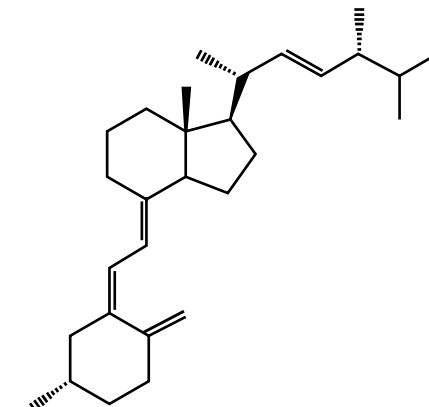


Cocoa: New Source of Vitamin D?

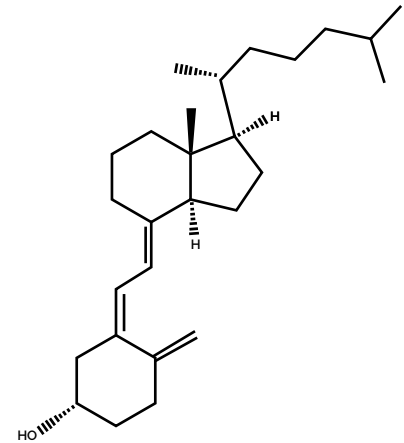
Literature describes vitamin D₂ to be formed during the fermentation of cocoa beans by sunlight from the precursor ergosterol.

A. What is vitamin D?

Vitamin D is a fat-soluble vitamin that can be divided into two forms. Vitamin D₂ (Ergocalciferol) is mostly found in plants, whereas vitamin D₃ (Cholecalciferol) is of animal origin. In summary, both forms are also referred to as calciferols. Vitamin D belonging to the group of secosteroids holds a special position among the vitamins, since – contrary to the historical definition of vitamins – it can be formed in the human body from precursors.



Ergocalciferol (vitamin D₂)



Cholecalciferol (vitamin D₃)

B. How is vitamin D formed?

Important for the formation of vitamin D₂ and D₃ are the respective precursors. In humans and animals, the body's own formation of vitamin D₃ in the skin takes place starting from 7-dehydrocholesterol (provitamin D₃). In the cell membrane, plants and fungi contain ergosterol (provitamin D₂), the precursor of vitamin D₂. Despite the different origin of the precursors, both are photochemically converted into the respective vitamin triggered by UV-B radiation (280–320 nm). The extent of vitamin D₃ formation in the human body depends on several factors, such as latitude, time of year and day, clothing, length of stay outdoors, skin type and the use of sunscreen. The endogenous synthesis covers 80–90% of the vitamin D requirement.

C. In which foods does vitamin D occur?

Vitamin D₃ can be ingested by eating animal foodstuffs. Especially fatty fishes such as herring or salmon as well as eggs and dairy products contain a lot of vitamin D₃. The supply of vitamin D₃ in the diet is 10–20%, very low compared to the endogenous synthesis in the body. The presence of vitamin D₂ is limited to plants and

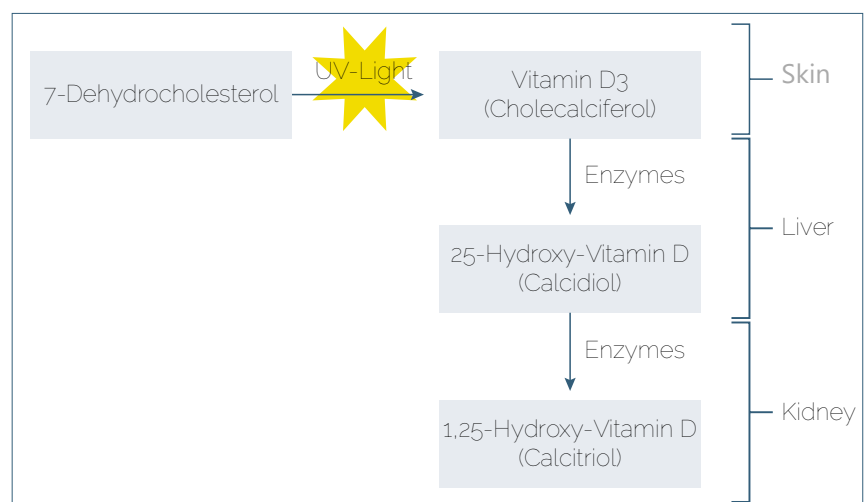
fungi, so that it only reaches the body through the consumption of plant foodstuffs. Especially mushrooms and chanterelles have a high content of vitamin D₂.

Recent studies have shown that dietary intake reflects a greater contribution to vitamin D supplementation than previously thought. Therefore, the occurrence of sources other than nutrition with known vitamin D-rich food is currently discussed. Literature describes vitamin D₂ to be formed during the fermentation of cocoa beans by sunlight from the precursor ergosterol. Thus, cocoa-

containing products and chocolate could contain vitamin D₂ in significant amounts.

D. How is vitamin D metabolized in the body?

The vitamin D formed or absorbed in the skin passes through the bloodstream to the liver, where it is converted by hydroxylation into the prohormone calcidiol (25-hydroxy-vitamin D). Subsequently, further hydroxylation in the kidney leads to the formation of calcitriol (1,25-hydroxy-vitamin D), the biologically active metabolite of vitamin D.





E. What functions does vitamin D have?

The main function of biologically active calcitriol involves the regulation of the calcium and phosphate balance and it is instrumental in bone metabolism. Thus, it promotes the absorption of calcium and phosphate from the intestine and leads to adequate bone mineralization. Furthermore, calcitriol is involved in impacting the transcription of more than 6,000 genes.

F. How high are our vitamin D needs and what are the consequences of a deficiency or an oversupply?

In the absence of endogenous synthesis, an estimate of adequate vitamin D intake of 20 µg/day is given for all ages above 1 year. However, the supply of vitamin D via the diet is not sufficient to reach this reference value. The difference must be compensated by the endogenous synthesis or the intake of vitamin D supplements. An enrichment of foods with vitamin D is not recommended. Primarily, the endogenous synthesis is to be stimulated by the influence of solar radiation. The dependence of the synthesis of the skin type, however, means that the vitamin D supply varies greatly per person. A human being with fair skin and blond hair will

produce significantly more vitamin D over the same period of time compared to a person of darker skin type and dark hair. But also the season has a big influence on its formation. In the winter months, the sun is not strong enough to ensure adequate vitamin D supply. However, the body accumulates vitamin D in fat and muscle tissue, a reservoir which can be replenished in the summer months by increased UV-B exposure. Thus, a proper vitamin D level is ensured even in winter-time. In infancy and childhood, vitamin D deficiency results in decreased bone mineralization and associated rachitis. In adults, the disruption of the bone metabolism leads to the development of osteomalacia or osteoporosis. Particularly at risk are people who spend less time outside. Since vitamin D synthesis decreases significantly with increasing age, older people are also classified into a risk group. Hypervitaminosis can be caused by an increased oral intake of dietary supplements (permanently > 100 µg/day) and leads to the formation of kidney stones.

G. How is the analysis of vitamin D performed?

The analysis of vitamin D takes place in several steps. First, saponification takes place to release the fatty ester bound vitamin D. After subsequent

liquid-liquid extraction and purification, a derivatization step with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) is performed. The adduct formation of vitamin D with PTAD achieves higher molecular masses and thus reduces the number of interfering components. At the same time, ionization efficiency and sensitivity are improved. The measurement is then carried out by LC-MS / MS.

LCI aims to demonstrate the relevance of cocoa and chocolate products in the delivery of vitamin D in human nutrition. For that reason, the development of a suitable and powerful analytical method to quantify vitamin D₂, D₃ and ergosterol by LC-ESI-MS / MS and including its validation is projected. In a second step, a monitoring of commercial cocoa and chocolate products is planned.



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