This is found naturally in the skin and is converted into cholecalciferol by UVB radiation. 90% of cholecalciferol is obtained this way, whilst the remaining 10% comes from dietary sources.

Cholecalciferol can be stored in the liver for many months and it serves as a source of active vitamin D for the winter months, when the skin is exposed to less UVB radiation.

Formation of the final compound occurs in the proximal tubules of the kidneys and is catalysed by 1α-hydroxylase, an enzyme that is activated by PTH secreted from the parathyroid glands. Calcidiol stores only last for a few weeks, so its synthesis from vitamin D₃ is carefully controlled by a negative feedback mechanism. The production of calcidiol inhibits further conversion of vitamin D₃.

25-OH₂-D binds to vitamin D receptors which are present on most cell membranes. This forms a complex with the retinoid-X receptor, which then binds to vitamin D response elements on DNA to mainly increase, but sometimes decrease gene expression.

- Increased intestinal absorption of calcium (via increased calbindin expression)
- Decreased renal excretion of calcium
- Increased mineralisation of bone through increased influx of calcium through osteoblastic/osteocytic cell membranes