

Table S1: Details of sequences use in the phylogenetic analysis.

Species	Description on Figure 2	Registered Name of Enzyme on NCBI	NCBI Accession Number	Number of Amino Acids
<i>Aedes aegypti</i>	DHCR24	delta(24)-sterol reductase	XP_001655874.2	504
<i>Bombyx mori</i>	BmDHCR24-1	delta(24)-sterol reductase	XP_004926865.1	506
<i>Bombyx mori</i>	BmDHCR24-2	delta(24)-sterol reductase	XP_004926210.1	531
<i>Caenorhabditis elegans</i>	DHCR24-1	Delta(24)-sterol reductase	NP_001362099.1	516
<i>Caenorhabditis elegans</i>	DHCR24-2	Delta(24)-sterol reductase	NP_510594.1	538
<i>Culex quinquefasciatus</i>	DHCR24	24-dehydrocholesterol reductase	EDS41303.1	504
<i>Danio rerio</i>	DHCR24	Delta(24)-sterol reductase	NP_001008645.1	516
<i>Homo sapiens</i>	DHCR24	24-dehydrocholesterol reductase	AAH11669.1	516
<i>Manduca sexta</i>	DHCR24	delta(24)-sterol reductase	XP_030025267.2	507
<i>Manduca sexta</i>	DHCR24-like	delta(24)-sterol reductase-like	XP_037295187.1	533
<i>Mus musculus</i>	DHCR24 precursor	delta(24)-sterol reductase precursor	NP_444502.2	516
<i>Tribolium castaneum</i>	DHCR24-like	PREDICTED: delta(24)-sterol reductase-like isoform X1	XP_001810773.1	505
<i>Tribolium castaneum</i>	predicted DHCR24	PREDICTED: delta(24)-sterol reductase	XP_966520.1	525
<i>Penaeus vannamei</i>	DHCR24-1 like	delta(24)-sterol reductase-like	XP_027224655.1	504
<i>Penaeus vannamei</i>	DHCR24-2 like	delta(24)-sterol reductase-like	XP_027238556.1	537
<i>Homo sapiens</i>	DHCR7	7-dehydrocholesterol reductase	NP_001157289.1	475

Table S2: Fragmentations of TMS derivatives of cholesterol, cholesterol-d7 and cholesterol-d6.

Annotation	Ions in TMS-Cholesterol (m/z)	Ions in TMS-Cholesterol-d7 (m/z)	Ions in TMS-Cholesterol-d6 (m/z)
M	458	465	464
M-CH ₃	443	450	449
M-TMSOH	368	375	374
M-TMSOH-CH ₃	353	360	359
M-C ₁ ,C ₂ ,C ₃ -TMSO	329	336	335
M-C ₁ ,C ₂ ,C ₃ -TMSO-CH ₂ CH ₂	301	308	307
C ₁ ,C ₂ ,C ₃ +TMSO	129	129	129

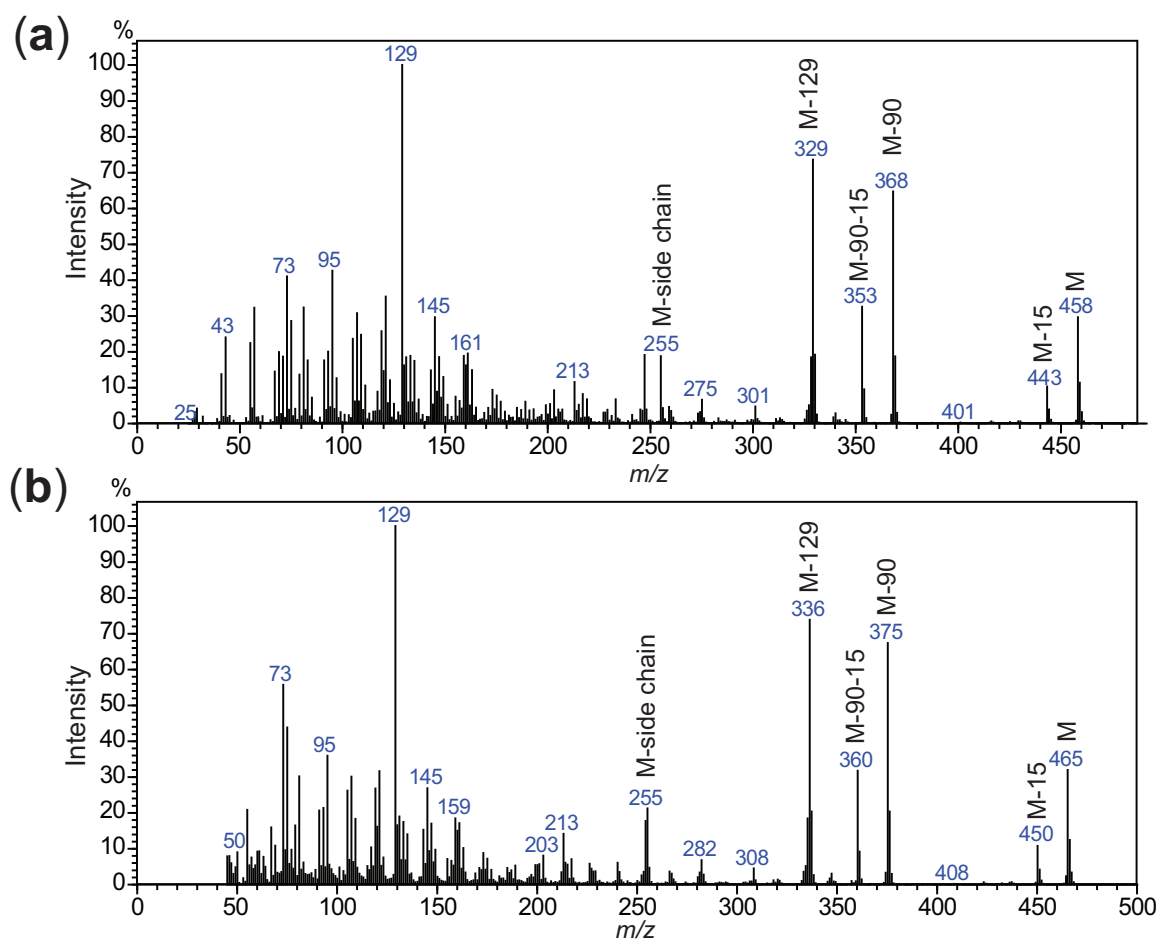


Figure S1: Mass spectra of TMS derivatives of commercial samples of (a) cholesterol and (b) cholesterol-d7.

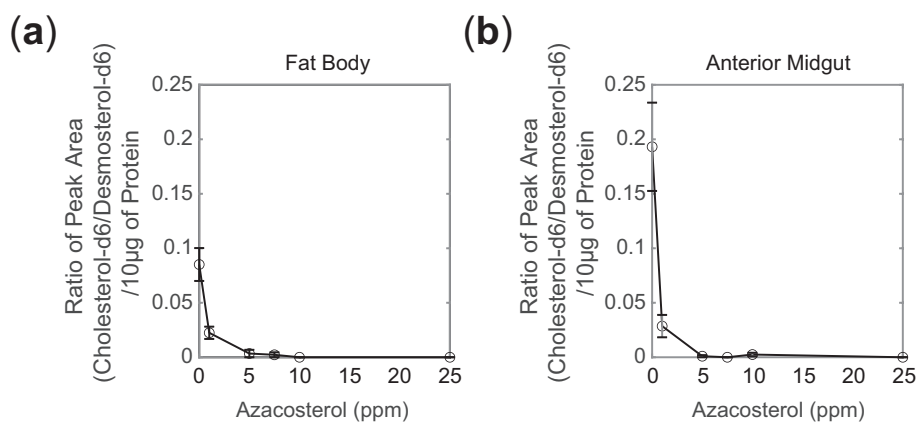


Figure S2: Effect of different amounts of azacosterol on the enzyme activity in the 15300 \times g-pellet fraction extracted from (a) fat body and (b) anterior midgut $n = 6-15$). Data is presented as mean \pm SEM.

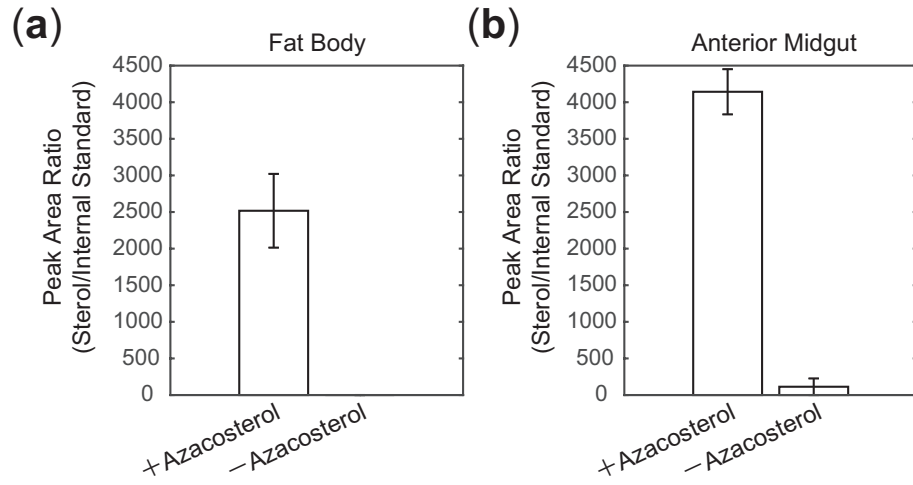


Figure S3: Effect of azacosterol on the amount of desmosterol in (a) fat body and (b) anterior midgut. Amount of desmosterol were standardized by the total amount of RNA. Data was derived from crickets feeding on diet with and without azacosterol for 18 hours ($n = 7$). Data is presented as mean \pm SEM.

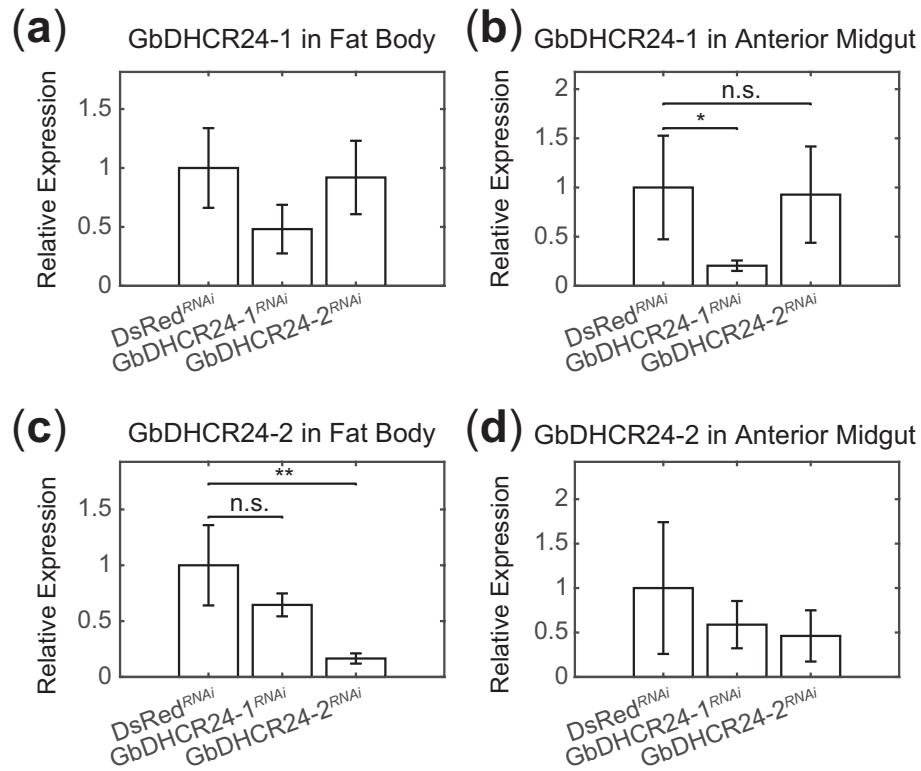


Figure S4: The efficiencies of the knockdown of GbDHCR24s were evaluated by qPCR. The average of relative expression levels of each GbDHCR24 to β -actin in the DsRed^{RNAi} crickets was standardized to 1. In order to normalise the data, the relative expression levels of GbDHCR24-1 to β -actin in the GbDHCR24^{RNAi} crickets were divided by the average of relative expression levels of each GbDHCR24 variant to β -actin in the DsRed^{RNAi} crickets. ($n = 6$). (a, b) Relative expression of GbDHCR24-1. (c, d) Relative expression of GbDHCR24-2. Data is presented as mean \pm SEM. Mann-Whitney U test was performed to analyse the statistical differences between the results obtained from DsRed^{RNAi} and GbDHCR24^{RNAi}. * $P < 0.05$ and ** $P < 0.01$.

GbDHCR24-1	1	---MASFD S ALEHVLVHYRWVEVCFFLLP I SLFYEVWLYTRNWI V FQLNSAPRQHD K KV
GbDHCR24-2	1	MTT T T T GFDRALQHLV V NYRWVE A SFFLLPL S FLYEV S YARNWL V FKL N TAP K QH N KKV
GbDHCR24-1	57	RNVQRQVKQWQD S GRRT K MCTARPGWQT S FRQPKY K TMFN V NVLVD I INVDEEK R TV
GbDHCR24-2	61	QYVQRQVRQWKE S SGN I PMCTARPGYQT S LRKPKY K NTMY N IHVNLVD V LEVNEEK Q TV
GbDHCR24-1	117	LV E PLVN M GQL S AT L TPLGWTIPV L PE I DDLT V GG M VMGT G IE S SSHKYGLFQHICK S Y E
GbDHCR24-2	121	R C EPLVT M GQL T AT L NPLGWTIPV V PE L DDLT V GG L VMGT G VE S T S HIYGLFQHIC V S Y E
GbDHCR24-1	177	LV L ADGSVV T CS K DNN S ELFY S IPWSY G SLG F LTA V E I Q I IPAT K YVK L EYK P VR S LD E A
GbDHCR24-2	181	LV M GDGSVV T CS K EN N PD L FY A VPWSY G TL G LTA A E I K I IPAK R YVK L HY Q PVY T LD D A
GbDHCR24-1	237	V S VFKEET L K T TQ N QFVEGL M F N K E EGVIMTGN M TELHEANK V NEIG M WYKPWF F EH V RS
GbDHCR24-2	241	V G LF A KES - K S KEH Q FVEGL M F S K N EGVIMTGD M V S FSKGET I NEIG K WYKPWF F LHV K K
GbDHCR24-1	297	FL K K G K S TE Y IP L RDY Y HR H TR S IFWE I QDIIPFG N NP I FR F ELGWMMPPKV S LL K L T Q T
GbDHCR24-2	300	FL K K G PGDE V IP L RDY Y HR H TR A L F WE V QDIIPFG N NI I FR Y ELGWMMPPKV S LL K L T ET
GbDHCR24-1	357	K A I K K L YEN N H I QD M L V PI S S M AE S IQMFHD A V Q VYPLWLC P F K L N ND P GL V H S S N NGD
GbDHCR24-2	360	K A I T D L YEN H MIQD F L I P V S A L K E A IT V S H K AVE I YPLWLC P F N L P ND P GM V H P A A NE
GbDHCR24-1	417	E I YVD I GVYGV P K A P N FHP V N T TRIE E YVR K V K GFQ M LYAD T Y A T R EE F E M FD F SLY K
GbDHCR24-2	420	E M YVD L GVYGV P K T G N FD A V K T TR H ME D F I A S V HGFQ M LYAD S FR T K D E F RQ M FD H SLY D
GbDHCR24-1	477	Q M R N K L NATEAF P DM Y D K V N K H V R E
GbDHCR24-2	480	I F R K K Y NATEAF P D V Y N K V S K S A R I

Figure S5: The alignment of GbDHCR24 showing the predicted phosphorylation sites. The phosphorylation sites predicted by NetPhos are shaded in blue. Identical amino acids at the same position are shaded in black, and similar amino acids at the same position are shaded in pale grey. The region matching the FAD binding domain on the Pfam database is indicated by the red line.