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Estrogen-induced gallstone formation in males. Relation to changes in serum and biliary lipids during hormonal treatment of prostatic carcinoma.

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Research Article

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Estrogen-induced Gallstone Formation in Males

Relation to Changes in Serum and Biliary Lipids during Hormonal Treatment of Prostatic Carcinoma

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Abstract

To assess if and by which mechanisms pharmacological estrogen treatment induces gallstone disease, we examined patients with recently diagnosed prostatic cancer randomly allocated to estrogen therapy (n = 37) or orchidectomy (n = 35). According to gallbladder ultrasonography, after 1 yr new gallstones had developed in 5 of 28 estrogen-treated patients, compared with 0 of 26 orchidectomized patients (P = 0.03).

Estrogen therapy for 3 mo increased the relative concentration of cholesterol and cholesterol saturation of bile by ~ 30% (n = 10). Serum LDL cholesterol was reduced by ~ 40%, and its relative change related inversely to that of bile cholesterol (R_s = −0.77). There were no changes in biliary or serum lipids after orchidectomy (n = 9).

Secretion rates of biliary lipids were measured with a duodenal perfusion technique. Patients on chronic estrogen therapy (n = 5) had ~ 40% higher biliary excretion rates of cholesterol than age-matched controls (n = 7). Phospholipid secretion was also higher, but no difference in bile acid secretion was found.

We conclude that an increased hepatic secretion of cholesterol results in increased cholesterol saturation of bile and an enhanced rate of gallstone formation during estrogen treatment. The changes in bile cholesterol seem to be related to the induced changes in serum lipoprotein metabolism.

Introduction

Cholesterol gallstones occur more frequently in women than in men in every population examined (1, 2). This difference between the sexes begins during puberty and continues through the fertile years, focusing attention on the effects of female sex hormones. Estrogens have been hypothesized to be one important factor in the formation of gallstones. The frequency of symptomatic gallstone disease is increased during therapy with exogenous estrogens in most (3–5), but not all (6), clinical studies. As silent gallstone disease is frequent, a correct prospective investigation of whether estrogen therapy induces gallstone formation requires information on pretreatment gallbladder status; such data are lacking in all previous studies.

The gallbladder bile of patients with cholesterol gallstones is generally supersaturated with cholesterol; i.e., it contains more cholesterol than can be solubilized by the available bile acids and phospholipids (7). Nucleation and growth of cholesterol crystals are additionally required for the actual formation of gallstones (8–10), but supersaturated bile is a necessary metabolic prerequisite for the development of cholesterol gallstones. Estrogen therapy (in combination with progestins) in women has been associated with an increase in bile cholesterol and bile cholesterol saturation (11, 12). The metabolic abnormalities underlying supersaturation of bile are not yet fully understood (1, 10). Abnormalities of serum lipoprotein metabolism are known to affect biliary cholesterol secretion (13), and considerable effects are induced in the composition of serum lipoproteins during estrogen therapy (14). Parallel determinations of serum and biliary lipids in response to estrogen treatment may thus serve as a model for studying their mechanistic interrelationships.

Antiandrogenic therapy with either exogenous estrogens or orchidectomy is currently the routine treatment of prostate cancer (15). The effects of exogenous estrogens in pharmacological doses can be expected to be more clear-cut in this situation compared with treatment of postmenopausal women. We therefore decided to examine the development of gallstone disease in men with prostate cancer randomized to estrogen therapy or orchidectomy. In addition, we determined the biliary lipid composition and cholesterol saturation of gallbladder bile in the two groups, and we have related the changes induced to those observed in serum lipoprotein pattern. Finally, to further understand the influence of estrogen therapy on biliary lipid metabolism, we investigated its effect on the secretion rates of biliary lipids.

Methods

Subjects. We studied 72 men with recently diagnosed prostatic cancer suitable for hormonal treatment as judged by a senior urologist. Patients with a history or signs of previous thromboembolism, cerebrovascular lesion, myocardial infarction, severe angina pectoris, severe intermittent claudication, or congestive heart failure were excluded. After informed consent the patients were randomly allocated either to orchidectomy (n = 35) or estrogen therapy (n = 37). Estrogen was given as polyestradiol phosphate, 160 mg i.m. monthly for the first 3 mo, followed by 80 mg i.m. every month. In addition to this, the patients were given ethinylestradiol, 1 mg per os daily for the first 2 wk and then 150 μg every day. This dosage is the lowest one recommended in Sweden for the treatment of prostatic cancer. The ethical aspects of the study were approved by the Ethical Committee at Huddinge University Hospital (29 September 1980).

Experimental procedure. Ultrasonography was performed before and after 1 yr of treatment in all patients except those with a history of cholecystectomy. The lipid composition (cholesterol, bile acids, and phospholipids) of stimulated fasting duodenal bile was determined in a subsample of 19 gallstone-free patients before and after 3 mo of therapy. 10 of these patients were in the estrogen group and 9 in the orchidectomy group. A detailed analysis of serum lipoproteins was performed simultaneously.

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In five patients with stable disease during long-term therapy (≥ 3 yr) with estrogen, and in seven male controls matched for age and weight, biliary lipid secretion rates were studied using a duodenal perfusion technique (see below).

Materials. 3 alpha-hydroxy steroid dehydrogenase (Sterognost) and cholesterol oxidase (Nyco-test cholesterol) were obtained from Nyegaard A/S, Oslo, Norway. Cholecystokinin was purchased from AB Kabi, Stockholm, Sweden.

Ultrasonography. All patients were examined after a 6-h fast. A Mark 500 sector scanner (Advanced Technology Laboratories) with a 3.5 MHz transducer was used. This system has a resolution of 1 mm and an accuracy of±2 mm per 140 mm. The patients were investigated in the left lateral decubitus and supine positions both horizontally and with the head elevated 45°. Longitudinal as well as transversal sections were obtained for measuring the thickness of the gallbladder wall as well as the size and number of gallstones. All examinations were performed, without knowledge of treatment, by the same experienced diagnostic radiologist according to the techniques described by Lawson (16) and Foster and McLaughlin (17).

Bile collection. Duodenal bile samples were obtained through a thin polyvinyl tube in the morning after an overnight fast. Gallbladder contraction was stimulated by intravenous injection of cholecystokinin, and ~ 5 ml of dark concentrated gallbladder bile was usually obtained. All samples of dilute bile were discarded. The bile was collected on ice and immediately transported to the laboratory for analysis.

Lipid analysis. Total serum cholesterol and triglyceride were determined with standard enzymatic techniques. HDL cholesterol was determined after precipitation with heparin-$MnCl_2$ and the concentration of LDL cholesterol estimated according to the formula of Friedewald (18).

To measure cholesterol and phospholipid concentration in the bile samples, an aliquot was immediately extracted with 20 vol of chloroform-methanol, 2:1 (vol/vol). Cholesterol was measured by an enzymatic method (19), and phospholipids were measured by the method of Rouser et al. (20). A 3 alpha-hydroxysteroid dehydrogenase assay was used to determine the total bile acid concentration (21). Carey's method was used to calculate cholesterol saturation assuming a total lipid content of 10 g/dl (22). Individual bile acids were analyzed by gas-liquid chromatography of a hydrolyzed bile sample (23).

Biliary lipid secretion. After an overnight fast a 3-lumen polyvinyl tube was positioned in the duodenum under fluoroscopic control. The infusion port was positioned at the ampulla of Vater, and the two collection ports 2 and 12 cm distally. A constant infusion of a glucose–amino acid infusion containing bromsulphalein as an unabsorbable marker was given through the proximal port at a rate of 5 ml/min for 8–10 h. After an equilibration period of 3–4 h aspirates (10 ml/h) were collected through the two distal ports using an infusion pump (Harvard Apparatus Co. Inc., South Natick, MA). The concentrations of cholesterol and bromsulphalein were determined in the samples from the distal port, and those of cholesterol, bile acids, and phospholipids in the samples collected from the proximal port. The secretion rates of cholesterol, bile acids, and phospholipids were calculated and expressed as micromoles of lipid secreted per hour. Details of this procedure have been given previously (24, 25).

Statistical analysis. The Wilcoxon signed rank test was used to test changes posttreatment compared with baseline. The comparisons between the estrogen and orchidectomy groups were done with the Wilcoxon-Mann-Whitney rank test or Fisher's exact test of probability. Correlations between biliary cholesterol and the different blood lipid fractions were evaluated with the Spearman rank correlation test. All data are presented as means±SEM.

Results

Gallstone formation. As shown in Table I, the randomization procedure was successful in that the estrogen group and the orchidectomy group were comparable with regard to baseline clinical data and serum lipids. There were two subjects in each group who had type II diabetes (treated with diet), and there was no evidence of renal or thyroid disease in any of the patients. Also, the distribution of elevated serum lipids was similar in the two groups (not shown). Before treatment, gallbladder disease had afflicted a quarter of the patients equally distributed between the estrogen and orchidectomy groups. There was a similar weight gain (mean, 1.5 kg) in the two groups. No biochemical evidence of cholestasis (e.g., elevations of alkaline phosphatase or bile acids in serum) was seen in any of the patients during the first year. After 1 yr of therapy with estrogen or after orchidectomy five patients without gallstones before treatment in the estrogen group developed new gallstones (Table II). No patient in the orchidectomy group produced new gallstones ($P = 0.03$, Fisher's exact test). Four patients in the estrogen group had increased number or size of gallstones compared with one in the orchidectomy group. One patient in the orchidectomy group had decreased gallstone size 1 yr after surgery. Thus, the comparison after 1 yr of treatment showed that nine patients in the estrogen group had progressive gallstone disease (defined as newly developed stones or stones clearly increased in number or size) compared with one in the orchidectomy group ($P = 0.01$, Fisher's exact test).

Table I. Basal Clinical Data and Gallbladder Status before Estrogen Treatment or Orchidectomy (Mean±SEM)

	Estrogen (n = 37)	Orchidectomy (n = 35)
Age (*yr*)	68±1	69±1
Body weight (*kg*)	75±2	74±2
Height (*cm*)	174±1	174±1
Relative body weight (%)*	101±2	100±3
Serum cholesterol (*mmol/liter*)	6.1±0.2	6.0±0.2
Serum HDL-cholesterol (*mmol/liter*)	1.3±0.1	1.3±0.1
Serum LDL-cholesterol (*mmol/liter*)	3.9±0.2	3.9±0.2
Serum triglycerides (*mmol/liter*)	1.8±0.1	1.6±0.1
Cholecystectomized	4 (11%)	5 (14%)
Gallstones	5 (14%)	4 (11%)
Gallbladder disease	9 (24%)	9 (26%)

To convert values in millimoles/liter to milligrams/deciliter, multiply cholesterol by 38.7 and triglycerides by 88.5.
* Calculated as body weight (kilograms)/(height [centimeters] − 100 × 100%).

Table II. Gallstone Formation and Progression as Assessed by Ultrasonography of the Gallbladder before and after 1 yr of Treatment in Patients without Previous Cholecystectomy (No. of Subjects)

	Estrogen group	Orchidectomy group	P*
New gallstones	5/28	0/26	0.03
Increased size or number of gallstones	4/5	1/4	

* Fisher's exact test.

Table III. Basal Clinical Data and Serum Lipid and Lipoprotein Concentrations before and 3 mo after Onset of Therapy in Patients Where Biliary Lipids Were Analyzed (Means±SEM)

Study group	Age	Relative body weight*		Serum concentration			
				Cholesterol	Triglycerides	HDL cholesterol	LDL cholesterol
	yr	%		*mmol/liter*			
Estrogen (n = 10)	66±1	103±4	Before	6.2±0.4	1.5±0.3	1.4±0.1	4.1±0.3
			During	5.4±0.2‡§	1.9±0.2	2.0±0.2‡‖	2.5±0.3§¶
Orchidectomy (n = 9)	66±2	103±3	Before	6.7±0.5	1.6±0.3	1.4±0.1	4.6±0.4
			After	7.0±0.4	1.6±0.3	1.5±0.1	4.8±0.4

To convert values in millimoles/liter to milligrams/deciliter, multiply cholesterol by 38.7 and triglycerides by 88.5. * Calculated as body weight (kilograms)/(height [centimeters] − 100) × 100%. ‡ $P < 0.01$ compared with orchidectomy group. § $P < 0.01$ compared with pretreatment value. ‖ $P < 0.002$ compared with pretreatment value. ¶ $P < 0.001$ compared with orchidectomy group.

Serum lipid analysis. There were no differences between the two subgroups with regard to baseline clinical data including serum lipids (Table III). The total serum cholesterol decreased during estrogen treatment compared with both baseline ($P < 0.01$) and the orchidectomy group ($P < 0.01$). The HDL cholesterol increased by 42% ($P < 0.002$), whereas the LDL cholesterol level decreased by 39% ($P < 0.01$). Neither total serum cholesterol, HDL cholesterol, nor LDL cholesterol differed significantly after surgical castration compared with baseline. The serum triglycerides did not change significantly in either group.

Biliary lipid analysis. As shown in Table IV and Fig. 1, the relative concentration of cholesterol as well as the cholesterol saturation of bile increased significantly during estrogen treatment ($P < 0.01$). Estrogen therapy induced no changes in bile acid or phospholipid concentration. There were no changes in any of the biliary lipids after orchidectomy. A comparison between the estrogen and orchidectomy groups during treatment showed a significantly higher relative bile concentration of cholesterol in the estrogen group, 7.2±0.9 M%, compared with 5.2±0.6 in the orchidectomy group ($P < 0.05$). The cholesterol saturation in the bile during estrogen treatment (110%) was significantly higher than in the orchidectomy group ($P < 0.05$). The proportion of chenodeoxycholic acid was lower in the estrogen-treated group (Table IV).

Correlation between serum and biliary lipids. There were significant correlations in the estrogen-treated group between the relative change in bile cholesterol saturation and LDL cholesterol ($R_s = -0.64$, $P < 0.05$) and between the relative change in bile cholesterol and LDL cholesterol ($R_s = -0.77$, $P < 0.01$). Concomitant with this, there was a significant correlation between the relative change in bile cholesterol and serum triglycerides ($R_s = 0.58$, $P < 0.05$). No significant correlation between the relative change in LDL cholesterol and serum triglycerides was found and no relation between the relative change in bile cholesterol and HDL cholesterol was seen ($R_s = 0.10$, NS). The changes in biliary bile acid composition were not related to those in cholesterol saturation.

Biliary lipid secretion rates. To further explore the possible causes for the increased cholesterol saturation of bile during estrogen therapy, we performed detailed studies of biliary secretion rates of lipids in a subset of hormone-treated males (Table V). Compared with age- and weight-matched male controls, the patients on long-term estrogen displayed ~ 40% higher biliary excretion rates of cholesterol. The secretion rate of phospholipids was also increased, whereas there was no difference in bile acid secretion. Of particular importance is the fact that there was no tendency to a decreased bile acid secretion in the estrogen-treated patients (Table V).

Discussion

The prevalence of gallstone disease before treatment (~ 25%) in our study of men with prostatic cancer was of expected magnitude. Thus, Lindström (26) reported a prevalence of ~ 27% in 60–69-yr-old males at autopsy. Already after 1 yr of treatment a significantly higher incidence of formation of new gallstones (~ 18%) and progressive gallstone disease (i.e., new gallstones or increased size or number of gallstones) was found in the estrogen group compared with the orchidectomy group. The findings suggest a rather dramatic increase in gallstone formation during estrogen treatment in males. This confirms and extends previous, nonprospective studies (3–5) and dis-

Table IV. Results of Biliary Lipid Analysis before and 3 mo after Onset of Therapy (Means±SEM)

	Study group			
	Estrogen (n = 10)		Orchidectomy (n = 9)	
	Before	During	Before	After
Biliary lipid classes				
Cholesterol (M%)	5.4±0.6	7.2±0.9*‡	5.6±0.6	5.2±0.6
Bile acids (M%)	74.7±1.5	71.2±2.0	73.3±1.8	75.9±2.7
Phospholipids (M%)	19.8±1.3	21.6±1.9	21.2±1.3	18.9±2.2
Cholesterol saturation (%)	80±9	110±11*‡	80±7	80±7
Biliary bile acid composition§				
Cholic acid (%)	39±4	43±5	35±4	36±3
Chenodeoxycholic acid (%)	36±4	29±2‡	41±3	43±2
Deoxycholic acid (%)	25±4	28±5	25±6	21±5

* $P < 0.01$ compared with pretreatment value.
‡ $P < 0.05$ compared with orchidectomy group.
§ Trace amounts (< 1%) of lithocholic acid and ursodeoxycholic acid were observed.

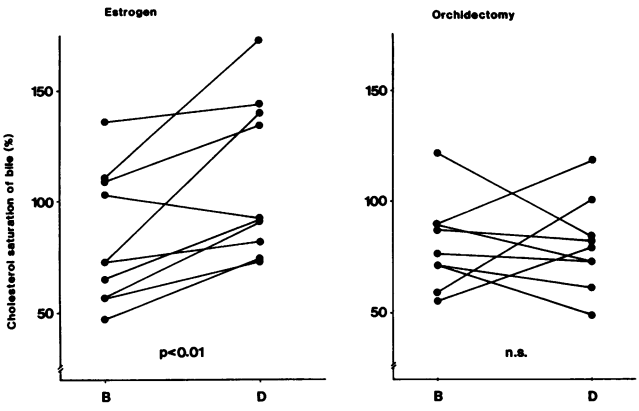

Figure 1. Cholesterol saturation of stimulated fasting duodenal bile before (*B*) and during (*D*) therapy; individual data.

agrees with the doubts that have recently been raised concerning estrogen therapy and gallstone disease (6).

We also demonstrated a clear increase in the cholesterol saturation of bile in the estrogen-treated group. This is in agreement with previous data on both females (11, 12) and males (27), and provides an explanation of the increased frequency of gallstones during estrogen therapy. It is important to note that these effects relate to treatment with exogenous estrogens, whereas no influence of endogenous estrogens on biliary lipids has been demonstrated (25, 28). In addition to the clear-cut changes in biliary lipids, other (additive) effects on gallstone formation may have been induced by the pharmacological doses of estrogen used in the present study. Such mechanisms may hypothetically influence the rate of nucleation of cholesterol crystals, gallbladder emptying, etc. At least in women, however, very limited effects on gallbladder motility are seen during treatment with estrogens (29).

The present study clearly demonstrates that the effect of exogenous estrogens on biliary lipid composition is the consequence of an increased secretion of cholesterol, and not of a decreased secretion of bile acids (Table V). Although a prospective trial was not possible, the difference between the estrogen-treated group and the well-matched controls was very distinct. This finding is in agreement with preliminary studies of various estrogen compounds in females (30). An interesting observation was the clear increase in phospholipid secretion, which may indicate that the secretion of the two lipid classes (cholesterol and phospholipids) is stimulated in a consonant way. A similar pattern in lipid secretion has recently been reported to occur during treatment with estrogens in a hamster model (31).

The observed decrease in the relative concentration of chenodeoxycholic acid in bile during estrogen therapy is in agreement with previous studies using contraceptive steroids (12, 32). In the latter situation, the explanation for the reduced percentage of chenodeoxycholic acid appears to be a simultaneous stimulation of cholic acid synthesis and repression of chenodeoxycholic acid formation (32). Although chenodeoxycholic acid feeding may induce unsaturation of gallbladder bile in humans (33, 34), it is unlikely that the reduction of this bile acid observed in the present study during estrogen therapy has any direct influence on biliary cholesterol saturation. Thus, there was no relationship between the change in bile acid composition and the increase in biliary cholesterol.

Concomitant with the changes in biliary cholesterol saturation, there were drastic increases of HDL and decreases of LDL cholesterol levels during estrogen therapy. Those changes in serum lipid composition are consistent with previous reports concerning estrogen therapy in men (35). Of particular interest are the negative correlations observed between the changes in LDL cholesterol and those in bile cholesterol and cholesterol saturation. The liver is a key organ in the regulation of LDL concentration (36). The catabolism of LDL is highly dependent on the expression of specific LDL receptors in the liver, and animal studies have indicated that this receptor capacity is increased during treatment with exogenous estrogen (37, 38). We have recently been able to demonstrate that the catabolism of LDL is also increased during estrogen therapy in man (39). It can thus be hypothesized that the increased inflow of lipoprotein cholesterol is a major determinant for the increased cholesterol concentration in bile in this situation. A similar hypothesis has been advanced from studies in estrogen-treated rats (40). It is of particular interest to consider the magnitude of changes in cholesterol excretion that are induced by estrogen therapy. From the difference between treated patients and matched controls (Table V) it can be estimated that an additional ~ 650 μmol (250 mg) of cholesterol

Table V. Biliary Lipid Secretion Rates in Patients on Estrogen Treatment

Patient No.	Treatment duration	Age	Weight	Relative body weight	Plasma cholesterol		Plasma triglycerides	Secretion rate		
					Total	HDL		Cholesterol	Bile acids	Phospholipids
	yr		*kg*	*%*		*mmol/liter*			*μmol/h*	
1	3	55	70	95	4.7	1.8	1.8	81	1,503	475
2	5	66	60	83	7.0	2.7	1.3	99	2,306	436
3	3	68	76	112	5.3	2.2	1.0	140	1,810	513
4	4	70	79	99	6.0	2.6	1.7	87	2,038	428
5	3	72	81	119	6.3	2.1	2.9	85	877	282
Mean±SEM		66±3	73±3	102±6	5.9±0.4	2.3±0.1	1.7±0.3	98±10*	1,707±220	427±35‡
Controls (n = 7)		64±3	80±2	101±3	5.2±0.2	ND	1.1±0.1	71±2	1,274±182	272±37
Range		54–71	72–87	87–108	4.4–6.0		0.9–1.4	65–78	830–2,279	133–435

* Significantly different from controls, $P < 0.005$; ‡ $P < 0.05$.

are secreted each day during estrogen therapy. From the data of Eriksson et al. (39) it can be calculated that an additional ~ 520 μmol (200 mg) of LDL cholesterol are cleared from the circulation in patients given a similar treatment regimen. Thus, particularly if some reabsorption of biliary cholesterol occurs (as is most probable), the increased catabolism of LDL cholesterol can fully explain the increased cholesterol secretion into bile during estrogen therapy. The stimulated LDL cholesterol uptake would in turn have to be explained by an increased cholesterol production, either in extrahepatic organs or in the liver (cf. reference 39).

A relation between HDL cholesterol and bile cholesterol saturation (41) and an association between reduced HDL cholesterol concentration and gallbladder disease (42) have been reported. We did not, however, find any relation between changes in bile and HDL cholesterol. Instead, we observed a significant correlation between the change in serum triglycerides and bile cholesterol. This is of great interest because it is known that hypertriglyceridemia is associated with supersaturated bile and an increase in the prevalence of cholesterol gallstones (43, 44).

In summary, we found an increase in gallstone formation during treatment with exogenous estrogen in males. This results from an enhanced saturation of fasting gallbladder bile with cholesterol, which is explained by an increased biliary secretion of cholesterol. Concomitant with this change there is a reduction in LDL cholesterol that correlates with the increase in bile cholesterol and bile cholesterol saturation. There are no changes in bile lipid composition or serum lipoprotein levels after orchidectomy, and there is no indication of gallstone formation. The present study provides further evidence, besides the risk of cardiovascular disease (45), in favor of orchidectomy in the treatment of prostatic carcinoma.

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