it is less reproducible and more time consuming, expensive and inconvenient. In addition, prior daily carbohydrate intake must be at least 150 g for two weeks to obtain valid test results.

- HbA_{1c} – global efforts to standardise the measuring and reporting of HbA_{1c} have seen this marker being included in the ADA criteria for the diagnosis of diabetes and the assessment of pre-diabetes.
- The gold standard for determining insulin resistance is the hyper-insulinaemic euglycaemic clamp study. Clamp studies specifically measure whole body insulin-mediated glucose uptake under controlled conditions of a combined glucose and insulin infusion.[9] However, these tests are too laborious for routine use and are rarely used in clinical practice. Various mathematical models have been used to determine formulas to act as surrogate markers of insulin resistance/sensitivity using fasting insulin and glucose measurements. One that is most often used is the homeostatic model assessment (HOMA) (Table 2).
- The measurement of insulin is hampered by the fact that it is an immunoassay and therefore the methods used and results generated may vary considerably from one laboratory to another (owing to a lack of assay standardisation).
- C-peptide – it ispart of the pro-insulin precursor molecule and released in equimolar amounts with insulin. C-peptide does not influence plasma glucose levels but is utilised as a marker of insulin production when measured in conjunction with glucose levels.[8]

Dyslipidaemia

The lipid profile associated with MetS is raised fasting triglycerides and low concentrations of HDL cholesterol. Other lipoprotein abnormalities, e.g. increased remnant lipoproteins, elevated apolipoprotein B, small LDL particles, and small HDL particles, have also been documented.[7] Although all of these have been implicated as being independently atherogenic, they do not form part of the diagnostic criteria for MetS and most are not routinely measured in laboratories.

Table 2. Some surrogate markers for determination of insulin resistance/ sensitivity[8]

HOMA1-IR (insulin resistance) = (FPI* × FPG)/22.5
HOMA1-%B (B cell function) = (20 × FPI*)/(FPG – 3.5)
Quantitative insulin sensitivity check index (QUICKI) = 1/[log(FPI) + log(FPG)]
*FPI – fasting plasma insulin.

Other

Microalbumin – the presence of urinary microalbumin is part of the WHO diagnostic criteria for MetS. It is measured using an early morning spot collection as a urine albumin:creatinine ratio or with a 24-hour urine collection and should be confirmed on at least two occasions. Microalbuminuria is a prognostic marker for CVD, a marker of incipient renal disease and a marker of inflammation.

C-reactive protein (CRP) – pre-diabetes and MetS are recognised as pro-inflammatory states. Obesity is related to increased production of pro-inflammatory cytokines and decreased production of the anti-inflammatory cytokine adiponectin by adipose tissue. Pro-inflammatory states are associated with increased cardiovascular risk and increased CRP levels. The measurement of high sensitivity CRP (hs CRP) has been recommended to assist in stratifying the risk for CVD.[7]

Uric acid – hyperuricaemia is commonly associated with MetS and has been postulated to play a causal role.

Diagnostic controversies

The criteria for pre-diabetes and MetS have evolved over the years and complete consensus regarding the definitions still has to be reached.

Overlap of IFG and IGT

There is controversy with regard to which test should be performed – fasting blood glucose (determining IFG) or OGTT (determining IGT). However, since the OGTT is a challenge test, it is likely that the detection rate will be higher. Generally, among subjects screened using IGT, only 20 - 25% have FPG levels that would indicate impaired fasting glucose. In subjects screened with IFG, $<50\%$ have a postprandial 2-hour glucose level of ≥ 7.8 mmol/l.[2] Also, the diagnosis of subjects screened using the HbA_{1c} criterion has been shown to not be completely consistent with IFG and IGT methods for the diagnosis/ screening of pre-diabetes.[9]

References available at www.cmej.org.za

Vitamin D in clinical practice

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Background

Vitamin D was first described in the 20th century when a cause for the high prevalence of rickets was sought. With the fortification of certain foods with vitamin D, the prevalence of rickets decreased and little attention was placed on vitamin D. However, in the past decade there has been a revived interest in vitamin D, as it is thought to be associated with various

nonskeletal disorders. Research has found that the prevalence of vitamin D deficiency worldwide is actually much higher than thought, and this has led to a massive increase in the demand for laboratory testing of vitamin D levels.

Metabolism[1,2]

Vitamin D is a steroidal hormone produced by the skin or absorbed by the gastrointestinal tract (GIT). Most comes from exposure to sunlight, and 5 - 10 minutes of sun exposure 2 - 3 times a week may be adequate to produce sufficient quantities of vitamin D. It is found in the bloodstream bound to vitamin-binding protein. Two hydroxylation steps occur: the first at position 25 in the liver and the second in position 1 in the kidney under the influence of 1a-hydroxylase, leading to the formation of 1,25-dihydroxy-vitamin D or calcitriol, which is the active form of vitamin D. This last step is tightly regulated by factors such as parathyroid hormone, low phosphate levels and low dietary calcium intake. Fig. 1 shows the metabolism of vitamin D.

1,25-dihydroxyvitamin D has a short half-life of about 4 hours and 25-hydroxyvitamin D has a long half-life of 2 - 3 weeks. For this reason, even though the latter form of vitamin D is about 400 times less potent than the former, the levels are about 500 - 1 000 times higher.

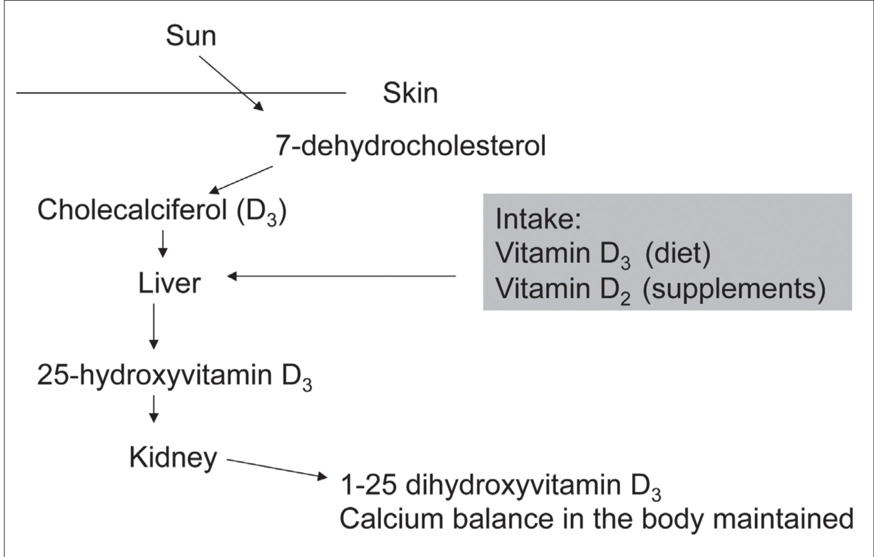

Fig. 1. The metabolism of vitamin D.

Skeletal actions [1,2]

For decades we have known of vitamin D's role in maintaining healthy bones. Vitamin D is known to facilitate the gastrointestinal absorption of calcium and phosphate. The main function of 1,25-dihydroxyvitamin D for bone mineralisation is to maintain the calcium-phosphate product in the circulation, which allows passive mineralisation of collagen bone matrix. Vitamin D deficiency is thus traditionally associated with deficiencies of bone mineralisation, namely rickets in children and osteomalacia in adults. Before the development of the latter two, the decreased ionised calcium levels due to inadequate gastrointestinal absorption induce an increase in parathyroid hormone levels. This leads to the differentiation of osteoclasts and the release of calcium and phosphate from bone. Parathyroid hormone also stimulates the production of 1,25 dihydroxyvitamin D in the kidneys.1,25-dihydroxyvitamin D also increases the expression of bone alkaline phosphatase and other markers of metabolism.

Non-skeletal actions

Besides the well-described skeletal effects of vitamin D, recent literature has also highlighted various non-skeletal effects of vitamin D. Vitamin D receptors have been isolated in brain, prostate, breast, colon, heart and immune cells and these cells respond directly to 1,25-dihydroxyvitamin D.[2]

Vitamin D deficiency has been implicated in the pathophysiology of diabetes mellitus, with countries that have little sunlight, such as Finland, having higher incidence of diabetes mellitus.[3]

Early in the 20th century it was described that sunlight helped for auto-immune disorders such as lupus vulgaris and infectious disorders such as tuberculosis. Recently, vitamin D has been described to be anti-inflammatory and necessary for antimicrobial action against intracellular organisms.[4] An association between low vitamin D levels, latitude and past sun exposure has also been found between vitamin D and multiple sclerosis,[5] rheumatoid arthritis and osteoarthritis. Certain cancers, such as breast, ovarian, colorectal and prostate have also been associated with low vitamin D levels.[6] Low vitamin D levels have also been associated with hypertension, most likely via the renin-angiotensin-aldosterone system.[7] Vitamin D insufficiency in pregnancy is associated with childhood disorders such as asthma and autism.[8,9] Vitamin D deficiency has also been linked to schizophrenia, depression

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and deterioration of mental function with age.[10]

Deficiency and 'desirable' serum levels

There is no consensus on optimal levels of 25-hydroxyvitamin D, although experts define deficiency as levels of <20 ng/ml (50 nmol/l). Vitamin D intoxication is defined as 25-hydroxyvitamin D levels >150 ng/ml (374 nmol/l) and is only found in individuals taking in excess of 40 000 IU per day. Using these levels, one billion people worldwide have vitamin D deficiency.[2]

Certain individuals are at increased risk of vitamin D deficiency:[11]

- people with pigmented skin
- those who lack exposure to sunlight
- people who wear skin-concealing garments or use sunscreen excessively
- children who are exclusively breast fed
- women who have multiple pregnancies with short intervals
- elderly, obese or institutionalised people
- vegetarians
- people who suffer from malabsorption, short bowel, liver or renal disease
- individuals who take certain pharmacological agents.

Vitamin D supplementation

Only few foods are a good source of vitamin D, namely fortified dairy products and breakfast cereals, fatty fish, beef liver and egg yolks. Besides sun exposure, the best way to get additional vitamin D is through supplementation. Traditionally, multivitamins contained about 400 IU of vitamin D, but newer ones contain 800 - 1000 IU. Two forms of vitamin D are available, namely D_2 (ergocalciferol) and D_3 (cholecalciferol). D_3 is the preferred form, as it is more effective. As it is a fat-soluble vitamin, it should be taken with meals. Corticosteroids can reduce calcium absorption, which impairs vitamin D metabolism. Lipase inhibitors such as Orlistat and cholestyramine may reduce its absorption, and phenobarbitol and phenytoin may increase the hepatic metabolism of vitamin D to inactive compounds and thereby decrease calcium absorption. Newer work has shown that adults may need a vitamin D intake exceeding 2 000 IU per day.[12]

Measurement of vitamin D

Vitamin D status is measured by 25-hydroxyvitamin D, as levels of 1,25-dihydroxyvitamin D in deficiency states may be normal, high or low. 25-hydroxyvitamin D is a better indicator of the body's vitamin D reserve and is therefore the analyte of choice to measure in the laboratory.[1] For the purpose of this article, we will be referring to the measurement of 25-hydroxyvitamin D to avoid confusion. The suggestion that vitamin D is associated with disease conditions other than calcium dysfunction, as mentioned above, has led to an increase in demand for the measurement of vitamin D.

Vitamin D can be measured separately as vitamin D_2 or D_3 or as a total value. Vitamin D_3 is mainly endogenously derived, and accounts for approximately 95% of vitamin D, whereas vitamin D_2 is derived from food sources and is usually the minor fraction. However, with supplementation, vitamin D_2 is given and assays that only measure vitamin D_3 may underestimate the efficiency of treatment.[13] Assays that mainly measure a specific fraction can thus mislead the physician. The most important value is the final total value, as this represents the total amount of 25-hydroxyvitamin D (D_2 and D_3) in the blood.

Previous assays were problematic, with >30% interlaboratory differences in results shown in 1995.[12] This variation in results at that time led to Heaney stating in 2000 that 'when ordering and interpreting serum 25-hydroxyvitamin D concentration, the physician needs, in virtually all cases, to ignore the laboratory's published reference range….'[14] Improvements in assay standardisation and the introduction of new automated assays for vitamin D led to an improvement in interlaboratory difference to 15% in 2011.[12]

Laboratories face various problems when measuring 25-hydroxyvitamin D, which exists in various molecular forms and is bound to vitamin-binding protein.[12,15] Reference ranges used by the laboratory depend on the reference population used, which in turn depends on season, altitude, latitude, age, skin colour and skin pigmentation, as all these factors may affect vitamin D levels.[2] The value also depends on the assay used, as standardisation has not yet been achieved due to methodological variability. This means that values may not be comparable between laboratories and may confuse clinicians.

Conclusion

Recent literature has highlighted the nonskeletal effects of vitamin D, which has led to an increase in demand for vitamin D assessment from the laboratory. This has forced the development of more rapid automated assays for the determination of vitamin D levels and attempts at standardisation of the assay to enhance clinician satisfaction and reduce confusion. As researchers discover more associations between vitamin D levels and disease, the demand for vitamin D measurement in clinical practice is bound to increase even further. It is therefore important that the clinician understands the basic physiology

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that can influence laboratory assays as well as the pitfalls of the assays to aid with interpretation of results.

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Laboratory tests in the diagnosis of cystic fibrosis

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Cystic fibrosis (CF) is the most common autosomal recessive disorder among white individuals, and occurs in all South African population groups. Recent evidence suggests a prevalence of 1 in 2 000 among white South Africans and 1 in 12 000 in the coloured population. In black South Africans carrier frequency estimates have been used to project the incidence of 1 in 4 624 live births.[1]

Generally, South Africa offers diagnostic services and expertise similar to those available worldwide for CF patients.[2]

The genetic defect

CF is caused by mutations in the CF transmembrane conductance regulator (CFTR) protein, a product of the CFTR gene located on chromosome 7. This gene encodes a cAMP-regulated chloride channel that regulates chloride transport at the apical membrane of epithelial surfaces, such as the airways, pancreatic ducts, biliary tree and sweat ducts.[3] Abnormal transport of chloride and/or other CFTR-affected ions leads to thick, viscous secretions in these organs. Consequently, the CF patient typically presents with multisystem disease, e.g. suppurative lung disease, pancreatic insufficiency, multifocal biliary cirrhosis, male infertility and high electrolyte loss in sweat.[4,5] The last mentioned forms the basis of the sweat test for the diagnosis of CF.[3]

Diagnosing CF

Current consensus is that the diagnosis of CF should be based on the presence of one or more characteristic clinical features, a history of CF in a sibling, or a positive newborn screening test *plus* laboratory evidence of an abnormality in the CFTR gene or protein. This can include biological evidence of channel dysfunction (abnormal sweat chloride or nasal potential difference) or identification of a CF disease-causing mutation in each copy of the CFTR gene. The vast majority of persons with CF are diagnosed based on classic signs and symptoms and corroborative laboratory results; however, in approximately 5 - 10% of patients the diagnosis is not clear-cut.[6]

Role of the laboratory in the diagnosis of CF

The laboratory plays a key role in the screening, diagnosis and follow-up of patients with CF. Screening tests include sweat conductivity measurement and newborn testing for immunoreactive trypsinogen (universal screening is not currently done in South Africa). Diagnostic tests include mutation analysis, and quantitative measurement of sweat chloride (the sweat test) – the gold standard. Other ancillary tests may also support the diagnosis of CF, e.g. faecal elastase and semen analysis. In addition, certain features such as chronic metabolic alkalosis and acute salt depletion may suggest the diagnosis.[6]

Genetic testing

Since the discovery of the CFTR gene in 1989, it has been possible to use gene mutation analysis as an adjunct to sweat testing for the diagnosis of CF.3 The most common mutation in the white South African population is the ΔF508 (delta F508) mutation and the most common in the black population is the 3120+G→A mutation. Both are present in the coloured population.[1,2] More than 1 200 mutations and polymorphisms have been identified, and as routine screening tests are not able to detect all CFTR gene mutations a negative genetic test does not ensure a normal CFTR genotype.5 Testing profiles may vary, from testing for the two most common above-mentioned mutations, to extended panels, including up to 50 of the most frequently observed CFTR mutations within populations of European origin. Using the êF508 only, about 80% of white carriers and 67.4% of coloured carriers will be identified. About 46% of black CF carriers will be detected using the 3120+GàA test.2 Requesting molecular analysis instead of performing a sweat test may confirm a diagnosis of CF, but cannot exclude it.1

Sweat testing is a measure of CFTR function and therefore remains an essential test for the diagnosis of CF, even in the genomic era.[3]

The sweat test

The measurement of sweat chloride concentration remains a pivotal test for the diagnosis of CF and needs to be performed to established guidelines to prevent pitfalls as well as false positive and false negative results. Sweat collection is generally performed in one of two ways: The Gibson Cooke method uses pilocarpine iontophoresis to stimulate sweat production, with subsequent collection of sweat onto gauze or filter paper for analysis, but more recently many laboratories have changed to using the Wescor® apparatus. This also employs pilocarpine iontophoresis, but the sweat is collected into microbore tubing.[7] Both collection methods are followed up by quantitative sweat chloride and sodium analysis.[5]

Generally, sweat tests are not performed until the subject is more than two weeks of age and weighs more than 3 kg. It can be attempted in term infants after 7 days of age if clinically important, but insufficient sweat collection is often a problem.[8] Sweat tests should never be performed on babies under 48 hours of age, as falsely high values may be obtained.[7,9] Testing should be postponed in acutely ill patients. If the patient is malnourished or dehydrated, has eczema, untreated Addison's disease, ectodermal dysplasia, certain types