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Selective ability of rat 7-Dehydrocholesterol reductase (DHCR7) to act on some 7-Dehydrocholesterol metabolites but not on lumisterol metabolites

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Abstract

7-Dehydrocholesterol reductase (DHCR7) catalyses the final step of cholesterol biosynthesis in the Kandutsch-Russel pathway, the reduction of 7-dehydrocholesterol (7DHC) to cholesterol. 7DHC can be acted on by a range of other enzymes including CYP27A1 and CYP11A1, as well as by UVB radiation, producing a number of derivatives including hydroxy-metabolites, some of which retain the C7–C8 double bond and are biologically active. These metabolites include lumisterol (L3) which is a stereoisomer of 7DHC produced in the skin by UVB radiation of 7DHC, as well as vitamin D3. The aim of this study was to test whether these metabolites could act as substrates or inhibitors of DHCR7 in rat liver microsomes. To initially screen the ability of these metabolites to interact with the active site of DHCR7, their ability to inhibit the conversion of ergosterol to brassicasterol was measured. Sterols that significantly inhibited this reaction included 7DHC (as expected), 20*S*(OH)7DHC, 27(OH) DHC, 8DHC, 20*S*(OH)L3 and 22(OH)L3 but not 7-dehydropregnenolone (7DHP), 25(OH)7DHC, L3 or vitamin D3 and its hydroxyderivatives. Sterols that inhibited ergosterol reduction were directly tested as substrates for DHCR7. 20*S*(OH)7DHC, 27(OH)DHC and 7-dehydridesmosterol were confirmed to be substrates, giving the expected product with the C7–C8 double bond removed. No products were observed from 8DHC or 20*S*(OH)L3 indicating that these sterols are inhibitors and not substrates of DHCR7. The resistance of lumisterol and 7DHP to reduction by DHCR7 in cells will permit other enzymes to metabolise these sterols to their active forms retaining the C7–C8 double bond, conferring specificity to their biological actions.

Keywords

7-dehydrocholesterol reductase; DHCR7; Lumisterol; Vitamin D3; 7-dehydropregnenolone

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Declaration of Competing Interest

The authors declare no conflict of interest.

1. Introduction

7-Dehydrocholesterol reductase (DHCR7) catalyses the final step of cholesterol biosynthesis in the Kandutsch-Russel pathway, the reduction of the C7–C8 double bond of 7-dehydrocholesterol (7DHC) to produce cholesterol [1,2]. It carries out a similar reaction on 7-dehydrodesmosterol to produce desmosterol as the second last step in cholesterol biosynthesis by the Bloch pathway [3]. Different tissues display preference for either one of these pathways with the Kandutsch-Russel pathway predominating in the skin [4]. Thus, in the skin, 7DHC is not only the primary substrate for DHCR7 but also for vitamin D3 formation where it is converted to pre-vitamin D3 by UVB radiation, with subsequent thermal isomerisation to vitamin D3 [5]. DHCR7 in the skin therefore appears to be an important control point for vitamin D3 formation, regulating the availability of 7DHC for its photoconversion [6,7]. This is supported by several studies on SNPs in the *7DHCR* gene which are associated with decreased levels of 25-hydroxyvitamin D3 (25(OH)D3) [6,8,9].

Homozygous or compound heterozygous mutations in the *7DHCR* gene give rise to Smith-Lemi-Opitz Syndrome (SLOS), characterized by growth retardation, intellectual disability and congenital abnormalities [6,10]. Patients with this syndrome display low cholesterol levels and elevated 7DHC [11]. The low cholesterol in utero appears to be the prime cause of the developmental abnormalities but a role for 7DHC and its metabolites is also suspected [11,12]. While patients with SLOS might be predicted to have elevated 25(OH)D3 levels, this was not observed in the one study likely due to sun avoidance, because of the skin sensitivity to ultraviolet light associated with this syndrome [13].

DHCR7, comprising 475 amino acids, is embedded in the endoplasmic reticulum and uses NADPH as a cofactor [1,6]. Its crystal structure has not been determined but a homology model has been built based on the crystal structure MaSR1 [14], a sterol reductase from *Methylomicrobium alcaliphilum* with which it shares 37 % identity [6]. It is predicted to have 10 transmembrane domains, an NADPH binding site in the catalytic region and a sterol sensing domain [6]. The activity of DHCR7 in microsomes is commonly measured using the non-natural substrate, ergosterol, a 7 sterol from fungi which the reductase converts to brassicasterol [15,16]. Neither ergosterol nor brassicasterol are found in liver microsomes and endogenous cholesterol does not interfere with their measurement by chromatography. Sheffer et al. examined the substrate specificity of DHCR7 in rat liver microsomes using lathosterol, and non-endogenous sterols including ergosterol, 7-dehydrositosterol and 7-dehydroepicholesterol (3 α OH group), as substrates and concluded that the presence of a C5–C6 double bond is required for the enzymatic reduction of the C7–C8 double bond [16].

A number of enzymatic and photochemical products of 7DHC metabolism have been characterized to date, summarised in Fig. 1. 7DHC can be converted to 8-dehydrocholesterol (8DHC) in a reversible reaction catalysed by 3 β -hydroxysteroid- Δ^8 , Δ^7 -isomerase [17,18]. 7DHC is metabolized to 25(OH)7DHC and 27(OH)7DHC by CYP27A1 [19,20] and to 24- and 25-(OH)7DHC by CYP46A1 [20]. CYP11A1 removes the side chain from 7DHC producing 7-dehydropregnenolone (7DHP) via 22(OH)7DHC and 20,22(OH)₂7DHC intermediates [21–23]. All these sterols retain the Δ^7 -double bond. 7DHP can in turn

be converted to some steroids which retain the 7 double bond [21,24–28]. UVB irradiation of 7DHC produces previtamin D3 from breakage of the sterol B-ring between carbons 9 and 10 (Fig. 1). This then undergoes spontaneous thermal isomerization to vitamin D3 [29]. Previtamin D3 can also undergo a further photochemical reaction producing lumisterol3 (L3) [30,31]. This involves resealing the B-ring, but in a 9 β ,10 α orientation rather than the 9 α ,10 β configuration seen in 7DHC, making lumisterol a stereoisomer of 7DHC. L3 can be converted to various hydroxy-derivatives by the actions of CYP11A1 and CYP27A1 (Fig. 1) [32–34]. The aim of the current study was to examine whether a range of these metabolites of 7DHC could act as either substrates or inhibitors of DHCR7 in rat liver microsomes.

2. Materials and methods

2.1. Materials

Glucose 6-phosphate and glucose-6-phosphate dehydrogenase (from *Leuconostoc mesenteroides*), NADPH, ergosterol, vitamin D3 and 7-dehydrocholesterol (7DHC) were from Sigma-Aldrich (Sydney, Australia). 25(OH)D3 was from Carbosynth Ltd (Compton, UK); lumisterol (L3) and 8-dehydrocholesterol (8DHC) was from Toronto Research Chemicals (Toronto, Canada), and 7-dehydodesmosterol (7DHD) was from Kerfaast Inc (Boston, MA). 20 S (OH)D3 was produced enzymatically from the action of bovine CYP11A1 on vitamin D3 as described before [29]. 27(OH)7DHC and 25(OH)7DHC were produced enzymatically from the action of human CYP27A1 on 7DHC [19] while 7-dehydropregnenolone (7DHP) was produced by the action of human CYP11A1 on 7DHC [21,22]. 20 S (OH)L3, 22(OH)L3, 24(OH)L3 and 20,22(OH)₂L3 were produced from the metabolism of lumisterol by bovine CYP11A1 [34] while 25(OH)L3, (25 R)-27(OH)L3 and (25 S)-27(OH)L3 were produced from the action of human CYP27A1 on lumisterol [33]. 20 S (OH)-7DHC was produced chemically [35]. Ergosterol and 7DHD were purified prior to use by HPLC using a C18 Alltima column (250 \times 4.6 mm, 5 μ m) (Grace, Hichrom, Berkshire, UK) with a 15 min 64–100 % methanol gradient followed by 55 min with 100 % methanol, at a flow rate of 0.5 mL/min.

2.2. Preparation of rat liver microsomes

Livers from Wistar rats (12 weeks old) were obtained through the UWA Program for use of Unwanted Tissues from Other Studies. Finely cut tissue fragments were homogenised using a Potter-Elvehjem homogeniser in 5 vol 0.25 M sucrose and microsomes prepared by differential centrifugation as described before [36,37].

2.3. Incubations of microsomes to measure DHCR7 activity

The activity of DHCR7 in rat liver microsomes was measured from the conversion of ergosterol to brassicasterol, measured at 205 nm by reverse-phase HPLC. The incubation procedure was based on that described by Shefer et al. [16]. Ergosterol (or other potential substrates) was initially dissolved in 45 % (by wt) 2-hydroxypropyl- β -cyclodextrin at final concentration of 600 μ M. Liver microsomes (0.5 mg) were incubated with 30 μ M ergosterol and 1.0 mM NADPH in 100 mM potassium phosphate buffer (pH 7.5) in a final volume of 0.5 mL for 1 h at 37 $^{\circ}$ C. Reactions were stopped by adding 0.5 mL 20 % (w/v) KOH in 50 % methanol, then stigmasterol (20 nmol) was added as an internal standard. Samples were

saponified by incubating samples at 37 °C for 1 h. Sterols were then extracted 3 times with 2 mL of hexane, extracts combined and dried under a stream of nitrogen at room temperature. Samples were made up in 0.35 mL 90 % methanol in water for HPLC analysis.

To screen for other possible substrates or inhibitors of DHCR7, competition experiments were carried out using ergosterol as substrate, as above, but with a competing sterol or secosteroid (30 μ M) also present. Competing sterols or secosteroids were initially made up as 600 μ M stocks in 45 % 2-hydroxypropyl- β -cyclodextrin. The final 2-hydroxypropyl- β -cyclodextrin concentration was kept at 3.27 % for all experiments.

2.4. HPLC analysis of incubation products

Samples were analysed on a Perkin-Elmer HPLC system (PE LC-250B Pump with Series 200 Autosampler) with a UV detector set at 205, equipped with a C18 Alltima column (250 \times 4.6 mm, 5 μ m) (Grace, Hichrom, Berkshire, UK). Metabolites were separated using a 15 min 64–100 % methanol gradient, followed by 65 min with 100 % methanol at a flow rate of 0.5 mL/min. The injection volume was set to 0.2 mL.

3. Results

3.1. Measuring DHCR7 activity with ergosterol as substrate

Ergosterol, only available from the diet, is a well characterized substrate for DHCR7 and was used in the current study. Both itself and its product (brassicasterol) are not detectable in rat liver microsomes making it more suitable than using 7DHC as substrate [15,16]. This is because cholesterol, which is the product of the reductase acting on 7DHC, is abundant in microsomes (Fig. 2) making it difficult to measure low rates of 7DHC metabolism. The metabolism of ergosterol by rat liver microsomes was measured by HPLC at 205 nm. Chromatograms of the extracted sterols displayed a curved baseline at 205 nm for the first 15 min due to the methanol gradient used, but the baseline was flat during the times that sterol substrates and products eluted facilitating peak area measurement (Fig. 2). Almost complete conversion of ergosterol, added dissolved in 2-hydroxypropyl- β -cyclodextrin, to brassicasterol was observed in the test incubation, with no conversion in the control lacking NADPH. 7DHC, the natural substrate for the enzyme, at the same concentration as ergosterol (both 30 μ M) was able to inhibit the conversion of ergosterol to brassicasterol by 93 %, confirming that the ergosterol and 7DHC compete for the active site of the reductase, with 7DHC having the higher affinity (Fig. 2; Table 1).

Using ergosterol as substrate, product formation was linear with time at least up to 70 min and linear with microsomal protein concentration up to 1.5 mg/mL under the conditions of our assay (Fig. 3). The K_m for NADPH measured at an ergosterol concentration of 30 μ M was 0.28 mM. We routinely assayed DHCR activity using a 1.0 h incubation with 1.0 mg/mL microsomal protein and a NADPH concentration of 1.0 mM (see Methods).

3.2. Competition of sterols and secosteroids with ergosterol

Competition experiments using ergosterol as substrate, similar to that described in Fig. 2 for ergosterol and 7DHC, were carried out with a range of different sterols and secosteroids to

screen for other possible substrates or inhibitors of DHCR7 (Table 1). All these sterols and secosteroids are produced endogenously in humans or are predicted to be present based on known enzymatic reactions (see Introduction). 7DHD is the natural substrate for DHCR7 in the Bloch pathway (where C7–C8 double bond reduction precedes reduction of the C24–C25 double bond) where it is converted to desmosterol. 7DHD inhibited ergosterol reduction by 57 % (Table 1). This was significantly less inhibition than was observed for 7DHC (93 %) in the same experiment (Table 1). Incubation of 7DHD alone with the microsomes resulted in substantial conversion to desmosterol from the action of DHCR7 with no 7DHC, the product of DHCR24 acting on 7DHD, being detected (Fig. 4).

3.3. Interaction of 7DHC-like metabolites with DHCR7

Analysis of hydroxy-derivatives of 7DHC revealed that 20*S*(OH) 7DHC inhibited ergosterol metabolism to a similar extent to 7DHC. In contrast, 27(OH)7DHC significantly inhibited brassicasterol formation by 25 %, much less than that observed with 7DHC, while no significant inhibition was observed for 25(OH)7DHC. Similarly, 7DHP which has the same B-ring configuration as 7DHC but lacks 6-carbons of the side chain, did not significantly inhibit brassicasterol formation.

To confirm that 20*S*(OH)7DHC and 27(OH)7DHC could serve as substrates for DHCR7, these were incubated with the enzyme in rat liver microsomes in the absence of ergosterol (Fig. 5). 20*S*(OH)7DHC was converted to the expected 20*S*-hydroxycholesterol, based on an identical HPLC retention time to authentic standard, by rat liver microsomes in the presence but not the absence of NADPH (Fig. 4A). 20*S*(OH)-7DHC was converted to the expected 20*S*-hydroxycholesterol based on its identical HPLC retention time to authentic standard, in the presence but not the absence of NADPH (Fig. 5A). Similarly, 27(OH) 7DHC was converted to the expected 27-hydroxycholesterol product by the microsomes in the presence but not the absence of NADPH (Fig. 5B). A small amount of 27(OH)7DHC product was also seen in competition with ergosterol (not shown), consistent with the small but significant inhibition of ergosterol reduction seen with this sterol (Table 1). Thus, 27(OH)7DHC is a substrate for DHCR7, but the reduction rate is lower than for ergosterol. 7DHP, missing six carbons from its side chain compared to 7DHC, was not converted to pregnenolone by DHCR7 (Fig. 6A), consistent with its lack of inhibition of ergosterol reduction (Table 1).

The reversible isomerization of 7DHC to 8DHC is catalysed by 3 β -hydroxysteroid-⁸,⁷-isomerase [17,18]. 8DHC, like 7DHC, accumulates in SLOS patients [6,11]. 8DHC decreased ergosterol reduction by DHCR7 by 34 % (Table 1). However, no product was detected by HPLC when it was tested alone, suggesting that it is a competitive inhibitor of DHCR7 but not a substrate, consistent with its lack of a double bond at C7–C8 for reduction (Fig. 6B).

3.4. Interaction of vitamin D3, lumisterol and their hydroxyderivatives with DHCR7

Vitamin D3 which has a broken B-ring did not significantly compete with ergosterol for binding to the reductase, nor did its hydroxyderivatives, 20*S*(OH)D3 or 25(OH)D3 (Table 1). L3 is a stereoisomer of 7DHC and is formed from pre-vitamin D3 by the UV-driven

resealing of the B-ring (Fig. 1). It has the C5–C6 and C7–C8 double bonds present as in 7DHC but the opposite configuration (9 β ,10 α orientation) for the reformed C9–C10 bond (Fig. 1). L3 did not significantly compete with ergosterol in the DHCR7 assay, nor did 20,22(OH)₂L3, 24(OH)L3, 25(OH) L3 or the two isomers of 27(OH)L3. However, both 22(OH)L3 and 20*S*(OH)L3 significantly inhibited ergosterol reduction, by 22 % and 64 % respectively.

To confirm that L3 is not a substrate for DHCR7, it was incubated with rat liver microsomes in the absence of ergosterol. No product was detected in the resulting chromatogram (Fig. 7A). No standard was available for the predicted product, 7-8-dihydrolumisterol, but based on the change in retention times for the other similar 7 sterols upon reduction of the C7–C8 double bond, it would be expected to have a slightly longer retention time (2–4 min) than lumisterol. In a similar experiment testing 20*S*(OH)L3 as a substrate, no product was observed from this sterol based on the absence of any peak with a retention time 1–2 min longer than the 20*S*(OH)L3 that was not present in the control (Fig. 7B). Thus, we conclude that lumisterol is not a competitive inhibitor of ergosterol metabolism by DHCR7 nor can it act as a substrate for the enzyme, while 20*S*(OH)L3 is an inhibitor of DHCR7 but cannot serve as a substrate.

4. Discussion

To date there have been limited studies on the range of sterols that can be reduced by DHCR7 and hence its substrate specificity. What has been reported is that DHCR7 requires a sterol with a double bond between C5 and C6 for it to be able to do the reduction at C7 and C8, converting the double bond in this position to a single bond [16]. Given that there are many natural metabolites of 7DHC (Fig. 1), particularly in skin cells [28,32], we were interested in whether any of these could act as substrates or inhibitors of DHCR7.

Modification of the side chain of 7DHC dramatically influenced the ability of DHCR7 to interact with the sterol, in a manner dependent on the site of modification. The presence of a C24–C25 double bond, as seen for 7DHD, reduced the ability of the sterol to compete with ergosterol compared to that seen for 7DHC. This indicates that 7DHC is the tighter binding substrate for the reductase, however relative maximum velocities for the enzyme acting on 7DHC and 7DHD remain to be established. Rat liver microsomes converted 7DHD to the expected product of DHCR7 action, desmosterol, with no detectable conversion to 7DHC suggesting low DHCR24 activity in the microsomes. DHCR7 and DHCR24 have been reported to physically interact in the endoplasmic reticulum [38].

The presence of the side chain of 7DHC is necessary for DHCR7 activity as cleavage of 6 carbons catalysed by CYP11A1, producing 7DHP, removed its ability to compete with ergosterol for reduction or to be acted on as a substrate. Hydroxylation of the side chain at C27 or C25 by CYP27A1 dramatically reduced or removed its ability to compete with ergosterol, but some product (27(OH)cholesterol) was observed for 27(OH)7DHC. The active site of DHCR7 can readily accommodate a hydroxyl group at C20, with 20*S*(OH)7DHC competing with ergosterol to a similar degree as 7DHC and being converted to the expected product, 20*S*(OH)cholesterol. Thus, metabolism of 7DHC by CYP11A1 or

other enzymes hydroxylating the side chain at C20 does not prevent metabolism by DHCR7, but removal of the side chain by CYP11A1 does. It should be noted that 20*S*(OH)7DHC has been detected in the human epidermis [32]. Hydroxylation near the end of the side chain by CYP27A1 [19,20] dramatically reduces the ability of the sterol to interact with DHCR7. In the case of 7DHP, it has been shown that it can be made from 7DHC by CYP11A1 in isolated mitochondria from rat skin [22] as well as in adrenal glands, human placenta and epidermal keratinocytes incubated *ex vivo* [21,28]. Furthermore, 7DHP has been detected in the human epidermis and serum [39]. Thus, in addition to SLOS patients, 7DHP can be produced under non-pathological conditions not only in the skin but also on the systemic level and can be hydroxylated to produce a number of unsaturated steroids so far detected in SLOS patients [12,25–27,40] and under *ex-vivo* physiological conditions [21,28,39], or *in vitro* [41]. 7DHP and some of its derivatives such as 20(OH)7DHP, 7,20(OH)₂7DHP have anti-proliferative activities in human keratinocytes, melanocytes and melanoma cells cultured *in vitro* [21,42–44]. The lack of activity of DHCR7 on 7DHP, and presumably its metabolites, enables them to maintain their 7 configuration without conversion to the corresponding more abundant steroids lacking the C7–C8 double bond. Thus, they retain their sensitivity to UVB which can potentially transform them to their corresponding secosteroid or lumisterol derivatives which have been reported to have biological activity on normal and malignant cells [24,28,42–46].

The favourable binding of sterols possessing a hydroxyl group on the side chain close to the d-ring, to DHCR7, was also seen with 20*S*(OH)L3 and to a lesser degree by 22(OH)L3, which both inhibited (presumably competitively) ergosterol metabolism by the enzyme. 20*S*(OH) L3 did not appear to be metabolized by DHCR7, indicating that sterol substrates require the 9 α ,10 β conformation in the B-ring, as seen in 7DHC and ergosterol, to carry out the C7–C8 reduction, with the 9 β ,10 α conformation present in lumisterol being unsuitable, even when binding is enhanced by the presence of a 20-hydroxyl group. L3 is produced from previtamin D3 in a UVB-mediated photochemical reaction (Fig. 1) and its formation was believed to be a mechanism to prevent excessive production of vitamin D3 [30]. More recent studies indicate that it is a prohormone converted to biologically active hydroxylumisterols by CYP11A1 and CYP11A1 [32,33,47–49]. Lumisterol is present in human skin at approximately 1% of the 7DHC concentration but is present in human serum at a concentration approximately equal to 7DHC [32]. Thus, its inability to be acted on by DHCR7 should be of physiological relevance, conversion of 7DHC to lumisterol protects it against the action of DHCR7, providing increased metabolic stability. Lumisterol can then be activated either by CYP11A1 or CYP27A1 to produce biologically active metabolites regulating epidermal functions [49]. 20*S*(OH) L3 has also been detected in human skin and serum and displays biological activity on normal and transformed skin cells [32]. In skin, its concentration is approximately 3 % of that of 7DHC so it is unlikely to be a major inhibitor of DHCR7 *in vivo*. However, it remains to be established if human DHCR7 has similar substrate/inhibitor specificity to the rat enzyme.

Vitamin D3, one of the major metabolites of 7DHC, does not appear to interact with DHCR7 based on its lack of inhibition of ergosterol reduction. This is not surprising given the alteration in structure that occurs with B-ring opening and isomerisation reactions involved in vitamin D synthesis (Fig. 1). This lack of interaction was also seen for major

monohydroxy-derivatives of vitamin D3, 20(OH)D3 and 25(OH)D3, so even the addition of the 20-hydroxyl group to the former did not facilitate binding to DHCR7. It has been reported that vitamin D decreases DHCR7 activity rapidly in keratinocytes, without altering the protein level [15]. Our data suggest that it is not due to competitive inhibition of the enzyme, consistent with their observation that there was no inhibition when vitamin D was added to cell lysates.

In conclusion, our identification of 8DHC and 20(OH)L3 as inhibitors of DHCR may aid in the further studies on the enzyme providing a structural basis for the development of tight binding inhibitors. Furthermore, resistance of lumisterol and 7DHP to DHCR7 enzymatic activity in cells will permit these compounds to be further metabolized to their active forms retaining the C7–C8 double bond which may confer specificity to their local and systemic actions.

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Abbreviations:

7DHC	7-dehydrocholesterol
8DHC	8-dehydrocholesterol
7DHD	7-dehydrodesmosterol
7DHP	7-dehydropregnenolone
L3	lumisterol3
SLOS	Smith-Lemi-Opitz Syndrome
20S(OH)D3	20S-hydroxyvitamin D3
25(OH)D3	25-hydroxyvitamin D3

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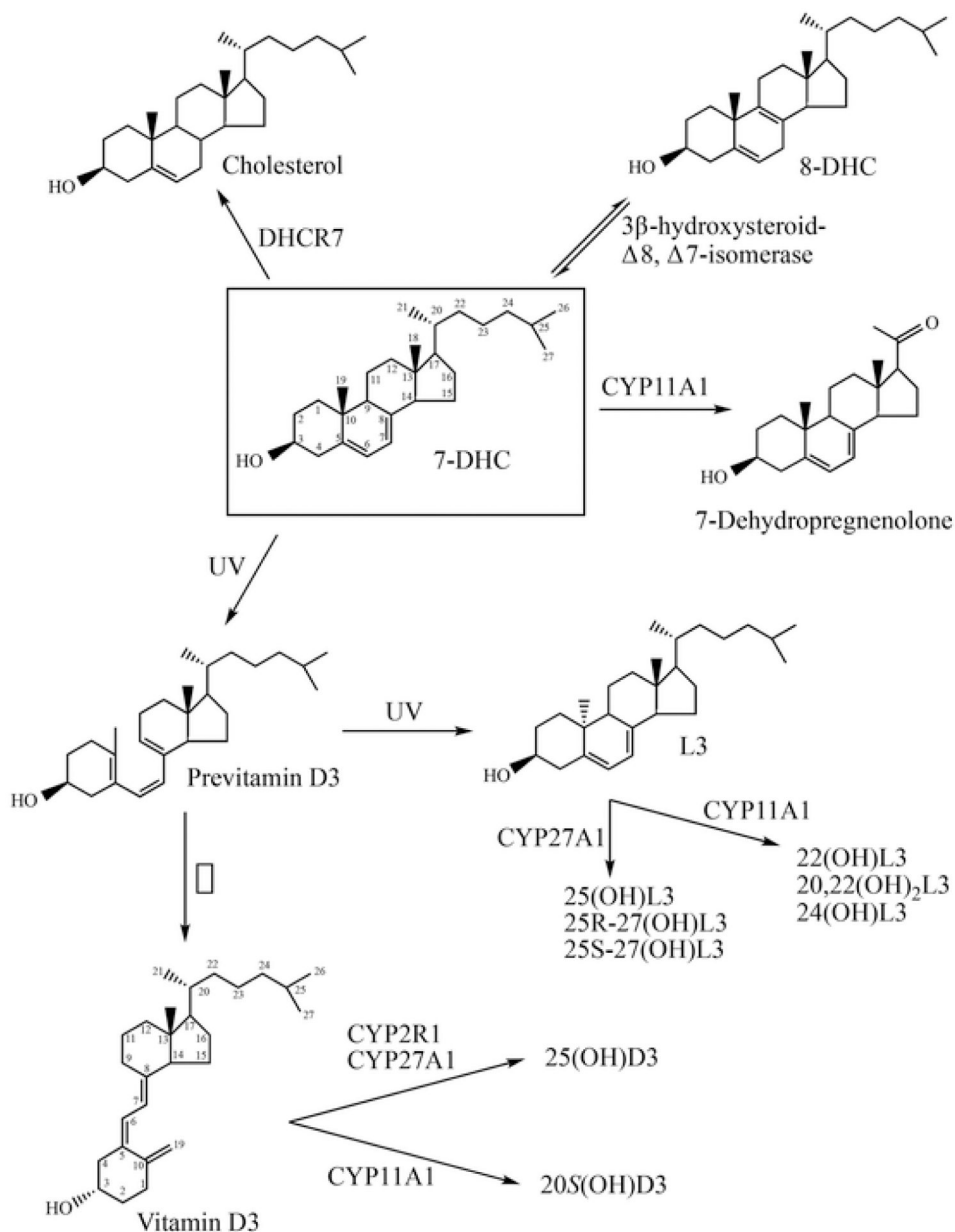


Fig. 1. Pathways for the biosynthesis of the major metabolites of 7DHC used on this study. The 7DHC structure is boxed. See text for references.

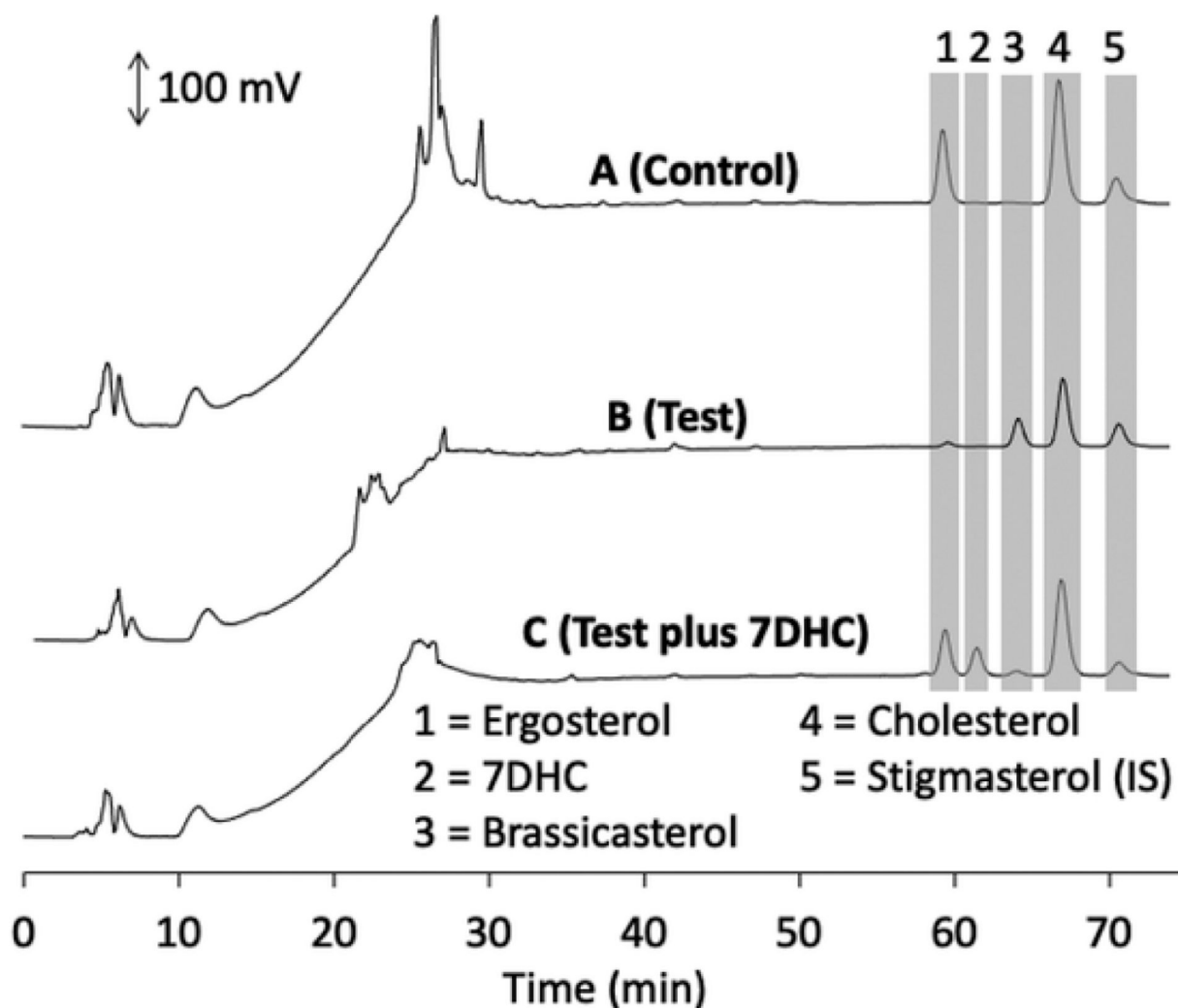


Fig. 2.

HPLC analysis of microsomal DHCR7 activity towards ergosterol in the absence and presence of 7DHC. Rat microsomes were incubated with 30 μ M substrates for 1 h at 37 $^{\circ}$ C, saponified and extracted 3 times with hexane. Stigmasterol was added prior to saponification as an internal standard. Samples were analysed by reverse phase HPLC at 205 nm on a C18 Alltima column using a methanol-water solvent system as described in the Methods. (A), Incubation with ergosterol in the absence of NADPH (negative control); (B), test incubation with ergosterol; (C), test incubation with ergosterol in the presence of 30 μ M 7DHC. Relevant peaks are highlighted with grey bars and numbered for identification from standards run separately.

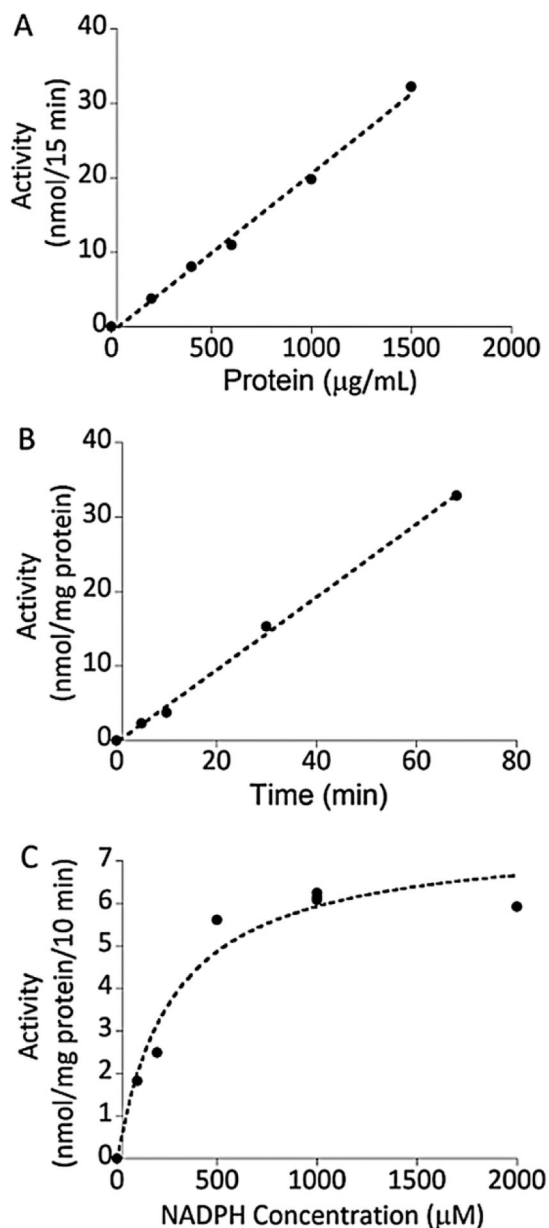


Fig. 3. Characteristics of the assay of DHCR7 in microsomes using ergosterol as substrate. (A), Time dependence of DHCR7 activity measured with 30 μ M ergosterol and a microsomal protein concentration of 1.0 mg/mL, as in Fig. 2. (B), Linearity of DHCR7 activity with microsomal protein concentration measured with 30 μ M ergosterol and an incubation time of 15 min. (C) K_m for NADPH measured with 30 μ M ergosterol, 0.5 mg/mL microsomal protein and an incubation time of 10 min.

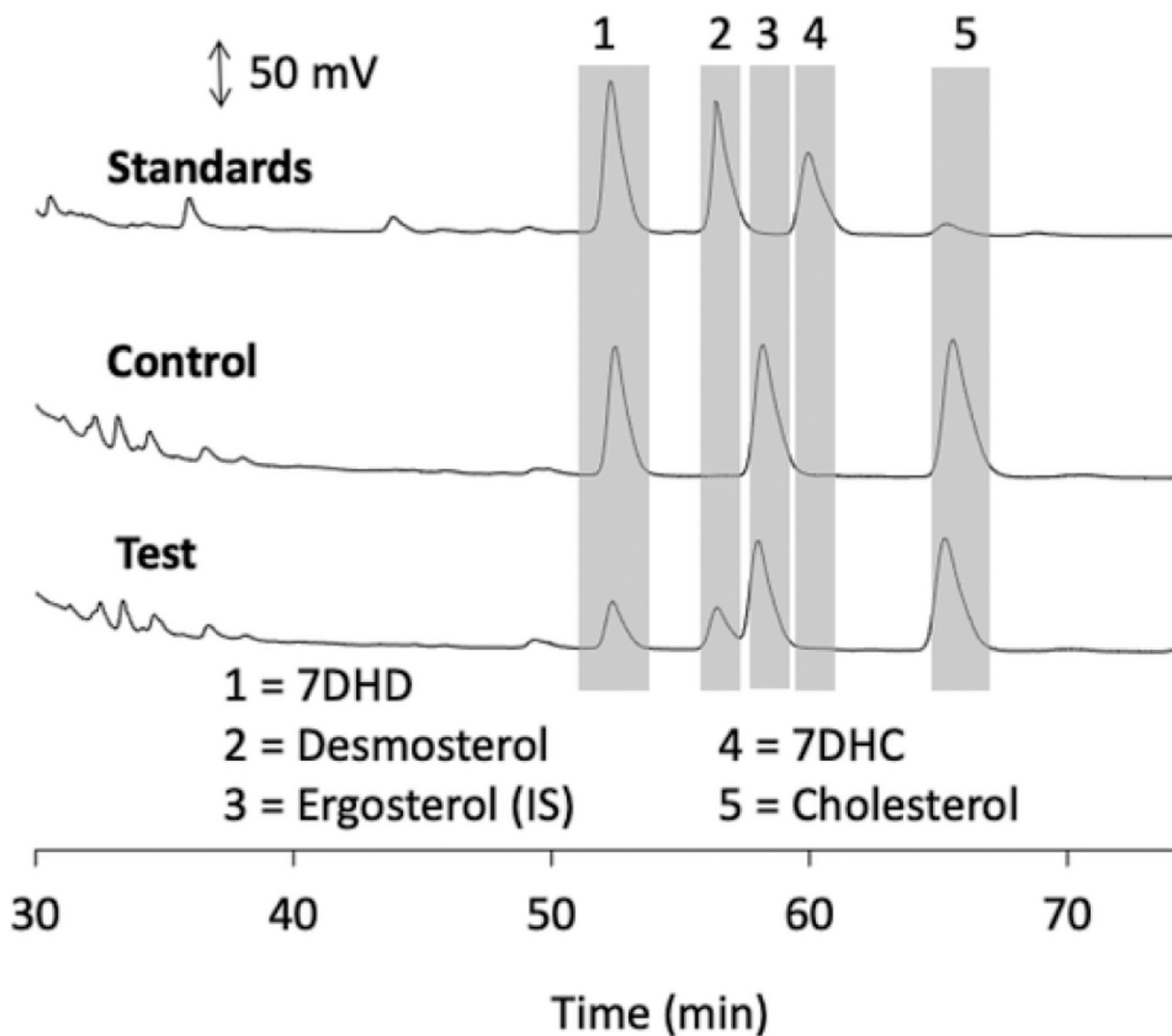


Fig. 4.

Metabolism of 7DMD by rat liver microsomes. Microsomes were incubated with 30 μ M 7DMD for 20 min at 37 $^{\circ}$ C and products analysed by HPLC as in Fig. 2. In this experiment however, ergosterol (15 nmol) was added as an internal standard at the end of the incubation, prior to extraction. (A), Chromatograms of standards, (B), control reaction lacking NADPH and (C), test reaction with NADPH. Chromatogram traces up to 30 min were similar to those in Fig. 2 and are not shown as no reactants nor products eluted during this period.

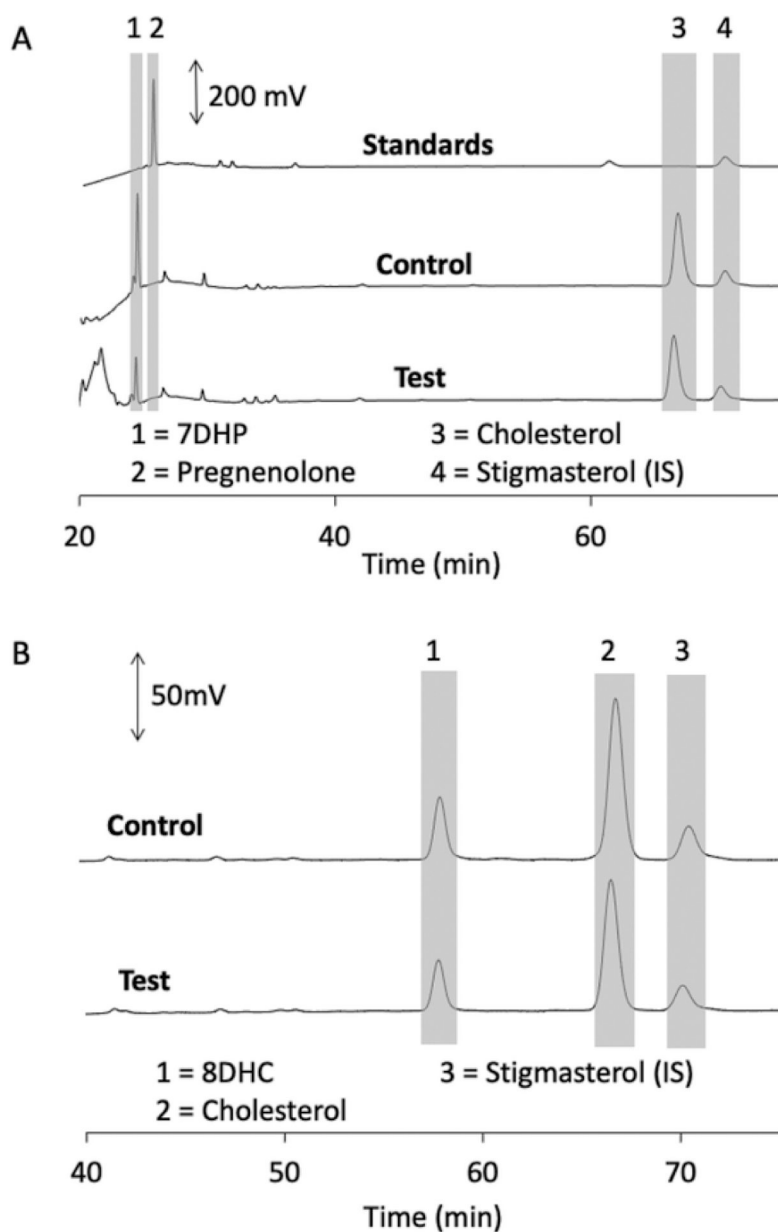


Fig. 6. 7DHP and 8DHC are not metabolised by DHCR7 in microsomes. Microsomes were incubated with 60 μ M 7-dehydropregnenolone (A) or 8DHC (B) for 1 h at 37 $^{\circ}$ C and products analysed by HPLC as in Fig. 2. Each panel shows chromatograms of standard(s), control reaction lacking NADPH and the test reaction with NADPH.

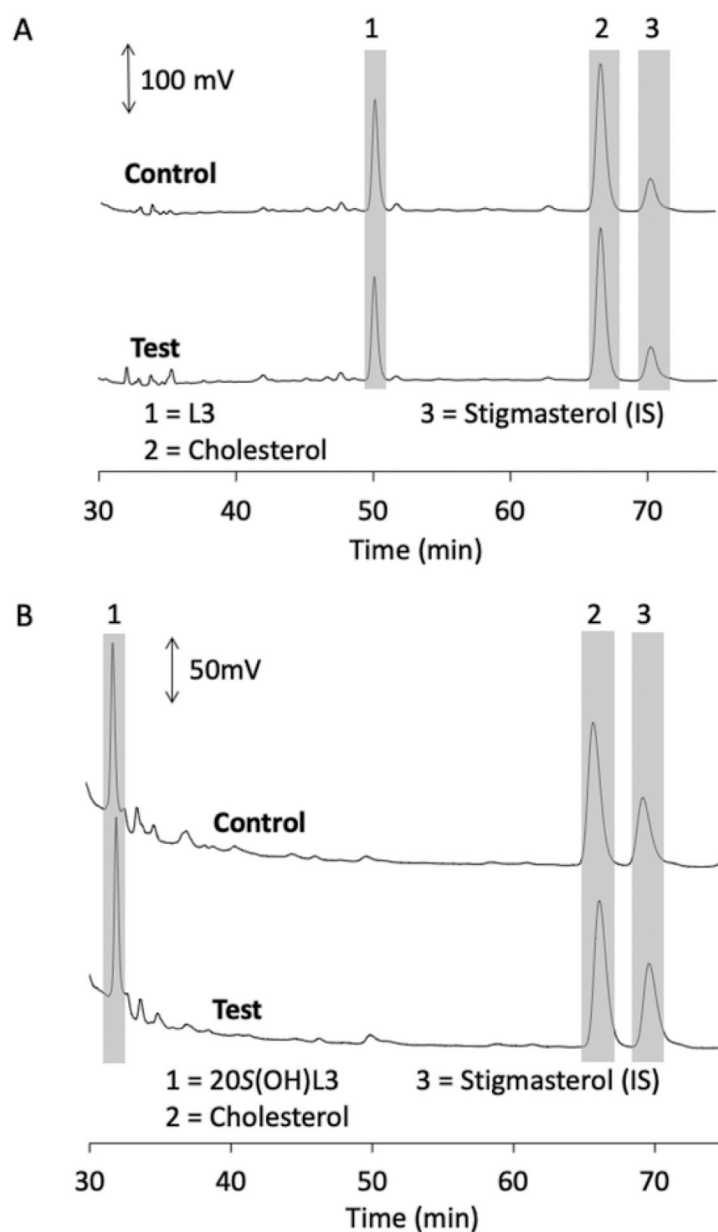


Fig. 7.

Lumisterol (L3) and 20S(OH)L3 are not metabolised by DHCR7 in microsomes. Microsomes were incubated with 60 μ M L3 (A) or 30 μ M 20S(OH) L3 for 1 h at 37 °C and products analysed by HPLC as in Fig. 2. Each panel shows chromatograms of the control reaction lacking NADPH and the test reaction with NADPH. Relevant peaks are highlighted with grey bars and numbered for identification from standards run separately.

Table 1
Competition of sterols and secosteroids for reduction of ergosterol by DHCR7.

The reduction of ergosterol to brassicasterol was measured with both ergosterol and the competing substrate at a final concentration of 30 μ M. The amount of brassicasterol produced in a 1 h incubation was measured by HPLC as in Fig. 1. Data are mean \pm SE of three replicates. Statistical significance for the difference in activity for each sterol tested to the normalized control (ergosterol only) for each experiment was evaluated by the Student's t-test. ns, not significant $p > 0.05$. Data were obtained from 7 separate experiments as indicated by the different SE for the normalised controls. The stereochemistry of the competing substrates is provided, where known.

Competing Substrate		Normalised activity Control (% control)	Normalised Activity Test (% control)	<i>p</i> -value
7DHC-like	7DHC	100 \pm 15.7	6.6 \pm 2.6 ^a	$p < 0.01$
	7DMD	100 \pm 15.7	42.6 \pm 9.2 ^a	$p < 0.05$
	20S(OH)7DHC	100 \pm 4.2	5.84 \pm 2.36	$p < 0.001$
	27(OH)7DHC	100 \pm 1.0	75.4 \pm 2.8	$p < 0.001$
	25(OH)7DHC	100 \pm 1.5	109.3 \pm 7.5	ns
	7DHP	100 \pm 5.8	105.7 \pm 7.3	ns
	8DHC	100 \pm 6.3	66.0 \pm 7.2	$p < 0.05$
	Secosteroids	vitamin D3	100 \pm 5.8	92.3 \pm 9.2
20S(OH)D3		100 \pm 6.3	96.5 \pm 3.4	ns
25(OH)D3		100 \pm 6.3	92.3 \pm 3.8	ns
Lumisterols	L3	100 \pm 5.8	106.8 \pm 9.8	ns
	20S(OH)L3	100 \pm 4.5	33.8 \pm 15.6	$p < 0.05$
	22(OH)L3	100 \pm 1.0	88.0 \pm 5.0	$p < 0.05$
	20,22(OH) ₂ L3	100 \pm 5.8	102.6 \pm 8.5	ns
	24(OH)L3	100 \pm 4.2	100.4 \pm 4.5	ns
	25(OH)L3	100 \pm 5.8	93.8 \pm 9.2	ns
	25R27(OH)L3	100 \pm 5.8	84.5 \pm 10.2	ns
25S27(OH)L3	100 \pm 5.8	92.0 \pm 8.8	ns	

^aSignificantly different from each other, $p < 0.001$.