Serum squalene and noncholesterol sterols before and after delivery in normal and cholestatic pregnancy

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Abstract Mechanisms of hyperlipidemia were studied by measurement of serum lipid concentrations and the ratios of cholesterol precursors (squalene, Δ^8-cholestenol, desmosterol, and lathosterol), plant sterols (campesterol and sitosterol), and cholestanol (a 5α-derivative of cholesterol) to cholesterol in nonpregnant women, and normal and cholestatic pregnancies near term, and a few days and 6 weeks after delivery. The ratios of the precursors are known to reflect cholesterol synthesis, those of plant sterols and cholestanol the absorption efficiency and biliary sterol secretion of cholesterol. In normal pregnancy, increased serum cholesterol was associated with up to 2-fold increases in squalene, desmosterol, and lathosterol proportions, and the values remained elevated, especially for desmosterol, during the lactation period. These findings suggest that pregnancy and lactation are associated with increased cholesterol synthesis. The proportions of plant sterols were slightly lower, but that of cholestanol was 2-fold that of the nonpregnant women. In contrast to the latter group, the cholestanol proportions were not related to those of plant sterols or the campesterol/sitosterol ratio. The values, especially of cholestanol, became normal during lactation. In cholestatic pregnancy the changes were basically similar, but the serum values of Δ^8-cholestenol increased more, and those of squalene, desmosterol and lathosterol less markedly, and the mean cholestanol proportion was 40% higher and the campesterol/sitosterol ratio 15% lower than in the normal pregnancy. Cholestanol was positively related to serum bilirubin and bile acids in cholestatic pregnancy, yet only one-third of the cholestatic pregnant women exhibited cholestanol values higher than in the healthy pregnant women.—**Nikkilä, K., S. Riikonen, M. Lindfors, and T. A. Miettinen.** Serum squalene and noncholesterol sterols before and after delivery in normal and cholestatic pregnancy. *J. Lipid Res.* 1996. **37:** 2687–2695.

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Normal pregnancy causes marked hyperlipidemia, mainly due to elevation of cholesterol and triglycerides in VLDL and LDL lipoproteins, whereas the rise in HDL is somewhat inconsistent (1–4, for review 5). Intrahepatic cholestasis of pregnancy, originally described in Swedish women in 1954 (6), is characterized by even higher serum levels of cholesterol and triglycerides (7). The pathogenesis of intrahepatic cholestasis of pregnancy is unknown, but several observations relate it to the effects of estrogens (8, 9). The main functional alteration is the inhibition of canalicular bile secretion (10). Impaired biliary cholesterol secretion obviously potentiates the hyperlipidemia caused by pregnancy so that the lipoprotein pattern in cholestasis of pregnancy resembles that found in cholestasis in general (11, 12).

In addition to cholesterol, normal serum contains small amounts of several other sterols that reflect cholesterol synthesis, sterol absorption, and liver function (13–19). For example, squalene and other cholesterol precursors, including Δ^8-cholestenol, desmosterol, and lathosterol, reflect hepatic cholesterol synthesis in many clinical conditions, whereas dietary plant sterols, such as sitosterol and campesterol, reflect intestinal cholesterol absorption efficiency. Cholestanol, a 5α-saturated derivative of cholesterol, directly reflects intestinal sterol absorption as well, in the same manner as plant sterols; serum values of cholestanol, similarly to those of plant sterols, are inversely related to cholesterol synthesis and thus to cholesterol precursors in serum. Markedly high serum levels of cholestanol and decreases in the campesterol/sitosterol ratio have been verified in various liver diseases with prolonged cholestasis, especially in primary biliary cirrhosis (PBC) (17–20). Although extensive studies on serum lipids in pregnancy exist, no similar investigations are available on noncholesterol sterols. To this end we considered that the squalene and

Abbreviations: ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high density lipoprotein; LDL, low density lipoprotein; PBC, primary biliary cirrhosis; VLDL, very low density lipoprotein.

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noncholesterol sterols might offer interesting information on cholesterol synthesis and absorption in women before and after delivery in uncomplicated pregnancy as compared to the situation of healthy nonpregnant women. Assuming that cholestanol and the sitosterol/campesterol ratio could distinguish women with cholestasis of pregnancy from those with normal pregnancy, serum squalene (the final nonsterol precursor of cholesterol) and noncholesterol sterols were quantitated in serum of normal nonpregnant women, and also before and after delivery in women with normal and cholestatic pregnancy.

SUBJECTS AND METHODS

Subjects

The present series comprised 40 subjects, including 28 patients with cholestasis of pregnancy, 13 healthy pregnant women, and a control group of 9 nonpregnant women. Some of their clinical data appear in **Table 1.**

The patients with cholestasis of pregnancy were from the Jorvi Hospital (n = 23) and from the Helsinki City Maternity Hospital (n = 5). Cholestasis of pregnancy, the inclusion criterion for the entry into the study, was defined by the symptom of itching, up to the time of delivery without dermatological disease, and by serum levels of bile acids above 6 μmol/l. The cholestatic patients were successive cases with no history of gallstones from our obstetrical department. They entered the prospective study during the third trimester of pregnancy and were followed for the present purposes for at least 6 weeks after delivery. The patients were on a standard hospital diet, with about 50% of the calories as carbohydrates, 35% as fat, and 150 mg/day of cholesterol.

The group of 13 healthy women with uncomplicated pregnancy without itching and normal serum bile acids (<6 μmol/l) were also successive cases from Jorvi and Helsinki City Maternity Hospitals. The group was age-matched with the cholestatic group. The mothers with a normal pregnancy were hospitalized for only a few days so that their pre-delivery dietary period in particular was mainly based on their home diet. The control group of 9 nonpregnant healthy nursing students were slightly younger than the pregnant groups (Table 1), and were receiving no medical treatment or estrogens. These control subjects continued with their regular dietary habits, their food originating mainly from the hospital kitchen so that its composition was generally similar to that of the pregnant women.

The ethics committees of Jorvi and Helsinki City Maternity Hospitals accepted the study protocols, and patients gave their informed consent to participate in the study.

Methods

Venous blood was collected once before delivery, and then 1 to 3 days and 6 weeks after delivery when mothers were again on their home diets. To avoid diurnal variation in serum sterol levels, each blood sampling occurred after a 10-h overnight fast and at the same hour in the morning (21). Serum was immediately separated by centrifugation at low speed and was stored at −20°C until analyzed.

TABLE 1. Clinical data for women with normal pregnancy (n = 13), women with cholestasis of pregnancy (n = 28) 1–7 days before delivery, and healthy nonpregnant women (n = 9)

Variable	Healthy nonpregnant women	Healthy pregnant women	Women with cholestasis of pregnancy
Age, years	25.3 ± 1.8	30.7 ± 0.9[a]	30.1 ± 1.0[a]
Bile acids, μmol/L	ND < 6	4.2 ± 0.5	31.3 ± 5.8[c]
Bilirubin, μmol/L	10.8 ± 2.1	5.4 ± 0.4	15.5 ± 2.8[c]
Alkaline phosphatase, U/L	139.3 ± 10.8	308.6 ± 26.1[a]	581.7 ± 31.0[b,d]
Aspartate aminotransferase, U/L	21.6 ± 1.6	20.8 ± 1.1	121.0 ± 17.0[b,d]
Alanine aminotransferase, U/L	11.0 ± 1.4	14.3 ± 1.6	201.9 ± 28.0[b,d]
Triglycerides, mmol/L			
Before delivery	0.72 ± 0.08	2.50 ± 0.38[b]	3.86 ± 0.27[b,d]
Post-delivery, 1–3 days		2.02 ± 0.20[b]	2.23 ± 0.24[b]
Post-delivery, 6 weeks		1.08 ± 0.17	0.91 ± 0.08
Cholesterol, mmol/L			
Before delivery	4.36 ± 0.09	6.96 ± 0.44[b]	8.02 ± 0.35[b]
Post-delivery, 1–3 days		5.61 ± 0.37	6.90 ± 0.27[b,c]
Post-delivery, 6 weeks		5.33 ± 0.27[a]	6.07 ± 0.16[b,d]
HDL-cholesterol, mmol/L			
Before delivery	1.40 ± 0.08	1.74 ± 0.12[a]	1.07 ± 0.06[a,d]

Values are reported as means ± SEM; ND, not determined.
[a] $P < 0.05$, [b] $P < 0.01$, compared with healthy nonpregnant women.
[c] $P < 0.05$, [d] $P < 0.01$, [e] $P < 0.001$, compared with healthy pregnant women.

Serum total triglycerides, and high density lipoprotein (HDL)-cholesterol after precipitation of apoB-containing lipoproteins with heparin-manganese, were measured with commercial kits from Boehringer Mannheim GmbH Diagnostica (Mannheim, Germany). Serum cholesterol, squalene, demethylated cholesterol precursor sterols (Δ^8-cholestenol, desmosterol, and lathosterol), plant sterols (campesterol and sitosterol), and cholestanol were analyzed from nonsaponifiable serum material by the gas–liquid chromatographic method on the 50 m-long SE-30 capillary column as described earlier (22–24).

Noncholesterol sterols in serum are transported by lipoproteins, mainly by low density lipoprotein (LDL) in a mixture of cholesterol. As pregnancy is known to increase serum lipids and lipoprotein, mainly LDL and very low density lipoprotein (VLDL) levels (1–5), it can be expected to increase noncholesterol sterol levels proportionally. To correct for differences in serum levels of different lipoprotein particles, the squalene and noncholesterol sterol values are here expressed in terms of $10^2 \times$ mmol/mol of cholesterol. These values are given subsequently in the text, when not otherwise mentioned, by the terms "proportions" or "ratios" to cholesterol rather than by concentrations in terms of μmol/l. Proportions clearly better reflect changes in synthesis and absorption of cholesterol than do the serum concentrations of these minor sterols and squalene (21, 25), e.g., plasma apheresis reduces serum cholesterol in familial hypercholesterolemia by several mmol/l within a few hours. The removal of LDL also dramatically lowers serum concentrations of squalene and noncholesterol sterols but their ratio to cholesterol remains unchanged because cholesterol synthesis or absorption are unchanged.

The routine automated methods of the Jorvi and Helsinki City Maternity Hospital laboratories served to estimate serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin. For the quantitation of total bile acids in serum, a commercial, colorimetric enzymatic method (Enzabile®, Nyegaard Diagnostica, Oslo, Norway) was used.

Exclusion of extrahepatic cholestasis was performed by ultrasound. The gallbladder and the liver were examined by ultrasound in the pregnant women during the postpartum period. Gallstones were detected in 8 of 28 women with a preceding cholestatic pregnancy, but in none of the noncholestatic pregnancies. None of the gallstones detected had caused cholestasis at the time of the ultrasound examination. One of the cholestatic patients underwent cholecystectomy before pregnancy. The patients with gallstones had not suffered abdominal-pain attacks.

The data were analyzed with BMDP programs (26). ANOVA (BMDP program 7D) was performed to assess the significance of the subgroup differences. In addition, the significance of the differences in the variables between the two groups and between repeated measurements within groups were evaluated with the non-parametric Mann-Whitney rank-sum test and the non-parametric Wilcoxon signed-rank-sum test (BMDP program 3D), respectively. Because of the lower age of the nonpregnant control group, comparisons between all groups were analyzed also using a stepwise regression method (BMDP program 2R), adjusted for age. The Pearson correlation coefficients and bivariate (scatter) plots were computed with BMDP programs 8D and 6D, respectively. All calculations are expressed as means ± SE.

RESULTS

In the patients with cholestasis of pregnancy, the clinical course was benign without adverse perinatal outcomes. Before delivery, their serum bile acid concentrations were about eight times as high as in the healthy pregnant group (Table 1). A minor proportion of the patients, 6 of 28 patients (**Fig. 1**), showed hyperbilirubinemia at prepartum, ranging from 24 to 55 μmol/l. At 6 post-delivery weeks all of their bile acid and bilirubin values were normal.

Alkaline phosphatase and transaminase activities were highest in the cholestatic group (Table 1). Alkaline phosphatase activity was higher also in the normal pregnancy group than in the nonpregnant controls

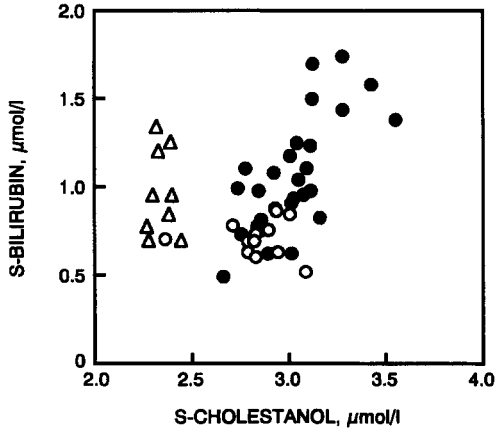

Fig. 1. Interrelationship between serum bilirubin and concentration of cholestanol; (△) nonpregnant controls (n = 9, $r = -0.042$); (○) normal pregnancy (n = 13, $r = 0.019$); (●) cholestasis of pregnancy (n = 28, $r = 0.591$). Note log values in figure for bilirubin and cholestanol concentrations.

TABLE 2. Serum noncholesterol sterols in healthy nonpregnant women, in healthy pregnant women, and in women with cholestasis of pregnancy

Variable	Healthy Nonpregnant Women (n = 9)	1–7 Days before Delivery: Healthy Pregnant Women (n = 13)	1–7 Days before Delivery: Women with Cholestasis of Pregnancy (n = 28)	1–3 Days after Delivery: Healthy Pregnant Women (n = 13)	1–3 Days after Delivery: Cholestasis of Pregnancy (n = 28)	6 Weeks after Delivery: Healthy Pregnant Women (n = 13)	6 Weeks after Delivery: Cholestasis of Pregnancy (n = 25)
Squalene	12.8 ± 0.9	26.6 ± 3.8[a]	13.0 ± 1.5[a]	25.5 ± 2.5[a]	9.6 ± 1.4[b]	25.2 ± 3.6[a]	8.20 ± 1.5[b]
	(21.2 ± 1.8)	(67.9 ± 8.5)[a]	(39.9 ± 5.1)[ab]	(51.9 ± 4.7)[a]	(24.6 ± 3.8)[b]	(48.7 ± 6.7)[a]	(18.6 ± 3.1)[b]
Δ^8-Cholestenol	15.7 ± 1.2	12.9 ± 1.5	22.8 ± 1.2[ab]	10.9 ± 1.8	24.1 ± 1.7[ab]	14.6 ± 1.9	27.4 ± 1.5[ab]
	(25.9 ± 2.1)	(34.0 ± 4.6)	(68.2 ± 3.9)[ab]	(22.8 ± 3.9)	(62.4 ± 4.5)[ab]	(30.3 ± 4.9)	(62.7 ± 3.7)[ab]
Desmosterol	43.2 ± 2.5	86.9 ± 6.9[a]	75.0 ± 3.8[a]	71.3 ± 4.7[a]	72.2 ± 5.1[a]	120 ± 12.1[a]	99.6 ± 6.1[a]
	(71.6 ± 5.0)	(234 ± 27.5)[a]	(227 ± 14.2)[a]	(152 ± 15.7)[a]	(187 ± 13.6)[a]	(239 ± 26.2)[a]	(227 ± 14.0)[a]
Lathosterol	83.5 ± 10.6	173 ± 15.1[a]	137 ± 7.70[ab]	154 ± 14.4[a]	131 ± 10.1[a]	153 ± 18.3[a]	105 ± 6.1[a]
	(139 ± 18.8)	(465 ± 57.1)[a]	(419 ± 32.1)[a]	(321 ± 35.9)[a]	(344 ± 32.3)[a]	(306 ± 42.7)[a]	(238 ± 14.2)[a]
Cholestanol	137 ± 7.4	277 ± 18.0[a]	388 ± 38.0[a]	256 ± 15.8[a]	275 ± 26.0[a]	150 ± 5.7	132 ± 6.0[b]
	(224 ± 10.6)	(739 ± 68.1)[a]	(1173 ± 127)[b]	(551 ± 51.1)[a]	(706 ± 66.0)[a]	(299 ± 16.1)[a]	(303 ± 16.4)[a]
Campesterol	298 ± 36.0	203 ± 17.8[a]	169 ± 13.1[a]	192 ± 17.5[a]	146 ± 10.7[a]	230 ± 23.5	195 ± 13.9[a]
	(485 ± 50.5)	(533 ± 57.6)	(528 ± 53.4)	(410 ± 45.0)	(385 ± 34.0)	(465 ± 57.0)	(448 ± 36.3)
Sitosterol	202 ± 18.2	139 ± 10.5[a]	140 ± 11.3[a]	127 ± 10.1[a]	120 ± 9.6[a]	146 ± 12.6[a]	140 ± 8.9[a]
	(330 ± 23.6)	(365 ± 35.9)	(432 ± 40.9)	(275 ± 30.4)	(312 ± 25.4)	(294 ± 29.1)	(320 ± 21.2)
Campesterol/ sitosterol	1.46 ± 0.07	1.47 ± 0.08	1.24 ± 0.05[ab]	1.52 ± 0.08	1.27 ± 0.06[b]	1.60 ± 0.09	1.40 ± 0.05

Values are given as means ± SEM. For each of the noncholesterol sterols, the values in the first line are in $10^2 \times$ mmol/mol; the values in the second line (in parentheses) are in μmol/l.

[a] $P < 0.05$ compared with healthy nonpregnant women.

[b] $P < 0.05$ compared with healthy pregnant women; analysis of variance.

(Table 1). At 6 weeks the alkaline phosphatase activity of the cholestasis group (249 ± 15 U/l) remained even higher when compared to the respective enzyme activity of the healthy mothers (177 ± 16 U/l, $P < 0.01$).

Serum cholesterol and triglycerides

Before delivery the mean serum total cholesterol and triglyceride concentrations were clearly higher in both pregnancy groups than in the control group (Table 1). In addition, serum triglyceride concentrations were significantly higher ($P < 0.01$) in the cholestasis group than in the normal pregnancy group (Table 1). Triglyceride values fell significantly within a few post-delivery days, and reached normal after 6 postpartum weeks. The cholesterol values also decreased in both pregnancy groups, but at 6 weeks the levels were still higher than for the controls, especially regarding the cholestasis group. Before delivery, HDL cholesterol was higher in the normal pregnant group and lower in the cholestatic group than any levels in the nonpregnant controls (Table 1).

Squalene and noncholesterol sterols

Normal pregnancy and early lactation. The proportions of squalene and noncholesterol sterols to cholesterol and their concentrations are shown in **Table 2** and in **Figs. 2–4.** The mean serum squalene, desmosterol, lathosterol, and cholestanol proportions of the healthy pregnant group were 2-fold the values of the healthy nonpregnant controls. Their concentrations exhibited over 3-fold increases, while because values for plant sterols were unchanged, the plant sterol proportions (ratios to cholesterol) were significantly lower than in the controls. The campesterol/sitosterol ratio remained unchanged. Values tended to decrease significantly for desmosterol and sitosterol at 1–3 days after delivery. At 6 weeks after delivery, when all but one of the mothers were breast feeding their infants, the proportions of squalene and lathosterol still exceeded the control nonpregnant values, those of desmosterol exceeded even the prepartum values, but that of cholestanol was already normal and that of sitosterol fell below nonpregnant values. It is peculiar that Δ^8-cholestenol was within the range of the nonpregnant women during and after pregnancy.

The proportion of cholestanol was positively related to proportions of plant sterols and the campesterol/sitosterol ratio (to campesterol, $r = 0.93$; to sitosterol, $r = 0.80$; to ratio 0.69) in the nonpregnant controls, but not in the healthy pregnant women (respectively, $r = 0.52$, 0.45, and 0.17). At 6 post-delivery weeks the correlations resembled more those in the control group.

Cholestatic pregnancy and early lactation. In contrast to normal pregnancy, the prepartum squalene proportion of the cholestatic mothers fell within control limits and decreased during the postpartum 6 weeks gradually even below pre-delivery values (Table 2, Fig. 3). All the prepartum and postpartum precursor sterol proportions were higher than in the control women (Table 2, Figs. 2,3). The prepartum proportion of Δ^8-cholestenol

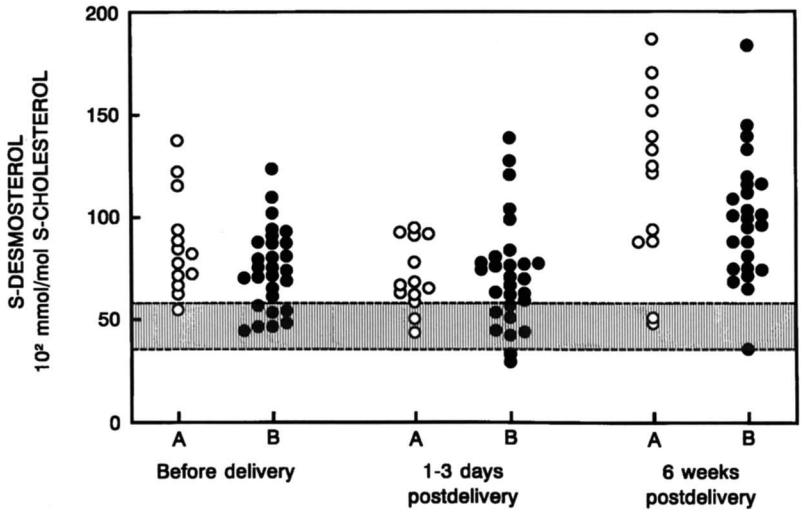

Fig. 2. Individual pre- and post-delivery serum proportions of desmosterol in healthy pregnant women (○ and A) and in women with cholestasis of pregnancy (● and B).

was clearly higher, while that of lathosterol was lower than in the normal pregnancy group. During the first days of lactation the sterol proportions were virtually unchanged, but at the 6th week of lactation (25 of 28 breast-fed) those of Δ^8-cholestenol and especially of desmosterol were again even higher than prepartum values, while that of lathosterol was below its normal postpartum value.

The cholestanol proportion was almost three times as high at prepartum as in the control nonpregnant women and 40% higher than in the normal pregnancies (Table 2, Fig. 4). The mean cholestanol concentration, on the other hand, was almost five times as high as in the control group and about 60% higher than in the normal pregnancy group. However, Fig. 1, showing a positive correlation between serum bilirubin and cholestanol concentrations, demonstrates that all but one of the cholestanol values in the normal pregnancies and all those in the cholestatic group exceed the upper nonpregnant control level, but only 32% of the cholestanol values exceeds the uppermost normal pregnancy concentration. In addition, all but one of the cholestanol values in the normal pregnancies fell within the limits of the values in the cholestasis group. Roughly similar results were obtained when the cholestanol proportions were used instead of concentrations.

The mean serum cholestanol proportion fell by about 30% during the first postpartum days to normal pregnancy levels but was still 2-fold that in the nonpregnant controls (Table 2, Fig. 4). At 6 weeks, cholestanol proportions in the three groups were similar. The prepartum plant sterol proportions were lower than in the control women, but similar to those in the normal pregnancy group (Table 2, Fig. 4). The campesterol/sitosterol ratio was 15% ($P < 0.05$) lower than in the nonpregnant or normal pregnancy women (Table 2). The plant sterol proportions had decreased by about 15% during the first postpartum days, and despite a slight increase in values during the first 6 weeks of the lactation period, the proportions were still below those of the controls (Table 2, Fig. 4). The campesterol/sitosterol ratio was not normalized before 6 weeks had passed (Table 2).

During cholestatic pregnancy the serum cholestanol proportions were positively correlated with the serum lathosterol ($r = 0.38$), bilirubin ($r = 0.74$), and serum bile acids ($r = 0.52$), and, in contrast to the nonpregnant women, negatively with the campesterol/sitosterol ratio ($r = -0.39$), but not significantly to alkaline phosphatase ($r = 0.29$). After delivery, especially at the 6th postpartum week, the correlations were similarly positive in both pregnancy groups, resembling those in the controls except that the correlation for the campesterol/sitosterol ratio was still negative.

DISCUSSION

Normal pregnancy and lactation

The new information from the present study is that up to 3-fold increases occurred in serum cholesterol precursor values for squalene, desmosterol, and lathosterol during normal pregnancies. This increase is only partly explained by the increase in serum lipoproteins transporting cholesterol and noncholesterol sterols, because the proportions of the latter to cholesterol were also increased roughly 2-fold. Thus, the pregnancy hyperlipidemia appears to be associated with increased cholesterol synthesis. It is somewhat exceptional, however, that Δ^8-cholestenol was not increased, even though

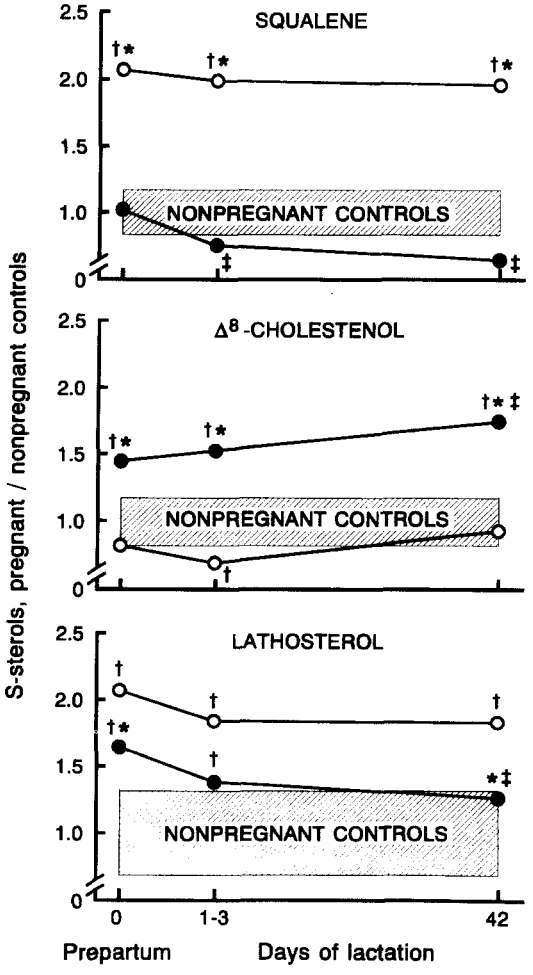

Fig. 3. Relative squalene, Δ^8-cholestenol, and lathosterol values in serum at prepartum and in early lactation of women with normal (○) and cholestatic (●) pregnancy. Mean sterol/cholesterol proportions of pregnant women divided by values for nonpregnant control women in Table 2. Shaded areas illustrate 95% confidence limits for nonpregnant women. $^{\dagger}P < 0.05$ vs. nonpregnant controls; $^{*}P < 0.05$ between pregnancy groups; $^{\ddagger}P < 0.05$ vs. pregnancy value.

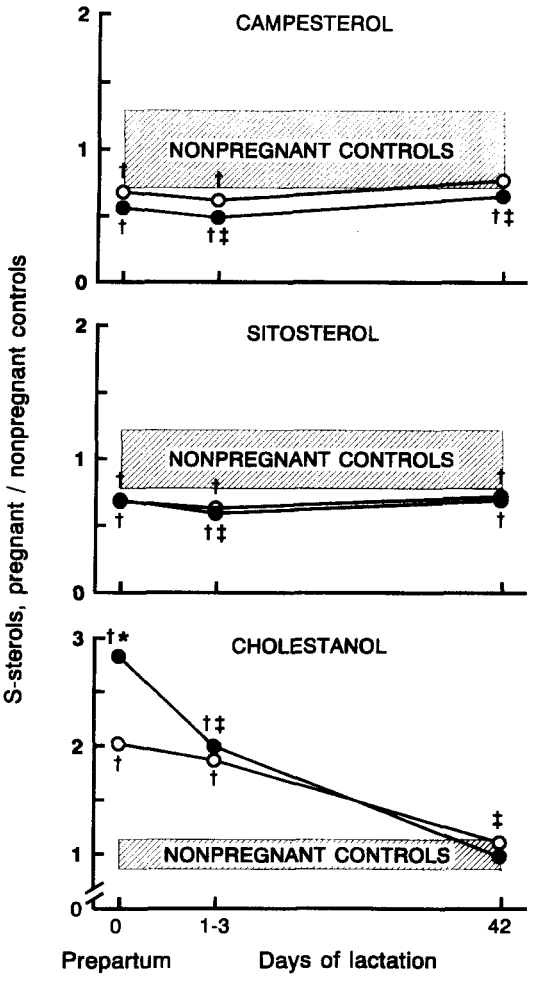

Fig. 4. Relative campesterol, sitosterol, and cholestanol values in serum at prepartum and in early lactation period of women with normal (○) and cholestatic (●) pregnancy. Mean sterol/cholesterol proportions of pregnant women divided by values for nonpregnant control women in Table 2. Shaded areas illustrate 95% confidence limits for nonpregnant women. $^{\dagger}P < 0.05$ vs. nonpregnant controls; $^{*}P < 0.05$ between pregnancy groups; $^{\ddagger}P < 0.05$ vs. pregnancy value.

its relative increase from stimulated cholesterol synthesis is usually higher than that of lathosterol (27). Activity of the enzyme converting Δ^8-cholestenol to Δ^7-cholestenol (lathosterol) may have increased more than other enzymes in the synthesis chain during cholestatic than during normal pregnancy. Moreover, the marked increase in squalene, the last nonsteroidal cholesterol precursor before cyclization to lanosterol, is interesting, because squalene appears to reflect acute (21) and, less consistently, chronic changes in cholesterol synthesis. As squalene is mainly transported by VLDL (21, 28) and, in contrast to the precursor sterols, quantitatively less by LDL, the proportionally higher increase in triglycerides and obviously also in VLDL may contribute to the increased squalene values in our normal pregnancies. Because cholesterol-precursor sterol proportions are related to hepatic HMG-CoA reductase activity (29), our findings suggest that increased lipoprotein levels are associated with enhanced hepatic cholesterol synthesis in normal pregnancy; however, the actual reason for this stimulation is unknown. Uterine and placental functions may require a higher lipid and cholesterol uptake, possibly contributing to the mother's enhanced cholesterol synthesis.

The precursor proportions remained elevated for up to 6 weeks after delivery, meaning that cholesterol synthesis was still high, probably due to cholesterol loss in milk during breast-feeding, causing a compensatory increase in cholesterol synthesis. In breast-feeding, mothers lose up to 200 mg/day of cholesterol in the milk,

especially when feeding their older infants (30). This lost cholesterol was judged to decrease the mothers' serum cholesterol, as compared to that of non-feeding mothers, to an extent corresponding to treatment of hypercholesterolemia by agents causing intestinal cholesterol malabsorption (31). Breast-feeding has been associated with low serum cholesterol values in mothers at 4–9 post-delivery months (30, 32). Thus, effective serum cholesterol lowering was most likely not yet achieved in the present study at 6 weeks. It is interesting to note that the desmosterol proportion became almost double the 1–3 day postdelivery proportions during the 6 subsequent weeks. This suggests that, as human milk is very rich in desmosterol (33), some of the cholesterol precursors may also originate from increased cholesterol synthesis in the breasts, in addition to the liver.

An additional new finding was that cholestanol exhibited surprisingly high serum values, 2- to 3-times as high during normal pregnancy as in the nonpregnant women, indicating that its synthesis was increased or that cholestasis contributed to its high values. Cholestanol is synthesized from cholesterol (34, 35), but normally the proportions of cholestanol in serum are negatively related to cholesterol synthesis, proportions of cholesterol precursors and biliary secretion, and positively to the plant sterol proportions and thus to cholesterol absorption efficiency (16). In clinical conditions with stimulated cholesterol synthesis, cholestanol proportions are not increased (16), and after delivery our values normalized within 6 weeks (see Fig. 4) despite enhanced cholesterol synthesis. Thus, as cholestanol absorption normally plays a minimal role (16, 34, 36) in normal pregnancy, impaired biliary secretion of cholestanol may be more important in regulating its serum value than enhanced cholesterol production-induced up-regulation of cholestanol synthesis.

Is normal pregnancy then associated with cholestatic features? Detection of mild cholestasis in pregnancy is difficult, and the diagnosis is made on clinical grounds, even though the diagnosis of cholestasis is frequently based upon increased bile acid values in serum (37, 38). Though increased bile acids excluded any women with cholestasis of pregnancy from the present normal pregnancy group, bromsulfphthalein-elimination studies have indicated delayed biliary secretion during the third trimester of normal pregnancy (39, 40). Thus, even though serum bile acids and bilirubin values were normal and were not related to cholestanol (Table 2), the proportion of the latter could be a very sensitive indicator of mild cholestasis in general and especially in normal pregnancy.

Serum plant sterols have not previously been studied in pregnancy. The campesterol/sitosterol ratio, an indicator of severe prolonged cholestasis (18, 19), was not affected by normal pregnancy, indicating that the cholestasis was actually mild. The reduced plant sterol proportions suggest, as shown in normal subjects (15, 41), that cholesterol and plant sterol absorptions would be decreased or that biliary secretion of plant sterols was increased. A third possibility would be a simple dilution of plant sterols by increased serum lipids. Increased cholesterol synthesis most likely resulted in enhanced biliary sterol secretion, decreasing the plant sterol proportions in serum.

Cholestasis of pregnancy and early lactation

The basic changes in the sterol patterns of cholestatic pregnancy are also new findings, and the changes resemble those of normal pregnancy, but *1*) increases in triglyceride and cholesterol values tended to be higher; *2*) the increase in cholesterol synthesis, indicated by the cholesterol precursors squalene, desmosterol, and lathosterol, tended to be less pronounced throughout the study; and *3*) the indicators of cholestasis, especially cholestanol but also the campesterol/sitosterol ratio, were more pronounced during the cholestasis pregnancies. Because cholestasis reduces biliary secretion of sterols in relation to serum values, including cholesterol (20), requirements for enhanced cholesterol synthesis may also be reduced.

The markedly high values for cholestanol suggest its use for diagnostic differentiation between normal and cholestatic pregnancies. The mothers, defined as having normal pregnancies by normal serum bile acids, actually had increased cholestanol in serum as a possible sign of mild cholestasis. On the other hand, only one-third of the cholestanol concentrations exceeded the maximum value for normal pregnancy, indicating that measurement of serum cholestanol can only partly distinguish women with a clinically significant gravidity cholestasis from those with a normal pregnancy. Indicators of cholestasis: cholestanol and the campesterol/sitosterol ratio had normalized at the 6 weeks postpartum, suggesting that disappearance of cholestasis normalized their values.

The low plant sterol proportions may not be related to bile acid retention-induced impairment in sterol absorption, because similarly low plant sterol values occurred in both normal and cholestatic pregnancy and even during lactation. Increased cholesterol synthesis, also during cholestatic pregnancy and the subsequent lactation period, was poorly related to cholestanol values, but this suggests that biliary cholesterol secretion and sterol secretion in general were also increased. As input of dietary plant sterols was hardly different in the pregnancy groups, the slightly increased biliary secretion of plant sterols and their simple dilution by increased serum lipids were responsible for their reduced

serum proportions also in the cholestatic pregnancy group.

Enhanced cholesterol synthesis with somewhat increased biliary cholesterol secretion could contribute to high manifestation of gallstones: 29% in our cholestatic pregnancy group. Serum squalene and noncholesterol sterol levels were, however, similar in pregnant women with and without gallstones. The bile apparently became supersaturated with cholesterol, especially because biliary secretion of bile acids was impaired (resulting more likely in the formation of cholesterol than of pigment stones). In the normal pregnancy group no gallstones occurred, suggesting that biliary bile acid secretion and sufficient biliary tract motility prevented gallstone formation. Gravidity-induced increase in clinically symptomatic gallstones is, however, well known, and in some studies the occurrence has been extremely high (42).

In conclusion, on the basis of serum squalene and noncholesterol sterol measurements, the combined hyperlipidemia of normal pregnancy appears to be associated with enhanced synthesis of cholesterol. Markedly increased cholestanol in serum may be a specifically sensitive indicator of mild cholestasis, possibly contributed by enhanced synthesis. In cholestatic pregnancy the changes are basically similar, although cholestasis reduces the campesterol/sitosterol ratio and cholesterol synthesis, and potentiates the increase of serum cholestanol, a fact that separates one-third of cholestatic mothers from those with normal pregnancy. The reduced plant sterol proportions in both groups throughout the study may more likely be related to increased biliary secretion and dilution by increased serum cholesterol than to reduced absorption. During the lactation period, cholestanol values become normalized despite continuously increased synthesis of cholesterol.

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