surgery, typically for thyroid, parathyroid, or other head or neck disorders. Autoimmune hypoparathyroidism may be isolated or associated with autoimmune polyglandular syndrome type I, which is also associated with chronic mucocutaneous candidiasis, pernicious anemia and other autoimmune conditions. Infiltrative disorders that can cause hypoparathyroidism include iron or copper overload, sarcoidosis, and metastatic malignancy affecting the parathyroid glands. Rare cases have been reported after radioactive iodine 131 (131-I) treatment for thyroid disease. In addition, hypomagnesemia may limit PTH secretion by normal parathyroid glands.”

Novel molecular biology techniques have improved understanding of parathyroid gland physiology and have led to the reporting of many different genetic defects responsible for familial hypoparathyroidism. These defects include gain-of-function mutations affecting the extracellular calcium-sensing receptor (CaSR), which causes autosomal dominant hypocalcemia, and defects affecting the guanine nucleotide-binding protein subunit alpha-11 (G-protein α-11). Additional defects include loss-of-function mutations affecting essential transcription factors or the PTH gene.

Dr. Clarke explains: “The clinical presentation varies widely from mild disease with tingling paresthesias and muscle cramps to more severe disease that may result in bronchospasm, laryngospasm, seizures, QT interval prolongation leading to cardiac dysrhythmias or, in extreme cases, sudden death. Onset of symptoms after surgery usually occurs within several days. Other causes may have subtle onset over years before they are finally recognized. Most patients have a moderate presentation with variable symptoms over time. Diagnosis of patients with suspected hypoparathyroidism requires assessment of fasting serum calcium, phosphorus, creatinine, PTH, 25-hydroxyvitamin D and magnesium. Once the diagnosis is confirmed, measurement of 24-hour urine calcium and creatinine is recommended to assess for baseline hypercalciuria.”

Dr. Clarke outlines: “Treatment with oral calcium and active vitamin D in divided doses each day is currently the standard of care, occasionally supplemented with magnesium, thiazide-type diuretics or phosphate binders, but this does not fully replace the functions of the missing PTH and can lead to long-term complications such as nephrocalcinosis, cal-

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calcium-containing kidney stones, renal dysfunction, basal ganglia calcifications and posterior subcapsular cataracts. Of particular concern are increased calcium losses in the urine in the absence of PTH, which are associated with nephrocalcinosis and impaired renal function in the long term. Several clinical studies have also reported decreased quality of life among patients with hypoparathyroidism."

Recent international guidelines published in *Journal of Clinical Endocrinology and Metabolism* in 2016 recommend six goals for chronic management therapy of patients with hypoparathyroidism, including:

- Prevention of signs and symptoms of hypocalcemia
- Maintenance of serum calcium slightly below normal (no more than 0.5 mg/dL below normal) or in the low-normal range
- Maintenance of the calcium x phosphate product below 55 mg²/dL² (4.4 mmol²/L²)
- Avoidance of hypercalciuria, maintaining urinary calcium excretion within normal limits
- Avoidance of hypercalcemia
- Avoidance of renal and other extraskeletal calcifications

Dr. Clarke highlights: “Novel treatments are therefore needed, and until recently hypoparathyroidism represented one of the few endocrine deficiency states not treated by replacement of the missing hormone. Recent trials demonstrating the efficacy of recombinant human (rh) PTH(1-84) for the treatment of hypoparathyroidism have led to the approval of rhPTH(1-84) for hypoparathyroidism in the United States in January 2015.”

The international guidelines published in *Journal of Clinical Endocrinology and Metabolism* in 2016 recommended consideration of treatment with rhPTH(1-84) in patients with:

- Variable and inconstant control of the serum calcium with frequent episodes of hypo- and hypercalcemia
- Nephrolithiasis
- Nephrocalcinosis, reduced creatinine clearance or eGFR < 60 mL/min
- Hypercalciuria or other biochemical indices of renal stone risk or both
- Persistently increased serum phosphate or calcium x phosphate product or both (> 55 mg²/dL² or 4.4 mmol²/L²)
- Excessive amounts of oral medications required to control symptoms, such as > 2.5 g of calcium or > 1.5 μg of active vitamin D or both
- A gastrointestinal tract disorder that might lead to variable calcium and vitamin D absorption
- Reduced quality of life

The decision to recommend rhPTH(1-84) should take into account the fact that this is currently an expensive drug.

Dr. Clarke notes: “The approval of this agent for treatment of hypoparathyroidism has stimulated research into new treatment modalities and development of PTH analogues. As new therapies become more widely available, evaluation of their effect on disease control, long-term complications and quality of life will be critically important.”

For more information

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**A Flushing Disorder: A Case From the Endocrine Teaching Clinics**

A 42-year-old man was referred to Mayo Clinic by his local physician for further evaluation of a flushing disorder. His symptoms began about seven years previously. At that time he would flush only if he had an alcoholic beverage and occasionally after a large meal or exercise.

About four years before coming to Mayo Clinic, he started flushing without obvious triggers and his symptoms worsened — by the time of presentation, he had three to four facial flushing episodes each day lasting 15 to 20 minutes and primarily involving his face and neck. With these episodes, he did not have any shortness of breath, diarrhea or sweating. He did not have hypertension.

Over the past four years, he had developed a persistent red rash over his back, chest and upper arms, which was reported unremarkable on skin biopsy performed two years previously. Over the past couple of years, he had also developed a sense of temperature dysregulation. He was not taking any regular medication, and there was no clinically significant family or social history.
On physical examination, he had a moderately flushed face (Figure 1A) and a diffuse red rash over his back. With his arms raised his facial flush did not change. The thyroid appeared normal on examination, without any nodules appreciated on palpation. The remainder of the physical examination was normal.

Normal laboratory studies included:

- Complete blood count and routine chemistries
- Serum serotonin; serum tryptase
- 24-hour urine for 5-hydroxyindoleacetic acid, N-methylhistamine, fractionated metanephrines and catecholamines

The 24-hour urine for 2,3-dinor-11beta-prostaglandin F2-alpha was mildly increased at 1,363 ng (normal, < 1,000 ng). Skin biopsies showed multiple telangiectasias and no sign of mast cell abnormalities. Tests for other potential causes of flushing disorder were obtained, which were remarkable for a serum calcitonin concentration markedly elevated at 552 pg/mL (normal < 16 pg/mL). Based on this information, thyroid ultrasound was obtained.

Ultrasound of the thyroid showed a 1.8-cm suspicious nodule in the midright thyroid lobe (Figure 2). Fine-needle aspiration biopsy findings were consistent with medullary thyroid carcinoma (MTC). Bidirectional sequence analysis was performed to test for the presence of a mutation in exons 10, 11, 13, 14, 15 and 16 of the RET proto-oncogene and was negative.

He underwent a near-total thyroidectomy, and pathology showed that the MTC formed a 1.8-by-1.4-by-1.1-cm mass. He also had an incidental 0.7-cm papillary thyroid carcinoma in the midportion of the right lobe of the thyroid. After surgery, he had complete resolution of his symptoms of flushing and the rash (Figure 1B). At his six-month follow-up visit, he had an undetectable serum calcitonin concentration and a normal serum concentration of carcinoembryonic antigen. Neck ultrasound showed no evidence of adenopathy.

Discussion
As highlighted in a review published in the Journal of the American Academy of Dermatology in 2006, flushing is a sensation of warmth accompanied by transient erythema that most commonly occurs on the face, but may also involve the neck, ears, chest, epigastrium, and arms or other areas. The most common etiologies for which a patient will present with flushing are fever, hyperthermia, primary gonadal failure such as menopause, emotional blushing and rosacea.

When an etiology is not identified by history and physical examination, initial laboratory evaluation includes:

- A complete blood count and liver function tests
- 24-hour urine for 5-hydroxyindoleacetic acid to screen for carcinoid syndrome
- Serum tryptase and 24-hour urine for
methylhistamine, 2,3-dinor-11beta-prostaglandin F2-alpha, and leukotriene E4 to screen for mast cell disease.

- Markers for other potential etiologies such as serum calcitonin

In this case, an elevated calcitonin led to further investigations, diagnosis and cure.

Blood concentrations of calcitonin are elevated in patients with medullary thyroid cancer. In patients with thyroid nodules, however, the routine assessment of serum calcitonin is controversial, and the American Thyroid Association guidelines published in *Thyroid* in 2016 do not recommend for or against routine measurement of serum calcitonin in patients with thyroid nodules. False-positive calcitonin results may be obtained in patients with hypercalcemia, hypergastrinemia, neuroendocrine tumors, renal insufficiency, papillary and follicular thyroid carcinomas, goiter, chronic autoimmune thyroiditis, and prolonged use of certain medications. A false-negative test result may be seen in rare medullary thyroid cancers that do not secrete calcitonin.

**For more information**
