

The Selective Estrogen Receptor Modulator Acolbifene Reduces Cholesterolemia Independently of Its Anorectic Action in Control and Cholesterol-Fed Rats¹

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ABSTRACT The cancer-preventing selective estrogen receptor modulator (SERM) acolbifene (ACOL) exerts a potent and pure antiestrogenic action in the mammary gland and uterus, yet it displays beneficial, estrogen-like actions on energy and lipid metabolism in rodents. The compound reduces food intake and strongly decreases cholesterolemia in rats fed a cholesterol-free diet. This study was designed to establish whether the anorectic effect of ACOL is involved in its cholesterol-lowering action, and whether the compound retains its ability to lower cholesterol concentrations in rats with diet-induced hypercholesterolemia. Female rats were fed a purified diet devoid of cholesterol (reference diet) or containing 2% cholesterol (C-diet); they were either not treated or treated daily with ACOL or not treated and pair-fed to the ACOL-treated rats. The C-diet did not affect food intake or weight and fat gains. ACOL reduced food intake (16%) and weight gain (45%, mainly fat) similarly in both dietary cohorts. ACOL, but not pair feeding, reduced cholesterolemia by 33% in rats fed the reference diet. As expected, the C-diet raised serum total cholesterol almost 3-fold and this increase was largely prevented by ACOL but not by pair feeding. Cholesterol was reduced by ACOL, mainly in the HDL fraction, in rats fed the reference diet, but only in the non-HDL fraction in those fed the C-diet. In livers of rats fed the reference diet, ACOL, but not pair feeding, increased protein abundance of the scavenger receptor, class B, type 1, and the LDL receptor, thought to be involved in ACOL-mediated cholesterol lowering. These findings demonstrate that the potent hypocholesterolemic action of ACOL is independent of the concomitant reduction in food intake and fat accretion, and that such action occurs in rats with overt diet-induced hypercholesterolemia. *J. Nutr.* 135: 2225–2229, 2005.

KEY WORDS: • *estrogen antagonist* • *diet-induced hypercholesterolemia* • *blood cholesterol*
• *scavenger receptor B1* • *LDL receptor*

There is increasing interest in the development of compounds that antagonize the effects of female sex steroids in the prevention and treatment of estrogen-dependent cancers. Several such compounds, including a variety of selective estrogen receptor modulators (SERMs),³ were developed in the past years; some of these compounds display, along with their strong antiestrogenic action in cancer-prone tissues, interesting estrogen-like actions on bone mineral density, body fat accretion, and the plasma lipid profile (1–5). Of particular interest in this context is acolbifene (ACOL, also known as EM-652), a 4th generation SERM with potent and pure anticarcinogenic properties in the mammary gland and uterus. ACOL was shown to reduce bone mineral mass loss and body fat accretion, as well as to improve the lipoprotein profile in rodents (6).

ACOL has consistently been found to dramatically reduce circulating cholesterol in rats (7–10). The fact that in these studies rats were fed a cholesterol-free diet strongly points to mechanisms related to hepatic cholesterol metabolism. Recently, we found (10) that ACOL upregulates the abundance of 2 key hepatic receptors responsible for a large fraction of cholesterol uptake by the liver: the scavenger receptor, class B, type 1 (SR-B1), which selectively takes up cholesterol from HDL (11–13) and plays a central role in reverse cholesterol transport; and the LDL receptor (LDLr), which internalizes mainly VLDL remnants, LDL particles, and apolipoprotein (apo)E-rich HDL (14–16). ACOL did not affect pathways of cholesterol synthesis (10), supporting the involvement of the clearance-related receptors in its hypocholesterolemic action.

As stated above, the hypocholesterolemic effect of ACOL was observed in rats fed a purified high carbohydrate diet virtually devoid of cholesterol (8–10). The downregulation of the LDLr by dietary cholesterol is well established (17,18), and a recent study demonstrated an identical effect of dietary cholesterol on the SR-B1 (19). These considerations raise the question whether ACOL retains its capacity to upregulate

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³ Abbreviations used: ACOL, acolbifene; apo, apolipoprotein; LDLr, LDL receptor; PMSF, phenylmethylsulfonyl fluoride; SERM, selective estrogen receptor modulator; SR-B1, scavenger receptor, class B, type 1.

these receptors and lower cholesterol in the presence of diet-induced hypercholesterolemia. In addition, estrogen, the SERM tamoxifen, and the steroidal antiestrogen ICI-182,780 affect whole-body energy balance (20–22). In rats, ACOL exerts an estrogen-like effect on food intake, which in turn reduces adipose tissue accretion (9). Although such negative energy balance is more likely to affect triacylglycerol rather than cholesterol metabolism, there is no a priori reason to reject the possibility that the anorectic effect of ACOL may participate in its hypocholesterolemic action.

The present study was designed to address both of these central issues related to the potency of ACOL to reduce cholesterolemia and to its mechanisms of action. Female rats were fed a purified diet without cholesterol or the same diet to which 2% cholesterol was added. Each of these 2 dietary cohorts was subjected to ACOL treatment, or alternatively to an imposed reduction in food intake matching that of the ACOL-treated rats. The paradigm allowed the assessment of the ability of ACOL to counteract the effects of dietary cholesterol as well as the contribution of changes in ingestive behavior to its hypocholesterolemic action.

MATERIALS AND METHODS

Animals and treatments. Female Sprague-Dawley rats ($n = 42$) initially weighing 175–200 g were purchased from Charles River Laboratories and housed individually in stainless steel cages in a room kept at $23 \pm 1^\circ\text{C}$ with a 12-h light:dark cycle (lights on at 1900 h). The rats were cared for and handled in conformity with the Canadian Guide for the Care and Use of Laboratory Animals, and the protocol was approved by our institutional animal care committee. The rats were acclimated to their environment for 1 wk and consumed tap water and a nonpurified rodent diet (Charles River Rodent Diet no. 5075, Ralston Products) ad libitum. Half of the rats were then fed an obesity- and hyperlipidemia-promoting purified diet to maximize the effect of ACOL treatment. The diet provided 50% of energy as carbohydrate, 30% as fat, and 20% as protein; its composition is given in Table 1. The other half of the rats were given the same diet to which 2% (wt:wt) cholesterol (ICN Biochemicals) was added. In each of the 2 dietary cohorts, 7 rats were assigned to the placebo (vehicle) group and another 7 rats were treated with ACOL. The compound was given once daily by oral gavage at a dose of 2.5 mg/kg body weight in a total volume of 0.5 mL of a 0.4% aqueous solution of methylcellulose. This dose of ACOL prevents tumor growth in rats (6). The remaining 7 rats in each dietary cohort were not treated and were pair-fed to the ACOL-treated group in the following manner: each rat was randomly paired with one of the ACOL-treated rats, and the amount of food provided was adjusted to the amount of food ingested the previous day by the ACOL-treated counterpart. To avoid intake of the restricted amount of food in 1 single large meal, two thirds of the food was provided to the pair-fed rats at the beginning of the dark active period, and the remaining third was given at the beginning of the light period. The placebo and pair-fed groups were administered the methylcellulose vehicle daily by gavage. Thus, there were 6 groups of 7 rats arranged in a 2×3 factorial design. Treatments were administered for 21 d. Food intake and body weight were monitored every day. Because a previous study showed that ACOL differentially affects SR-B1 abundance depending on nutritional status (10), food-deprived rats were studied. Hence, the day before the completion of the study, food was removed after the last vehicle or ACOL gavage at 2100 h, and rats were killed by decapitation the next day between 0900 and 1100 h.

Blood and tissue collection. At the time of killing, blood was collected from the neck wound and was immediately centrifuged at $1500 \times g$ at 4°C for 15 min. Serum was stored at -80°C for later biochemical measurements. The liver was excised and weighed, and a sample of liver was immediately frozen in liquid nitrogen and stored at -80°C for later determination of cholesterol content. Retroperitoneal and inguinal white adipose tissues were excised and weighed.

Serum and tissue measurements. The HDL fraction was isolated by precipitation of apoB-containing lipoproteins with sodium phos-

TABLE 1

Composition of the reference diet¹

Nutrient	Amount
	g/kg diet
Cornstarch	265
Sucrose	265
Soybean oil	40
Beef tallow	100
Casein	215
DL-Methionine	4
Vitamin mix ²	11
Mineral mix ³	50
Cellulose ⁴	50

¹ The C-diet had the same composition and was supplemented with 20 g/kg cholesterol.

² Teklad no. 40060 Vitamin Mix (Teklad Test Diets). Composition (g/kg mix): *p*-aminobenzoic acid, 11.0; ascorbic acid, coated (97.5%), 101.7; biotin, 0.04; vitamin B-12 (0.1% trituration in mannitol), 3.0; calcium pantothenate, 6.6; choline dihydrogen citrate, 349.7; folic acid, 0.2; inositol, 11.0; menadione, 5.0; niacin, 9.9; pyridoxine-HCl, 2.2; riboflavin, 2.2; thiamin-HCl, 2.2; dry vitamin A palmitate (500,000 U/g), 4.0; dry cholecalciferol (500,000 U/g), 0.4; dry vitamin E acetate (500 U/g), 24.2; cornstarch (carrier), 466.7.

³ AIN-93G Mineral Mix (ICN Biochemicals). Composition (g/kg mix): calcium carbonate, 357.0; potassium phosphate, monobasic, 196.0; potassium citrate, monohydrate, 70.8; sodium chloride, 74.0; potassium sulfate, 46.6; magnesium oxide, 24.3; ferric citrate, 6.1; zinc carbonate, 1.6; manganese carbonate, 0.6; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenate, 0.01; ammonium paramolybdate, 0.008; sodium meta-silicate, 1.4; chromium potassium sulfate, 0.3; lithium chloride, 0.02; boric acid, 0.08; sodium fluoride, 0.06; nickel carbonate, hydroxide, tetrahydrate, 0.03; ammonium vanadate, 0.007; sucrose, finely ground (carrier), 220.7.

⁴ Alphacel (ICN Biochemicals).

photungstate-magnesium chloride. Total and HDL cholesterol were quantified using a reagent kit (Total Cholesterol) from Wako Diagnostics. Non-HDL cholesterol was obtained by difference. Frozen liver samples were thawed; total lipids were extracted according to the method of Folch et al. (23), solubilized in isopropanol, and liver cholesterol concentration quantified in these lipid extracts using the above-mentioned reagent kits.

Western blot analysis of SR-B1 and LDLr protein. The liver content of these proteins was determined in liver extracts from rats fed the reference, cholesterol-free diet, after electrophoretic separation, by RIA using a commercially available anti-SR-B1 antibody (Novus Biologicals) and an anti-LDLr antibody kindly provided by Dr. Joachim Herz (University of Texas Southwestern Medical Center, Dallas, TX). To extract the SR-B1 protein, a liver sample (~50 mg) was homogenized in buffer A containing 20 mmol/L Tris-HCl (pH 7.5), 2 mmol/L MgCl₂, 0.2 mol/L sucrose, 5 mmol/L phenylmethylsulfonyl fluoride (PMSF), 10 μg/L leupeptin, 20 μg/L aprotinin, and 5 μg/L pepstatin A. The crude extract was centrifuged at $10,000 \times g$ for 10 min at 4°C , and the supernatant was then ultracentrifuged at $100,000 \times g$ for 45 min at 4°C . The membrane fraction was resuspended in buffer B containing 62.5 mmol/L Tris-HCl (pH 6.8), 2% (v:v) SDS, 10% (v:v) glycerol and 5% (v:v) β-mercaptoethanol. To extract the LDLr protein, a liver sample (~100 mg) was homogenized in 1 mL of buffer C containing 50 mmol/L Tris (pH 7.5), 2 mmol/L CaCl₂, 0.5% Triton X-100, and 1 mmol/L each of leupeptin, PMSF, and phenanthroline. The crude extract was centrifuged at $10,000 \times g$ for 10 min at 4°C and the supernatant was then ultracentrifuged at $100,000 \times g$ for 60 min at 4°C . The supernatant fractions were frozen at -80°C until further processing. Protein concentration of the liver extracts was determined by the method of Lowry et al. (24). SR-B1 (5 μg) and LDLr (10 μg) protein extracts per lane were loaded onto 7.5% polyacrylamide gels and separated by electrophoresis under nonreducing con-

ditions. The protein bands were transferred onto polyvinylidene fluoride membranes, exposed to 1:1500 anti-SR-B1 or 1:10,000 anti-LDLr, and revealed with 1:10,000 horseradish peroxidase-linked anti-rabbit IgG (Amersham Biosciences).

Statistical analysis. Data are expressed as means \pm SEM; except for SR-B1 and LDLr protein concentration, data were analyzed by factorial ANOVA with 2 factors: diet, with 2 levels (cholesterol-free, cholesterol 2%), and a factor termed "drug treatment" for convenience, with 3 levels (placebo, ACOL, pair-fed). Main and interactive effects of treatments were determined and are reported within ANOVA tables. Pairwise comparisons were also carried out to identify individual between-group differences using Fisher's post-hoc Least Significant Difference test. The liver SR-B1 and LDLr data, which were obtained from the cohort fed the reference diet, were analyzed by one-factor ANOVA. Differences reaching the confidence level of $P < 0.05$ were considered significant.

RESULTS

Consumption of the C-diet did not affect final body weight, weight gain, food intake, or adipose tissue weights (Table 2). ACOL had its expected anorectic effect (-16%), with a consequent decrease in body weight (-8%), weight gain (-41%), as well as white adipose tissue weights (-45%). Pair feeding untreated rats had the same effects on these variables as did treatment of rats with ACOL (Table 2). Differences in weight gain were largely explained by changes in white adipose mass ($r = 0.80$, $P < 0.0001$). Liver weight was increased by the C-diet, partly because of cholesterol accumulation (see below), whereas ACOL and pair feeding reduced liver weight in proportion to body weight. The C-diet vastly increased liver cholesterol concentration. In fact, in the placebo-treated groups, the C-diet increased liver total cholesterol content from 15 to 440 mg cholesterol, which explained 28% of the C-diet-induced increase in liver weight. Neither ACOL treatment nor pair feeding affected liver cholesterol concentration.

Diet and ACOL treatment had interactive effects on total and lipoprotein cholesterol, i.e., the drug did not exert the same effect, or did so but to a different extent, depending on diet (Fig. 1). As expected, the C-diet greatly increased (275%) serum total cholesterol concentration (Fig. 1A), which in turn was reduced by ACOL treatment, but relatively more so in the C-fed rats (-56%) than in those fed the reference diet (-33%). Pair feeding did not affect cholesterolemia. The C-diet reduced HDL cholesterol in placebo-treated and pair-fed rats to approximately one third of the level in rats fed the reference cholesterol-free diet, whereas HDL cholesterol was unaffected by diet in ACOL-treated rats (Fig. 1B). Conversely,

the C-diet dramatically increased non-HDL cholesterol (19-fold), and ACOL treatment, which did not affect rats fed the reference diet, dampened the diet-induced elevation in non-HDL cholesterol to less than half that in placebo-treated and pair-fed rats (Fig. 1C). In the cohort fed the reference diet, the ACOL-induced reduction in total cholesterol was due almost entirely to a reduction in HDL cholesterol, whereas the decrease in non-HDL cholesterol (Fig. 1C) totally explained the drug-induced decrease in cholesterol in C-fed rats.

To gain insight into the reasons why ACOL reduced cholesterolemia independently of its anorectic action, protein levels of SR-B1 and the LDLr were quantified in livers of rats fed the reference diet. ACOL, but not pair feeding, tended (+54%, $P = 0.1$) to increase liver SR-B1 concentration (Fig. 2A), and increased that of the LDLr by 170% ($P < 0.0001$; Fig. 2B). Serum total and HDL cholesterol concentrations, but not those of non-HDL cholesterol, were inversely correlated with those of liver SR-B1 ($r = 0.56$, $P < 0.02$, and $r = 0.70$, $P < 0.003$, respectively) and the LDLr ($r = 0.59$, $P < 0.005$, and $r = 0.65$, $P < 0.002$, respectively).

DISCUSSION

The 2 major goals of the present study were to assess whether the reduction in food intake elicited by treatment with the SERM ACOL plays a role in its hypocholesterolemic action, and whether the latter is maintained in diet-induced hypercholesterolemia. The findings demonstrate that ACOL reduces cholesterol independently of changes in food intake, and that the SERM maintains its hypocholesterolemic potential under cholesterol feeding.

The study confirms previous findings regarding the effects of ACOL on body weight, food intake, and fat deposition (8-10). The decrease in body weight, mainly in the form of fat stores, was associated with a reduction in food intake. The drug therefore shares some actions on energy and fat metabolism with natural estrogen and other antiestrogens (20,21,25-27).

In the cohort fed the cholesterol-free reference diet, the hypocholesterolemic action of ACOL described previously (7-10) was confirmed. Importantly, the 16% decrease in food intake did not contribute to the ACOL-induced reduction in cholesterolemia because the latter was identical in untreated rats that were pair-fed and those that consumed food ad libitum. ACOL therefore acts upon cholesterol metabolism through mechanisms that are independent of ingestive behavior and the effect of the latter on lipid metabolism. As ex-

TABLE 2

Body and tissue weights, food intake, and liver cholesterol concentration in rats fed a purified diet or the same diet containing 2% cholesterol, treated or not with acolbifene, or untreated and pair-fed to acolbifene-treated rats for 3 wk¹

	Purified diet			Purified + 2% Cholesterol diet			ANOVA		
	Placebo	Acolbifene	Pair-fed	Placebo	Acolbifene	Pair-fed	Diet	Drug	D \times D
Body weight, g	238 \pm 7	218 \pm 6*	212 \pm 4*	237 \pm 6	220 \pm 5*	215 \pm 3*	NS	0.0003	NS
Weight gain, g/21 d	49 \pm 5	27 \pm 3*	22 \pm 2*	49 \pm 5	31 \pm 6*	29 \pm 3*	NS	<0.0001	NS
Food, g/21 d	313 \pm 12	253 \pm 11*	259 \pm 7*	308 \pm 12	268 \pm 9*	273 \pm 3*	NS	<0.0001	NS
White adipose, g	3.2 \pm 0.3	1.7 \pm 0.1*	1.8 \pm 0.1*	2.7 \pm 0.2	1.4 \pm 0.2*	2.0 \pm 0.2*	NS	<0.0001	NS
Liver, g	6.9 \pm 0.3	5.4 \pm 0.2*	5.4 \pm 0.2*	8.5 \pm 0.4 [†]	7.4 \pm 0.3* [†]	6.9 \pm 0.2* [†]	<0.0001	<0.0001	NS
Liver cholesterol, μ mol/g	5.6 \pm 0.2	6.3 \pm 0.4	6.0 \pm 0.3	134 \pm 7 [†]	131 \pm 13 [†]	137 \pm 8 [†]	<0.0001	NS	NS

¹ Values are means \pm SEM, $n = 6-7$ rats. * Different from placebo group; [†] different from corresponding drug treatment group fed the purified diet.

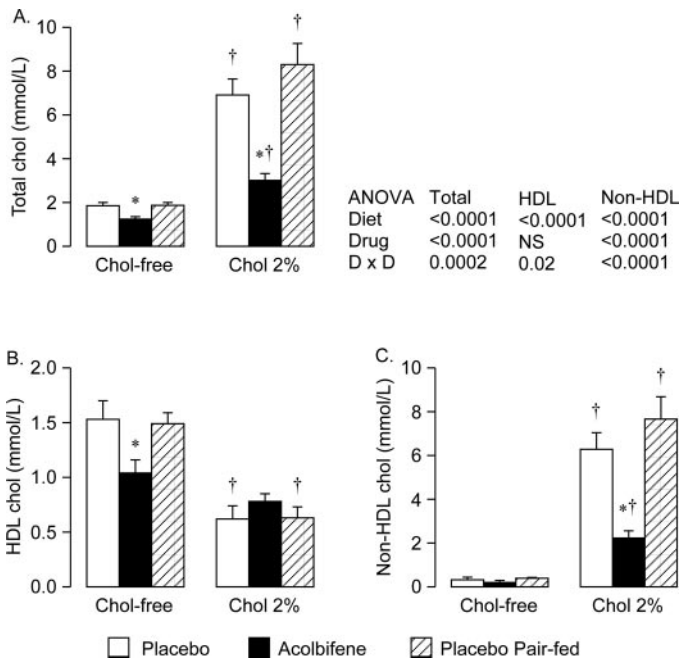


FIGURE 1 Serum total (A), HDL- (B), and non-HDL (C) cholesterol concentrations in rats fed a purified diet or the same diet containing 2% cholesterol, treated or not with acolbifene, or untreated and pair-fed to acolbifene-treated rats for 3 wk. Bars represent the means \pm SEM, $n = 6-7$ rats. *Different from the placebo group; †different from the corresponding drug treatment group fed the purified diet.

pected, because HDL transports more than two thirds of circulating cholesterol in rats fed a cholesterol-free diet, its decrease explained most of the hypocholesterolemic effect of ACOL in rats fed the reference diet.

Further confirming a previous study (10), cholesterol lowering in rats fed the reference diet was associated with a tendency for increased hepatic SR-B1 ($P = 0.1$) and a robust elevation in LDLr protein concentrations. In these rats, most of the reduction in cholesterol occurred in the HDL fraction, which correlated strongly with both liver SR-B1 and LDLr concentrations. The ACOL-induced increase in SR-B1 protein levels was more variable and somewhat less robust than in our previous study (10); this may have been due to the differing lipid composition of the diets because dietary lipids were shown to modulate SR-B1 expression (28,29). The LDLr may have contributed to the small, nonsignificant reduction in the non-HDL cholesterol fraction, as well as to the clearance of apoE-containing HDL. Interestingly, pharmacologic amounts of natural estrogen (mostly estradiol) strongly upregulate liver LDLr expression, which explains most of its hypocholesterolemic effect (30–35); at the same time, however, the hormone completely blunts SR-B1 expression (19,36,37), in clear contrast to ACOL. The reasons why ACOL exerts either pro- or antiestrogenic actions in a target-dependent fashion are likely linked to conformational determinants of the ligand-receptor complex that differentially affect the recruitment of coactivators and corepressors (6). Although the involvement of the SR-B1 and LDLr in the cholesterol-lowering action of ACOL awaits direct confirmation, a mechanism of action related to the clearance of plasma cholesterol is highly likely because ACOL does not seem to affect hepatic cholesterol synthesis (10) and acts efficiently even in the absence of dietary cholesterol. It should be noted that in the present study, ACOL did not affect liver cholesterol concentration,

strongly suggesting that the liver was able to dispose of the additional cholesterol cleared from the circulation.

The cholesterol-enriched diet brought about the expected shift in cholesterol lipoprotein distribution, the bulk of which was found in the non-HDL fraction, along with a decrease in the absolute amount of cholesterol transported by HDL. Dietary cholesterol exerts these actions by favoring the hepatic assembly and secretion of cholesterol-enriched VLDL particles, with a concomitant increase in intravascular intermediate density lipoprotein formation, and by decreasing HDL formation through modulation of apolipoprotein production (38–40). Cholesterol feeding also lowers the liver abundance of the SR-B1 and LDLr (19,41). Remarkably, in the present study ACOL was able to blunt by approximately two thirds the 3-fold increase in cholesterolemia brought by cholesterol feeding, again independently of its effect on food intake. As expected, the non-HDL fraction explained all of the ACOL-induced cholesterol lowering in this cholesterol-fed cohort. It should be mentioned that this fraction also contains some cholesterol-rich HDL; however, the contribution of the latter to the cholesterol content of the fraction termed non-HDL is minimal (39). Because livers from C-diet-fed rats were unavailable for receptor quantitation, it remains to be demonstrated whether ACOL can prevent the reduction in SR-B1 and LDLr that would be expected with cholesterol feeding. Similarly, whether the hypocholesterolemic action of ACOL in diet-induced hypercholesterolemia is exerted through the same pathways as in normolipemic rats is unknown. Preliminary studies suggest that ACOL may modulate the expression of the cholesterol transporters ABCG5/8 (C. Lemieux and Y. Deshaies, unpublished observations), which may in turn affect its intestinal absorption, a contributor of potential importance under cholesterol feeding conditions. Further investigations of these major modulators of cholesterolemia are therefore clearly warranted to identify the precise mechanisms of the hypocholesterolemic action of ACOL in cholesterol-fed rats.

The consequences of the HDL cholesterol-lowering action of ACOL in rats fed a cholesterol-free diet must be considered in the context of the fundamental differences that exist between human and rodent lipoprotein metabolism. As stated above, HDL is the major cholesterol carrier in rats, and a robust lowering of total cholesterol seldom occurs without a decrease in this fraction. Gene manipulation studies point to clearly beneficial consequences of high SR-B1 and reverse cholesterol transport activities (42–44). Therefore, in the context of human physiology, the ACOL-mediated tendency

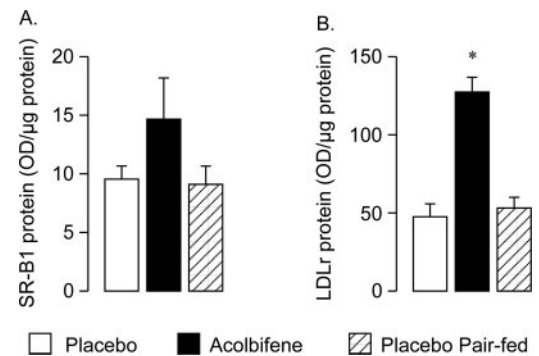


FIGURE 2 Liver concentration of SR-B1 (A) and LDLr (B) protein in rats fed a purified diet without cholesterol, treated or not with acolbifene, or untreated and pair-fed to acolbifene-treated rats for 3 wk. Bars represent the means \pm SEM, $n = 5-7$ rats. *Different from the placebo group, $P < 0.05$.

to increase liver SR-B1 abundance, along with the increase in the LDLr, would both be considered to be positive effects in terms of cardiovascular risk associated with lipoprotein metabolism.

In summary, the present findings demonstrate that ACOL exerts its potent hypocholesterolemic action independently of the concomitant reduction in food intake and body fat accretion, possibly because the compound increases the abundance of key liver lipoprotein receptors that are not affected by a reduction in food intake equivalent to that elicited by the compound. The study also highlights the potency of ACOL as a hypocholesterolemic agent, because its action is largely maintained in rats with severe diet-induced hypercholesterolemia.

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LITERATURE CITED

- Reckless, J., Metcalfe, J. C. & Grainger, D. J. (1997) Tamoxifen decreases cholesterol sevenfold and abolishes lipid lesion development in apolipoprotein E knockout mice. *Circulation* 95: 1542–1548.
- Nuttall, M. E., Bradbeer, J. N., Stroup, G. B., Nadeau, D. P., Hoffman, S. J., Zhao, H., Rehm, S. & Gowen, M. (1998) Iloxifene: a novel selective estrogen receptor modulator prevents bone loss and lowers cholesterol levels in ovariectomized rats and decreases uterine weight in intact rats. *Endocrinology* 139: 5224–5234.
- Shibata, J., Toko, T., Saito, H., Lykkesfeldt, A. E., Fujioka, A., Sato, K., Hashimoto, A., Wierzba, K. & Yamada, Y. (2000) Estrogen agonistic/antagonistic effects of miproxifene phosphate (TAT-59). *Cancer Chemother. Pharmacol.* 45: 133–141.
- Clarkson, T. B., Anthony, M. S. & Wagner, J. D. (2001) A comparison of tibolone and conjugated equine estrogens effects on coronary artery atherosclerosis and bone density of postmenopausal monkeys. *J. Clin. Endocrinol. Metab.* 86: 5396–5404.
- Sbarouni, E., Flevari, P., Kroupis, C., Kyriakides, Z. S., Koniavitou, K. & Kremastinos, D. T. (2003) The effects of raloxifene and simvastatin on plasma lipids and endothelium. *Cardiovasc. Drugs Ther.* 17: 319–323.
- Labrie, F., Labrie, C., Bélanger, A., Simard, J., Gauthier, S., Luu-The, V., Mérand, Y., Giguère, V., Candas, B., et al. (1999) EM-652 (SCH 57068), a third generation SERM acting as pure antiestrogen in the mammary gland and endometrium. *J. Steroid Biochem. Mol. Biol.* 69: 51–84.
- Luo, S., Sourla, A., Labrie, C., Bélanger, A. & Labrie, F. (1997) Combined effects of dehydroepiandrosterone and EM-800 on bone mass, serum lipids, and the development of dimethylbenz(A)anthracene-induced mammary carcinoma in the rat. *Endocrinology* 138: 4435–4444.
- Picard, F., Deshaies, Y., Lalonde, J., Samson, P., Labrie, C., Bélanger, A., Labrie, F. & Richard, D. (2000) Effects of the estrogen antagonist EM-652.HCl on energy balance and lipid metabolism in ovariectomized rats. *Int. J. Obes. Relat. Metab. Disord.* 24: 830–840.
- Lemieux, C., Picard, F., Labrie, F., Richard, D. & Deshaies, Y. (2003) The estrogen antagonist EM-652 and dehydroepiandrosterone prevent diet- and ovariectomy-induced obesity. *Obes. Res.* 11: 477–490.
- Lemieux, C., Gélinas, Y., Lalonde, J., Labrie, F., Cianflone, K. & Deshaies, Y. (2005) The hypolipidemic action of the selective estrogen receptor modulator Acolbifene is associated with decreased liver MTP and increased SR-B1 and LDL receptors. *J. Lipid Res.* 46: 1285–1294.
- Ji, Y., Jian, B., Wang, N., Sun, Y., Moya, M. L., Phillips, M. C., Rothblat, G. H., Swaney, J. B. & Tall, A. R. (1997) Scavenger receptor BI promotes high density lipoprotein-mediated cellular cholesterol efflux. *J. Biol. Chem.* 272: 20982–20985.
- Stangl, H., Hyatt, M. & Hobbs, H. H. (1999) Transport of lipids from high and low density lipoproteins via scavenger receptor-BI. *J. Biol. Chem.* 274: 32692–32698.
- Gu, X., Kozarsky, K. & Krieger, M. (2000) Scavenger receptor class B, type I-mediated [³H]cholesterol efflux to high and low density lipoproteins is dependent on lipoprotein binding to the receptor. *J. Biol. Chem.* 275: 29993–30001.
- Seo, T., Al-Haideri, M., Treskova, E., Worgall, T. S., Kako, Y., Goldberg, I. J. & Deckelbaum, R. J. (2000) Lipoprotein lipase-mediated selective uptake from low density lipoprotein requires cell surface proteoglycans and is independent of scavenger receptor class B type 1. *J. Biol. Chem.* 275: 30355–30362.
- Strickland, D. K., Gonias, S. L. & Argraves, W. S. (2002) Diverse roles for the LDL receptor family. *Trends Endocrinol. Metab.* 13: 66–74.
- Nimpf, J. & Schneider, W. J. (2000) From cholesterol transport to signal transduction: low density lipoprotein receptor, very low density lipoprotein receptor, and apolipoprotein E receptor-2. *Biochim. Biophys. Acta* 1529: 287–298.
- Horton, J. D. & Shimomura, I. (1999) Sterol regulatory element-binding proteins: activators of cholesterol and fatty acid biosynthesis. *Curr. Opin. Lipidol.* 10: 143–150.
- Edwards, P. A., Tabor, D., Kast, H. R. & Venkateswaran, A. (2000) Regulation of gene expression by SREBP and SCAP. *Biochim. Biophys. Acta* 1529: 103–113.
- Fluiter, K., van der Westhuijzen, D. R. & van Berkel, T. J. (1998) In vivo regulation of scavenger receptor BI and the selective uptake of high density lipoprotein cholesteryl esters in rat liver parenchymal and Kupffer cells. *J. Biol. Chem.* 273: 8434–8438.
- Wade, G. N. & Heller, H. W. (1993) Tamoxifen mimics the effects of estradiol on food intake, body weight, and body composition in rats. *Am. J. Physiol.* 264: R1219–R1223.
- Wade, G. N., Blaustein, J. D., Gray, J. M. & Meredith, J. M. (1993) ICI-182,780: a pure antiestrogen that affects behaviors and energy balance in rats without acting in the brain. *Am. J. Physiol.* 265: R1392–R1398.
- Wade, G. N., Powers, J. B., Blaustein, J. D. & Green, D. E. (1993) ICI 182,780 antagonizes the effects of estradiol on estrous behavior and energy balance in Syrian hamsters. *Am. J. Physiol.* 265: R1399–R1403.
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497–509.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275.
- Gray, J. M., Schrock, S. & Bishop, M. (1993) Estrogens and antiestrogens: actions and interactions with fluphenazine on food intake and body weight in rats. *Am. J. Physiol.* 264: R1214–R1218.
- Sato, M., Rippey, M. K. & Bryant, H. U. (1996) Raloxifene, tamoxifen, nafoxidine, or estrogen effects on reproductive and nonreproductive tissues in ovariectomized rats. *FASEB J.* 10: 905–912.
- Ke, H. Z., Paralkar, V. M., Grasser, W. A., Crawford, D. T., Qi, H., Simmons, H. A., Pirie, C. M., Chidsey-Frink, K. L., Owen, T. A. et al. (1998) Effects of CP-336,156, a new, nonsteroidal estrogen agonist/antagonist, on bone, serum cholesterol, uterus and body composition in rat models. *Endocrinology* 139: 2068–2076.
- Dorfman, S. E., Wang, S., Vega-Lopez, S., Jauhainen, M. & Lichtenstein, A. H. (2005) Dietary fatty acids and cholesterol differentially modulate HDL cholesterol metabolism in Golden-Syrian hamsters. *J. Nutr.* 135: 492–498.
- Loison, C., Mendy, F., Serougne, C. & Lutton, C. (2002) Increasing amounts of dietary myristic acid modify the plasma cholesterol level and hepatic mass of scavenger receptor BI without affecting bile acid biosynthesis in hamsters. *Reprod. Nutr. Dev.* 42: 101–114.
- Kovanen, P. T., Brown, M. S. & Goldstein, J. L. (1979) Increased binding of low density lipoprotein to liver membranes from rats treated with 17 alpha-ethinyl estradiol. *J. Biol. Chem.* 254: 11367–11373.
- Chao, Y. S., Windler, E. E., Chen, G. C. & Havel, R. J. (1979) Hepatic catabolism of rat and human lipoproteins in rats treated with 17 alpha-ethinyl estradiol. *J. Biol. Chem.* 254: 11360–11366.
- Windler, E. E., Kovanen, P. T., Chao, Y. S., Brown, M. S., Havel, R. J. & Goldstein, J. L. (1980) The estradiol-stimulated lipoprotein receptor of rat liver. A binding site that membrane mediates the uptake of rat lipoproteins containing apoproteins B and E. *J. Biol. Chem.* 255: 10464–10471.
- Erickson, S. K., Jaeckle, S., Lear, S. R., Brady, S. M. & Havel, R. J. (1989) Regulation of hepatic cholesterol and lipoprotein metabolism in ethinyl estradiol-treated rats. *J. Lipid Res.* 30: 1763–1771.
- Di Croce, L., Bruscalupi, G. & Trentalunga, A. (1996) Independent behavior of rat liver LDL receptor and HMGCoA reductase under estrogen treatment. *Biochem. Biophys. Res. Commun.* 224: 345–350.
- Bertolotti, M. & Spady, D. K. (1996) Effect of hypocholesterolemic doses of 17 alpha-ethinyl estradiol on cholesterol balance in liver and extrahepatic tissues. *J. Lipid Res.* 37: 1812–1822.
- Landschulz, K. T., Pathak, R. K., Rigotti, A., Krieger, M. & Hobbs, H. H. (1996) Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. *J. Clin. Invest.* 98: 984–995.
- Graf, G. A., Roswell, K. L. & Smart, E. J. (2001) 17 α -Estradiol promotes the up-regulation of SR-BI in HepG2 cells and in rat livers. *J. Lipid Res.* 42: 1444–1449.
- Krause, B. R. & Newton, R. S. (1985) Apolipoprotein changes associated with the plasma lipid-regulating activity of gemfibrozil in cholesterol-fed rats. *J. Lipid Res.* 26: 940–949.
- DeLamatre, J. G. & Roheim, P. S. (1981) Effect of cholesterol feeding on apo B and apo E concentrations and distributions in euthyroid and hypothyroid rats. *J. Lipid Res.* 22: 297–306.
- Fungwe, T. V., Cagen, L., Wilcox, H. G. & Heimberg, M. (1992) Regulation of hepatic secretion of very low density lipoprotein by dietary cholesterol. *J. Lipid Res.* 33: 179–191.
- Lopez, D. & Ness, G. C. (1997) Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase unmask transcriptional regulation of hepatic low-density lipoprotein receptor gene expression by dietary cholesterol. *Arch. Biochem. Biophys.* 344: 215–219.
- Krieger, M. (1999) Charting the fate of the “good cholesterol”: identification and characterization of the high-density lipoprotein receptor SR-BI. *Annu. Rev. Biochem.* 68: 523–558.
- Trigatti, B. L., Krieger, M. & Rigotti, A. (2003) Influence of the HDL receptor SR-BI on lipoprotein metabolism and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 23: 1732–1738.
- Rigotti, A., Miettinen, H. E. & Krieger, M. (2003) The role of the high-density lipoprotein receptor SR-BI in the lipid metabolism of endocrine and other tissues. *Endocr. Rev.* 24: 357–387.