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The cholesterol metabolite cholest-4-en-3-one and its 3-oxo derivatives suppress body weight gain, body fat accumulation and serum lipid concentration in mice

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Abstract: Based on the findings that cholest-4-en-3-one, an intestinal metabolite of cholesterol, has an anti-obesity effect on animals, the structure-effect relationship of its 3-oxo derivatives and related compounds were investigated. Cholesten-3-ones, which possesses an enone structure with a carbonyl group at C3, markedly inhibit body weight gain and body fat accumulation, as well as the levels of serum triglyceride and cholesterol in animals without any clinical abnormalities.

Cholesterol is catabolized into various substances in the intestinal tract. There are two main pathways by which cholesterol catabolized by intestinal bacteria as follows[1]: (a) cholesterol → cholest-4-en-3-one **1** → 5β-cholestan-3-one (coprostanone) **14** → 5β-cholestan-3-ol (coprostanol) **23**; and (b) cholesterol → **1** → 5α-cholestan-3-one **13** → 5α-cholestan-3-ol (cholestanol) **22**. We studied the biological effects of intestinal catabolites of cholesterol and found that 0.5% dietary exposure to **1** markedly inhibited body fat accumulation in CDF1 mice without any anorexia or clinical abnormalities[2]. In consideration of the C3 carbonyl and olefinic groups in **1**, we designed a series of enones and related steroids as shown in Table 1, and investigated the structure-effect relationship of these compounds to body weight, abdominal fat weight and serum lipid concentrations in mice.

Experimental

Chemistry

1, cholest-5-en-3-one **2**, **13**, cholesta-5,7-dien-3β-ol **19**, 24-ethylcholest-5-en-3β-ol (β-sitosterol) **20**, 24-ethylcholesta-5,22-dien-3β-ol (stigmasterol) **21**, **22**, cholesteryl acetate **26**, cholesteryl benzoate **27**, cholesteryl pelargonate **28** and cholest-5-en **29** were obtained from Sigma Chemical Co., St. Louis and purified as required. Synthesis of cholesta-1,4-dien-3-one **3**, cholesta-4,6-dien-3-one **4**, cholesta-1,4,6-trien-3-one **5**, 4-bromocholest-4-en-3-one **6**, 4-phenylthiomethylcholest-4-en-3-one **7**, cholest-4-en-3,6-dione **8**, 6-hydroxycholest-4-en-3-one **9**, 6β-bromocholest-4-en-3-one **10**, **14**, 4,5β-epoxycholestan-3-one **15**, 5α,6β-dibromocholestan-3-one **16**, 5α,6β-dibromocholestan-3-one **17** and cholest-4-en-3-ol **18** from compound **1**, 24-ethylcholest-4-en-3-one **11** from **20**, 24-ethylcholesta-5,22-dien-3-one **12** from **21**, **23** from **14**, 3-acetoxycholesta-3,5-diene **24** and 3-(N-pyrrolidyl)cholesta-3,5-diene **25** from cholesterol was carried out by appropriate dehydrogenation, carboxylation, halogenation, hydroxylation, epoxidation or esterification. They were purified by silica gel chromatography and/or recrystallization.

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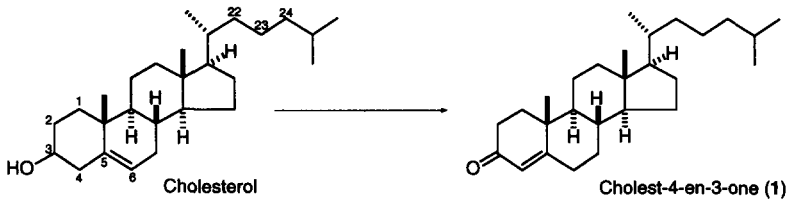
Table 1. Effect of cholesten-3-one derivatives and related compounds on body weight gain, feed consumption and feed efficiency of CDF1 mice



Compound	Substituent at C-3	Unsaturation in A ring	Unsaturation in B ring	Additional feature	Body weight gain (g/day/mouse)[a]	Feed consumption (g/day/mouse)[b]	Feed efficiency (*a*/*b*)
No chemical					0.57	5.92	0.10
1	O	Δ4			0.35	5.43	0.06
2	O	Δ5			-0.10	3.62	-0.03
3	O	Δ1,4			0.15	4.94	0.03
4	O	Δ4	Δ6		0.32	4.71	0.07
5	O	Δ1,4	Δ6		0.27	5.41	0.05
6	O	Δ4		4-Br	0.52	6.58	0.08
7	O	Δ4		4-$C_6H_5SCH_2$	0.56	6.23	0.09
8	O	Δ4		6=O	-0.36	6.00	-0.06
9	O	Δ4		6-OH(α:β=2:1)	0.28	5.70	0.05
10	O	Δ4		6β-Br	0.26	4.20	0.06
11	O	Δ4		24α-C_2H_5	0.40	4.94	0.08
12	O		Δ5	Δ22, 24α-C_2H_5	0.52	6.37	0.08
13	O			5α-H	0.50	5.39	0.09
14	O			5β-H	0.28	4.60	0.06
15	O			4, 5β-Epoxy	0.46	6.14	0.08
16	O			5α-OH, 6β-OH	0.58	5.39	0.11
17	O			5α-Br, 6β-Br	0.43	5.72	0.08
18	OH(α:β=1:6)				0.52	5.83	0.09
19	OH				0.58	6.48	0.09
20	OH			24α-C_2H_5	0.49	5.43	0.09
21	OH			Δ22, 24α-C_2H_5	0.57	6.30	0.09
22	OH			5α-H	0.55	6.10	0.09
23	OH			5β-H	0.38	5.45	0.07
24	CH_3COO	Δ3	Δ5		0.33	5.64	0.06
25	C_4H_8N	Δ3	Δ5		-0.18	6.10	-0.03
26	CH_3COO		Δ5		0.52	5.28	0.10
27	C_6H_5COO		Δ5		0.46	5.57	0.08
28	$C_8H_{17}COO$		Δ5		0.52	5.28	0.10
29			Δ5		0.56	6.95	0.08

Assay for the structure-effect relationship

Male CDF1 mice (BALB/c x DBA, Charles River Japan, Inc. Atsugi), 5 weeks of age, were divided into 17 and 14 groups of six mice each for experiments A and B, respectively. Mice were housed with 6 animals per cage and maitained at 24 ± 1°C and 50 ± 5% relative humidity with a 12h/12h light/dark schedule. Commercial purified feed (type modified AIN, Oriental Yeast Co., Tokyo; calories, 1,523 kj; 22.8% protein; 6.0% soy bean oil; 54.1% nitrogen-free extract; 22.8% fiber; 2.9% ash; 9.0% water) was used as the basal diet. The experimental diet was prepared by addition of test agents at 0.5% w/w to the basal diet and stored at 4°C until use. Animals were given the diet and water ad libitum. Clinical signs were observed every day and body weights were determined twice a week. Animal care and the experiments were carried out according to the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science. After feeding, the animals in each experiment were dissected, whole blood was collected from the vena cava for lipid measurements and organs were necropsied. Abdominal adipose tissue were removed surgically and weighed. The upper jejunum was collected and fixed in buffered formalin. The specimens were sectioned by freezing with a cryostat. Frozen sections were stained with Oil-red O for microscopic study. Serum (0.1 ml) was diluted with 10 volumes of 1M ethanolic KOH and hydrolyzed at 80°C for 1 hr, followed by extraction twice with 2 ml of n-hexane. Steroids were analyzed by GC using 5α-cholestan (Sigma) as an internal standard. GC was performed using GC-14ASC (Shimadzu Co., Kyoto) equipped with a flame ionization detector and TC-1 capillary column (corresponding to OV-1, 30 m x 0.25 mm., df: 0.25 mm, GL Science Inc., Tokyo). Chromatography conditions were as follows: carrier gas, helium; flow rate of carrier gas, 1 ml/min; split ratio, 1/50; column temperature, 293°C; and temperature of injection point and detector, 320°C. A GC-mass spectrometer (M-80, Hitachi Co., Tokyo) was used for identification of metabolites. Triglyceride was measured using a quantitation kit (Wako Pure Chem. Ind., Osaka).

Biological results

Marked inhibition of body weight was observed in animals fed compounds **1-5**, **8-10**, **14** and **25** (Table 1 & Fig. 1). In other words, body weight gain were inhibited by enones possessing a common 3-ketone and one or two double bonds in the A or B ring, except for compounds **14** and **25**. It was assumed that compound **25** showed their activity via **1** since cholestanol, a metabolite of **1**, was detected in the serum of each group. In the enone group, compounds **6** and **7** with a modified A ring, and compounds **11** and **12** with a modified side chain had no or only weak effects on body-weight inhibition. However, enone compounds with a modified B ring such as compounds **8-10** possessed such activity. Cholestan-3-ones without a double bond in the A and B rings, namely compounds **13** and **15-17**, had no effect. Compounds **18** to **29** without the carbonyl group at C3 also had no effect except for **24** and **25**. Among the active cholestan-3-ones, the only exception was **14** that has an A/B cis juncture and no double bond. Compound that possesses inhibitory effects on body weight gain showed a marked decrease both feed consumption and feed efficiency (**2-5**, **10** and **14**) or only feed efficiency (**1**, **5**, **8**, **9** and **25**)(Table 1). They are closely associated with decreases in the amounts of adipose tissue, triglyceride and/or total cholesterol concentrations in serum (Table 2). Compound **22** or **23** was detected in the serum in animals fed compound **1**, **4**, **10**, **13**, **14** or **23-25**. However, in animal fed compound **2**, **3**, **5**, **8**, **9** , **11**, **15** or **29** the test compounds and their metabolites were not detected in the serum.

In a microscopic examination of the villi of the jejunum, the resynthesis of lipid particles, followed by the formation of chylomicrons and their excretion into the central lacteal, was observed in untreated controls after feeding of the diet. Conversely, lipid particles were accumulated, but not well developed to form chylomicrons in the mucosal cells of the mice administered cholesten-3-ones with the diet.

All animals were healthy without any clinical abnormalities and had normal hair, skin, eyes and mucus; respiratory, alimentary and nervous systems, and activity and behavioral patterns. No obvious anomaly due to consumption of chemicals was found by necropsy.

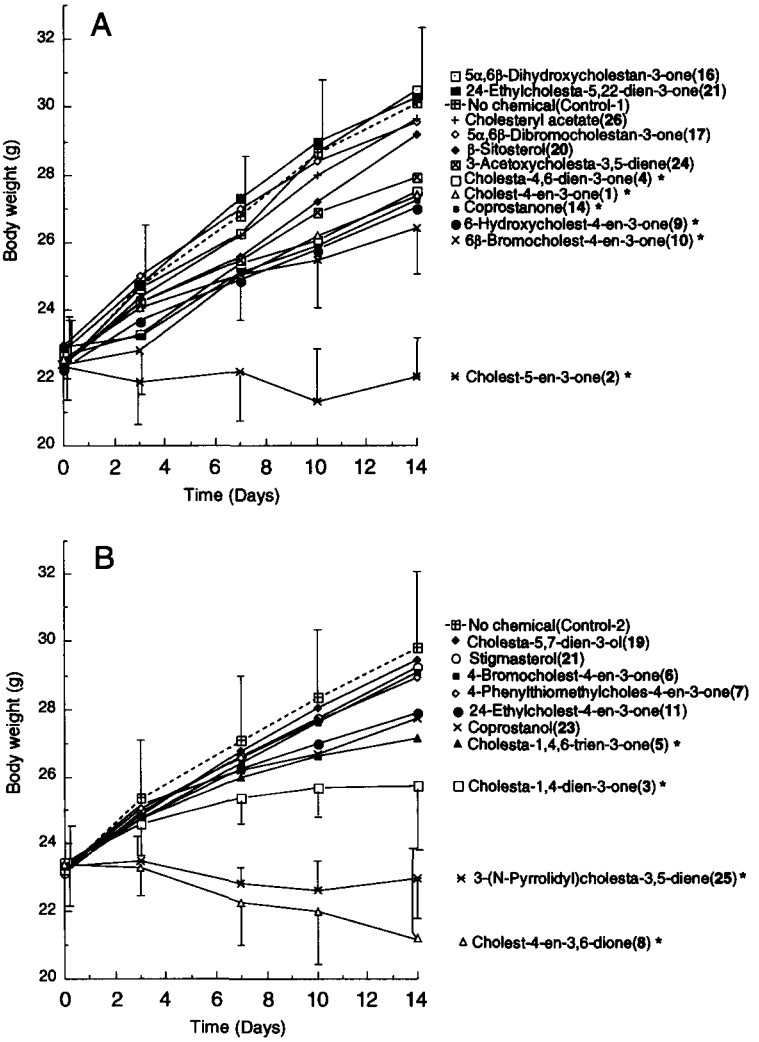


Fig.1. Time-course of body weight of CDF1 mice administered cholesten-3-one derivatives and related compounds. Each point represents the mean ± SD for 6 mice in each group. The SD is given only for compounds **2**, **3**, **8**, **25** and controls because the others overlapped. Growth curves of the groups given compounds **12**, **13**, **15**, **18**, **22** and **27-29** were very close to those of controls and are omitted from figures A and B to avoid overlapping. Data were subjected to statistical analysis of two-way ANOVA. Significantly different from the control values at * $p < 0.05$.

Table 2. Abdominal fat weight and serum lipid concentration of CDF1 mice administered cholsten-3-one derivatives and related compounds

Compound	Abdominal fat (g)	Serum cholesterol (mg/dl)	Serum triglyceride (mg/dl)	Serum metabolite (mg/dl)
No chemical	1.29 ± 0.09	174.5 ± 8.2	290.3 ± 23.7	0
1	0.68 ± 0.04 *	115.8 ± 8.6 *	218.3 ± 12.5	**22** (48.0 ± 3.6)
2	0.48 ± 0.07 *	122.1 ± 4.8 *	87.5 ± 8.0 *	0
3	0.61 ± 0.06 *	165.4 ± 12.8	266.9 ± 33.3	0
4	0.90 ± 0.06 *	112.7 ± 2.9 *	172.6 ± 7.9 *	**22** (34.6 ± 2.8)
5	–	206.3 ± 4.4 *	390.4 ± 30.3	0
8	0.30 ± 0.05 *	110.0 ± 1.2 *	133.6 ± 14.0 *	0
9	0.80 ± 0.07 *	136.7 ± 6.2 *	120.0 ± 9.2 *	0
10	0.81 ± 0.07 *	176.8 ± 8.5	257.3 ± 30.3	**22** (6.3 ± 1.6)
11	1.00 ± 0.11	164.6 ± 8.0	188.3 ± 17.5 *	0
13	1.25 ± 0.07	161.3 ± 6.3	291.2 ± 27.9	**22** (32.5 ± 1.3)
14	0.92 ± 0.08 *	134.1 ± 9.0 *	201.7 ± 14.6 *	**23** (23.1 ± 6.8)
15	1.12 ± 0.07	141.6 ± 2.4 *	218.0 ± 17.1	0
23	0.90 ± 0.08 *	148.1 ± 3.6	212.7 ± 25.7	**23** (12.7 ± 1.4)
24	1.03 ± 0.14	187.5 ± 9.3	119.9 ± 4.7 *	**22** (1.7 ± 0.1)
25	0.41 ± 0.04 *	158.1 ± 5.3	277.7 ± 32.8	**22** (27.3 ± 5.9)
29	–	165.8 ± 10.3	253.6 ± 26.0	0

No investigation was carried out on animals fed compound **6**, **7**, **12**, **16-22** or **26-28** which showed no inhibition of body weight gain. Values represent mean ± SEM (n = 6). Data were subjected to statistical analysis using one-way ANOVA. *, $p < 0.05$. –, not measured; 0, not detectable.

Discussion

In the study of the structure-effect relationship of cholesten-3-ones and related steroids, 10 of the 29 chemicals tested showed inhibitory effects on body weight gain, body fat accumulation and serum lipid concentration in animals. All active compounds except for compound **14** have an enone structure possessing a common 3-ketone and one or two double bonds in A or B ring. If the A ring was modified by another group, the activities were lost. From the above data, it appears that the mode of action of cholesten-3-one derivatives may be related to a twisted planar cyclic structure possessing a hydrophobic tail and low polarity of the head.

The microscopic observations indicate that cholesten-3-ones induced malabsorption of fat as a

consequence of inhibition of chylomicron formation. It is important to establish whether these compounds inhibit the activities of apolipoprotein B-48 and/or microsomal triglyceride transfer protein (MTP) which are essential factor for chylomicron assembly.

Since all animals were healthy without any clinical abnormalities, cholesten-3-ones appeared to have very weak toxicity in test animals. However, whether large doses or long-term administration of these compounds induce diarrhea or fat soluble vitamin deficiencies similar to the side effects of lipase inhibitors is still detected in the serum. If these metabolites accumulate in the tissue, some cumulative damage may be induced.[3] Compounds **2**, **3**, **5**, **8**, **9** and **11** may be nonabsorbable or completely decomposed in the body because these compounds and their metabolites were not detected in the serum. This explanation is supported by the fact that **1** induced hypertrophy of the adrenals which may be due to accumulation of **22** when it was administered in large doses to rats, although **2** did not induce such damage (data not shown).

The effect of cholesten-3-ones was also recognized in different species. When Watanabe heritable hiperlipidemic (WHHL)-rabbits[4,5] which are models of familial hyperlipidemia, dietary obesity Sprague-Dawley (SD) rats, and normal SD and Fischer F344 rats, were administered **1** or **2**, the animals showed significant inhibition of body weight gain and body fat accumulation as well as marked decreases of serum cholesterol and triglyceride.[6]

We clarified that cholesten-3-one steroids which possess enone structure with carbonyl group at position C3 markedly inhibits not only body weight gain and body fat accumulation but also amounts of cholesterol and trygliceride in serum without obvious clinical abnormalities. Chemical and physiological character of cholesten-3-one shows that these steroids are a new class of metabolic regulators of lipids. Particularly promising is **2** and **8**, whose physiological activity on lipids dynamics was strong, and which produced no degradation-resistant metabolite and gave low toxic effect on the general condition and internal organs. Further investigation is remained to clarify the mode of action of these compounds and screening agents which is more effective and less toxic. The pharmacology of these compounds on human may leads to the new therapeutic concept that dietary restriction is of limited significance in the therapy of obesity and hyperlipidemia.

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