patient-oriented and epidemiological research

Elevated serum squalene and cholesterol synthesis markers in pregnant obese women with gestational diabetes mellitus[1]

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Abstract We examined serum cholesterol synthesis and absorption markers and their association with neonatal birth weight in obese pregnancies affected by gestational diabetes mellitus (GDM). Pregnant women at risk for GDM (BMI >30 kg/m^2) were enrolled from maternity clinics in Finland. GDM was determined from the results of an oral glucose tolerance test. Serum samples were collected at six time-points, one in each trimester of pregnancy, and at 6 weeks, 6 months, and 12 months postpartum. Analysis of serum squalene and noncholesterol sterols by gas-liquid chromatography revealed that in subjects with GDM (n = 22), the serum Δ8-cholestenol concentration and lathosterol/sitosterol ratio were higher ($P < 0.05$) than in the controls (n = 30) in the first trimester, reflecting increased cholesterol synthesis. Also, subjects with GDM had an increased ratio of squalene to cholesterol (100 × μmol/mmol of cholesterol) in the second (11.5 ± 0.5 vs. 9.1 ± 0.5, $P < 0.01$) and third (12.1 ± 0.8 vs. 10.0 ± 0.7, $P < 0.05$) trimester. In GDM, the second trimester maternal serum squalene concentration correlated with neonatal birth weight ($r = 0.70$, $P < 0.001$). 🔓 In conclusion, in obesity, GDM associated with elevated serum markers of cholesterol synthesis. Correlation of maternal serum squalene with neonatal birth weight suggests a potential contribution of maternal cholesterol synthesis to newborn weight in GDM.—Miettinen, H. E., K. Rönö, S. Koivusalo, B. Stach-Lempinen, M. Pöyhönen-Alho, J. G. Eriksson, T. P. Hiltunen, and H. Gylling. **Elevated serum squalene and cholesterol synthesis markers in pregnant obese women with gestational diabetes mellitus.** *J. Lipid Res.* 2014. **55:** 2644–2654.

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Gestational diabetes mellitus (GDM), i.e., glucose intolerance diagnosed for the first time during pregnancy, is associated with unfavorable pregnancy outcomes for both the mother and the fetus (1). These GDM-related risks, including fetal macrosomia, are mediated, at least partly, via disturbed glucose and/or lipid metabolism. The association of high maternal glucose levels with fetal macrosomia is well-documented (1), but macrosomia is also observed in diabetic pregnancies with satisfactory glycemic control (2). It has been suggested that maternal plasma lipids might be stronger determinants for fetal growth than plasma glucose levels (3) [reviewed in (4)]. In some studies, concentrations of maternal serum triglycerides have associated positively (5) and HDL cholesterol negatively with neonatal birth weight (6).

Hyperlipidemia of pregnancy is a well-characterized phenomenon and considered necessary to satisfy the needs of the developing fetus (7) [reviewed in (4)]. However, excessive hyperlipidemia may not solely be a benign condition. High levels of maternal plasma cholesterol have been associated with increased expression of proteins involved in cholesterol metabolism in the placenta (8), which could potentially modulate fetal development. Hypercholesterolemia of the mother may also trigger pathogenic events in the fetal aorta and determine susceptibility to atherosclerosis later in life (9). Similarly, the offspring

Abbreviations: ALAT, alanine transaminase; GDM, gestational diabetes mellitus; GLC, gas-liquid chromatography; GLM, general linear model; OGTT, oral glucose tolerance test.
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of GDM mothers have an increased risk for obesity and T2D later in life (10, 11).

The lipid changes associated with T2D, i.e., low HDL cholesterol, elevated serum triglycerides, and small dense LDL, are well-described (12). Furthermore, we have shown that T2D (13) as well as obesity (14) associate with increased cholesterol synthesis and decreased intestinal cholesterol absorption efficiency. As GDM is proposed to mimic T2D in many ways (insulin resistance, increase in body fat), similar lipid changes would also be expected to occur in GDM. However, studies investigating serum lipids and lipoproteins in GDM have been controversial, showing an increase, a decrease, or no change in serum triglyceride, total cholesterol, or LDL cholesterol levels (8, 15, 16) [reviewed in (4)].

In addition to cholesterol, serum contains cholesterol precursors [squalene, lanosterol (14α-trimethyl-5α-cholest-8,24-dien-3β-ol), Δ8-cholestenol (5α-cholest-8-en-3β-ol), desmosterol (cholest-5,24-dien-3β-ol), and lathosterol (5α-cholest-7-en-3β-ol)], which in many conditions, such as T2D, reflect cholesterol synthesis, cholestanol (a derivative of cholesterol), and plant sterols (campesterol, sitosterol, and avenasterol), which reflect cholesterol absorption efficiency (17). Serum noncholesterol sterols in pregnancy have been examined previously in only two studies. The first compared normal and cholestatic pregnancy just before delivery and 6 weeks postpartum (18), and the latter the effect of consuming plant stanol ester margarine on sterols in normal pregnancy in the first and third trimester and 1 month postpartum (19). To our knowledge, no studies exist that explore squalene and noncholesterol sterols in subjects with GDM, nor is there data available about potential associations of maternal noncholesterol sterols with neonatal birth weight.

Thus, the present study was undertaken to examine serum cholesterol precursors, cholestanol, and plant sterols in obese subjects with GDM or normal glucose tolerance during pregnancy and the following 12 months postpartum. Furthermore, as maternal lipids might be determinants of fetal growth, we investigated whether these maternal sterols associate with neonatal birth weight. We assumed that serum squalene and noncholesterol sterols could be the most sensitive markers reflecting potential changes in cholesterol metabolism during GDM even when glycemic control is satisfactory.

MATERIALS AND METHODS

Subjects

All study subjects were participants of the Finnish RADIEL study, described previously in detail (20), and consisted of all eligible subjects from the RADIEL study's control arm. Pregnant women (n = 52) classified as at risk for developing GDM (BMI >30 kg/m²) were enrolled in the present study at the time of their first visit to the maternity clinics in Southern Finland. With the exception of two subjects (Estonian and Russian origin) all study subjects were of Finnish origin. All participants signed an informed consent form.

Exclusion criteria for all subjects included diabetes, undiagnosed thyroid disease (abnormal serum thyroid-stimulating hormone concentration), anemia, use of insulin, age <18 years, oral corticosteroid treatment, alcohol or drug abuse, multiple pregnancy, physical disability, significant cooperation difficulties (e.g., insufficient language skills), or history of severe psychiatric disorder or hypertension.

An oral glucose tolerance test (OGTT) (75 g) was performed on all subjects at weeks 22–29 of pregnancy, with the exception of three subjects with OGTT performed at weeks 31–33, and was considered diagnostic for GDM if any of the measures were pathological. The following diagnostic thresholds were used: fasting plasma glucose >5.3 mmol/l, 1 h plasma glucose ≥10.0 mmol/l, or 2 h plasma glucose ≥8.6 mmol/l (21). The GDM group (n = 22) consisted of women having a pathological OGTT, while the control subjects (n = 30) had a normal OGTT during pregnancy and no history of previous GDM.

Weight and height were measured at the time of enrollment and at each maternity clinic visit. The prepregnancy weight was self-reported. The newborns were weighed directly after the delivery on a digital baby scale. The relative weight was calculated using Finnish standards adjusted for sex and gestational age (22).

As all study subjects were obese, they received dietary and exercise information leaflets (20, 23, 24). In addition, subjects who were diagnosed for GDM received dietary counseling and information of diabetes in the primary health care center. In case plasma glucose concentrations in home measurements repeatedly exceeded 5.5 mmol/l before breakfast or 7.8 mmol/l 1 h after a meal, insulin treatment was initiated. If insulin was started before the third trimester, samples were taken and subjects were excluded from further analysis (n = 1) due to potential interference of insulin therapy on lipid metabolism. One subject was treated with metformin (starting at week 15).

The study was performed according to the principles of the Declaration of Helsinki and was approved by the Ethical Committee of the Helsinki University Central Hospital.

Methods

Serum samples were collected from all subjects during pregnancy (at I, II, and III trimester corresponding to pregnancy weeks 10–14, 22–25, and 34–36) and at 6 weeks, 6 months, and 12 months postpartum. All samples were stored at −80°C until analyzed. Fasting plasma glucose, alanine transaminase (ALAT), serum insulin, and thyroid-stimulating hormone concentrations were determined by routine hospital laboratory methods. Serum cholesterol and cholesterol precursors (squalene, lanosterol, Δ8-cholestenol, desmosterol, and lathosterol), cholestanol, and plant sterols (campesterol, sitosterol, and avenasterol) were assayed by gas-liquid chromatography (GLC), as described (25). Shortly, serum squalene and noncholesterol sterols were quantified from nonsaponifiable serum-based materials by capillary GLC (Agilent 6890N Network GC system; Agilent Technologies, Wilmington, DE) equipped with a 50 m long nonpolar Ultra 2 capillary column (5% phenyl-methyl siloxane; Agilent Technologies) with 5α-cholestane as internal standard (25). The serum values of squalene and noncholesterol sterols are expressed as concentrations (μg/dl) or ratios to cholesterol (100 × μmol/mmol of cholesterol) by dividing squalene and noncholesterol sterol concentrations with the cholesterol value of the same GLC run in order to eliminate the effect of different cholesterol levels (26). We also calculated the lathosterol/sitosterol ratio, which reflects cholesterol synthesis (26).

Serum phospholipids, triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol concentrations were determined by enzymatic methods, phospholipids with DiaSys Diagnostic

System (Holzheim, Germany), and the others with Thermo Konelab GO (Thermo Scientific, Vantaa, Finland).

Statistics

Statistical analyses were performed with IBM SPSS 19.0 statistics program. Normality was tested before further analyses. Blom's formula was used to normalize skewed distributions (27). Student's *t*-test was used to analyze differences in BMI and weight between groups, and paired *t*-test for serum lipid and sterol changes within groups during pregnancy and postpartum. General linear model (GLM) for repeated measures was used for testing differences between the groups in the function of time using age and prepregnancy BMI as covariates. When significant differences ($P < 0.05$) were detected, GLM univariate variance analysis was applied to examine differences between the groups at each time-point using age and the corresponding BMI as covariates. Spearman's correlation was used to analyze correlation between parameters. Multiple stepwise regression analysis was performed separately at each trimester to determine if maternal parameters associated with neonatal birth weight. Independent variables in the analyses included maternal prepregnancy BMI; weight gain (kg); age; trimester-specific plasma glucose and serum insulin concentrations; serum total cholesterol, HDL cholesterol, and LDL cholesterol; serum triglycerides; phospholipids; and squalene and noncholesterol sterols (concentration and ratio to cholesterol, analyzed separately). Multicollinearity between the covariates was not detected during analyses. $P < 0.05$ was used as an inclusion criterion in the model. $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the study subjects at the time of enrollment in their first trimester are shown in **Table 1**. The GDM and control groups did not differ in age, nor were there any differences in plasma ALAT; serum total, HDL, and LDL cholesterol; or serum total triglyceride or phospholipid levels. Plasma glucose was higher in the GDM group than in the control group ($P < 0.001$), but insulin levels or insulin/glucose ratios did not differ between the groups. All study subjects were obese (BMI >30 kg/m^2), as called for in the inclusion criteria. Subjects with GDM had higher BMI than the controls, i.e., 36.9 versus 33.3 kg/m^2 ($P < 0.01$), and this difference remained significant throughout pregnancy. Therefore, in all further GLM analyses BMI was used as a covariate. Subjects with GDM gained less weight during pregnancy than the control subjects, i.e., 3.6 ± 0.7 (SE) kg versus 7.6 ± 0.9 kg, respectively ($P < 0.01$).

The groups did not differ in terms of smoking (three control subjects smoked before pregnancy, two of them stopped when pregnant), history of preeclampsia (in three control subjects), use of pregnancy vitamins (Multitabs Raskaus, Pfizer Oy) or vitamin D supplements (73% vs. 85%, GDM vs. control, NS), development of hypertension (three subjects in both groups), preeclampsia (two subjects in controls), or hepatogestosis (none) during pregnancy. None of the subjects had a previous or newly diagnosed lipid disorder, or used statins.

Neonatal birth weights (3,753 ± 118 g vs. 3,566 ± 82 g), gestational age (39.7 ± 0.27 weeks vs. 40.3 ± 0.24 weeks),

TABLE 1. Characteristics of the control and GDM study groups and their serum and lipoprotein cholesterol, serum triglyceride, phospholipids, insulin, and plasma glucose and ALAT concentrations during pregnancy and postpartum and 6 weeks, 6 months, and 12 months postpartum

	First Trimester		Second Trimester		Third Trimester		6 Weeks Postpartum		6 Months Postpartum		12 Months Postpartum	
	Control (n = 30)	GDM (n = 22)	Control (n = 30)	GDM (n = 22)	Control (n = 27)	GDM (n = 21)	Control (n = 27)	GDM (n = 17)	Control (n = 28)	GDM (n = 20)	Control (n = 28)	GDM (n = 20)
Age (years)	32.1 ± 1.0	32.2 ± 0.8	—	—	—	—	—	—	—	—	—	—
BMI (kg/m^2)	33.3 ± 0.5	36.9 ± 1.0^b	34.2 ± 0.6	37.4 ± 1.0^b	35.8 ± 0.5	38.3 ± 1.1^a	NA	NA	33.9 ± 05	36.1 ± 1.3	33.1 ± 0.7	36.6 ± 1.5^a
Weight (kg)	91.7 ± 1.6	101.8 ± 3.3^b	94.4 ± 1.6	103.0 ± 3.1^a	98.5 ± 1.5	105.9 ± 3.4^a	NA	NA	93.4 ± 1.4	99.5 ± 4.0	91.2 ± 1.9	101.6 ± 4.6^b
ALAT (IU/ml)	16.7 ± 1.7	25.1 ± 4.5	18.0 ± 1.7	21.3 ± 4.4	13.9 ± 1.3	31.9 ± 9.5	50.3 ± 16.4	33.2 ± 5.9	30.0 ± 6.1	27.3 ± 9.6	22.3 ± 2.4	21.0 ± 1.9
Total cholesterol (mmol/l)	4.75 ± 0.13	4.84 ± 0.19	5.70 ± 0.18	5.65 ± 0.19	6.29 ± 0.19	6.27 ± 0.25	5.25 ± 0.19	5.29 ± 0.28	4.52 ± 0.13	4.81 ± 0.19	4.56 ± 0.15	4.44 ± 0.19
HDL cholesterol (mmol/l)	1.64 ± 0.06	1.61 ± 0.08	1.77 ± 0.10	1.83 ± 0.07	1.76 ± 0.07	1.66 ± 0.11	1.53 ± 0.07	1.51 ± 0.11	1.42 ± 0.06	1.53 ± 0.08	1.41 ± 0.05	1.35 ± 0.06
LDL cholesterol (mmol/l)	2.46 ± 0.10	2.40 ± 0.14	3.10 ± 0.15	2.94 ± 0.17	3.57 ± 0.19	3.25 ± 0.22	3.21 ± 0.18	3.08 ± 0.29	2.61 ± 0.139	2.81 ± 0.15	2.61 ± 0.13	2.42 ± 0.14
Triglycerides (mmol/l)	1.41 ± 0.11	1.49 ± 0.16	1.84 ± 0.15	1.99 ± 0.16	2.44 ± 0.17	2.76 ± 0.24	1.39 ± 0.20	1.58 ± 0.20	1.22 ± 0.21	1.20 ± 0.18	1.26 ± 0.10	1.34 ± 0.15
Phospholipids (mg/dl)	207.1 ± 5.4	207.4 ± 5.3	231.7 ± 6.1	229.2 ± 5.6	246.6 ± 5.3	245.7 ± 6.5	205.2 ± 4.9	203.5 ± 7.4	180.3 ± 6.1	191.1 ± 5.6	181.0 ± 5.3	179.0 ± 6.2
Glucose (mmol/l)	4.75 ± 0.05	5.24 ± 0.09^c	4.69 ± 0.05	5.20 ± 0.11^c	4.67 ± 0.06	5.04 ± 0.07^a	5.01 ± 0.07	5.31 ± 0.09^a	5.01 ± 0.05	5.4 ± 0.08^a	5.09 ± 0.06	5.38 ± 0.10^a
Insulin (mU/l)	9.0 ± 0.9	12.2 ± 2.3	10.6 ± 0.9	12.7 ± 1.6	14.7 ± 1.3	15.1 ± 1.7	6.8 ± 0.6	7.7 ± 1.5	NA	NA	9.1 ± 1.0	12.3 ± 2.2

Values are expressed as mean ± SE. Student's *t*-test was used for BMI and weight, GLM univariate analysis for others using BMI and age as covariants when available. NA, not available.

^{a} $P < 0.05$, GDM versus control at the respective time-point.

^{b} $P < 0.01$, GDM versus control at the respective time-point.

^{c} $P < 0.001$, GDM versus control at the respective time-point.

or relative birth weights did not differ significantly between the newborns of GDM or control mothers. None of the newborns were small or large for age (i.e., weight <2 SD or >2 SD).

Plasma glucose, serum insulin, and lipids in pregnancy and postpartum

During pregnancy, plasma fasting glucose concentration was significantly higher in the GDM group than in the control group (GLM repeated measures, $P < 0.001$) and remained higher during the follow-up period 12 months postpartum (GLM repeated measures, $P < 0.05$) (Table 1). All plasma glucose values were indicative of good glycemic control. Serum insulin levels did not differ between the groups at any measured time-point, and neither did the insulin/glucose ratio reflecting insulin sensitivity.

Serum total cholesterol increased equally in both groups by 30% and serum triglycerides almost doubled during pregnancy (Table 1). The concentration of HDL cholesterol reached its peak in the second trimester in both groups, while that of LDL cholesterol reached its peak in the third trimester. Serum phospholipids increased by 20% in both control and GDM pregnancies ($P < 0.05$). All measured lipids, i.e., serum total, LDL, HDL cholesterol, triglycerides, and phospholipids, were higher during pregnancy than 12 months postpartum, and did not differ significantly between the groups.

In the control group, but not in the GDM group, the change in BMI during pregnancy (from prepregnancy to third trimester) correlated with the change in serum insulin concentration ($r = 0.624$, $P = 0.006$), and with the change in plasma glucose concentration ($r = 0.433$, $P < 0.05$). The serum insulin and insulin/glucose ratio in the third trimester correlated with weight change in both groups ($r = 0.681$, $P < 0.01$ in controls, and $r = 0.498$, $P < 0.05$ in GDM). There was no significant correlation between pregnancy weight change (prepregnancy to third trimester) and change in serum total, HDL, or LDL cholesterol, or serum triglyceride or phospholipid levels, but the weight change from the first to the third trimester correlated to the change in serum phospholipids in controls ($r = 0.422$, $P < 0.05$).

Squalene and noncholesterol sterols in pregnancy

In the first trimester, serum concentrations of squalene and noncholesterol sterols, as well as their ratios to cholesterol, were similar in both groups, with the exception of slightly higher serum Δ8-cholestenol concentration and ratio to cholesterol ($P < 0.05$) in the subjects who were later diagnosed for GDM than in the control subjects (**Table 2**, **Fig. 1**). Also, their ratio of serum lathosterol to that of sitosterol, reflecting cholesterol synthesis, was higher ($P < 0.05$).

During pregnancy, the serum concentrations of lanosterol, desmosterol, cholestanol, campesterol, sitosterol, and avenasterol increased from the first to the third trimester in both groups (Table 2). On the contrary to the controls, serum concentrations of Δ8-cholestenol and lathosterol did not increase significantly in the GDM group. Serum lathosterol
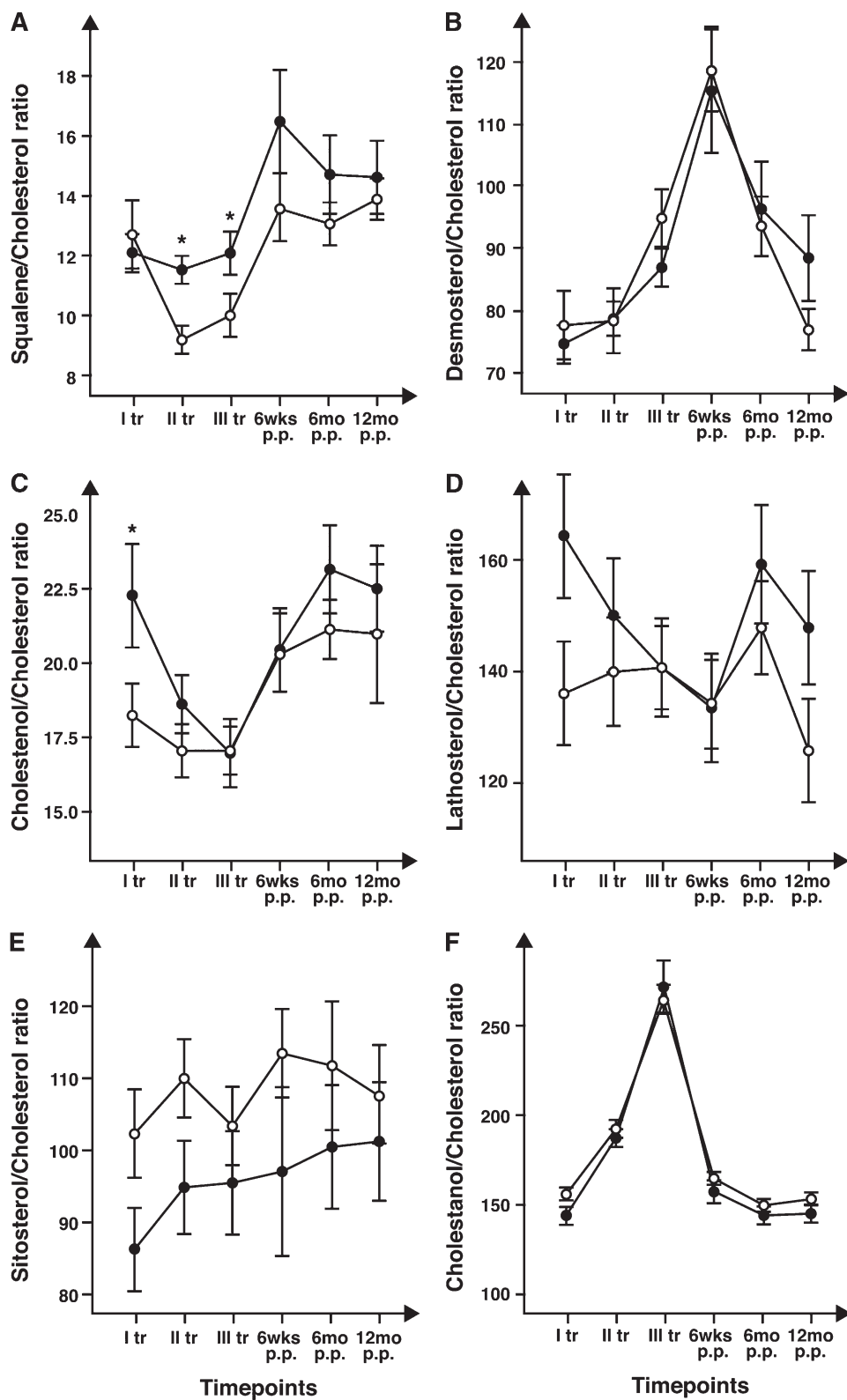
TABLE 2. Serum squalene and noncholesterol sterol concentration during pregnancy and 6 weeks, 6 months, and 12 months postpartum in control and GDM study groups

Variable	First Trimester Control (n = 28)	First Trimester GDM (n = 21)	Second Trimester Control (n = 30)	Second Trimester GDM (n = 22)	Third Trimester Control (n = 27)	Third Trimester GDM (n = 21)	P^a	6 Weeks Postpartum Control (n = 27)	6 Weeks Postpartum GDM (n = 17)	6 Months Postpartum Control (n = 28)	6 Months Postpartum GDM (n = 20)	12 Months Postpartum Control (n = 28)	12 Months Postpartum GDM (n = 20)
Squalene (μg/dl)	22.1 ± 2.2	21.7 ± 1.5	18.9 ± 1.0	24.0 ± 1.3[b]	23.1 ± 1.8	27.3 ± 2.0	0.040	25.8 ± 2.2	31.6 ± 3.7	21.6 ± 1.3	26.7 ± 2.6	23.4 ± 1.4	24.4 ± 1.4
Lanosterol (μg/dl)	24.0 ± 1.8	26.8 ± 2.6	28.1 ± 1.8	32.8 ± 2.9	29.1 ± 2.0	33.0 ± 3.0	0.168	27.6 ± 2.2	31.0 ± 3.3	26.8 ± 2.0	30.0 ± 2.3	24.1 ± 1.9	28.3 ± 2.3
Δ8-Cholestenol (μg/dl)	32.0 ± 2.2	38.8 ± 2.8[b]	35.1 ± 1.9	38.9 ± 2.7	38.9 ± 2.0	38.7 ± 3.0	0.050	39.0 ± 2.9	39.3 ± 3.7	35.0 ± 2.1	41.8 ± 3.3	34.5 ± 3.2	35.8 ± 2.4
Desmosterol (μg/dl)	138 ± 13	134 ± 7	165 ± 13	164 ± 8	218 ± 13	198 ± 11	0.688	228 ± 16	223 ± 25	155 ± 10	174 ± 17	130 ± 8	146 ± 15
Lathosterol (μg/dl)	239 ± 18	288 ± 17	292 ± 23	314 ± 27	323 ± 22	322 ± 23	0.045	262 ± 20	259 ± 26	248 ± 17	288 ± 23	214 ± 18	236 ± 17
Lathosterol/Sitosterol	1.5 ± 0.18	2.2 ± 0.25[b]	1.4 ± 0.14	1.8 ± 0.17	1.5 ± 0.13	1.7 ± 0.14	0.051	1.3 ± 0.13	1.6 ± 0.28	1.5 ± 0.13	1.9 ± 0.30	1.4 ± 0.10	1.7 ± 0.21
Cholestanol (μg/dl)	272 ± 9	261 ± 17	401 ± 16	396 ± 24	614 ± 28	639 ± 58	0.874	315 ± 13	306 ± 26	247 ± 10	261 ± 14	257 ± 11	238 ± 15
Campesterol (μg/dl)	332 ± 20	309 ± 27	432 ± 26	395 ± 36	441 ± 32	419 ± 35	0.630	404 ± 25	385 ± 53	350 ± 34	361 ± 35	351 ± 32	331 ± 34
Sitosterol (μg/dl)	177 ± 10	157 ± 14	230 ± 13	201 ± 17	239 ± 15	220 ± 20	0.310	218 ± 14	187 ± 27	185 ± 16	182 ± 19	181 ± 14	168 ± 17
Avenasterol (μg/dl)	53.3 ± 2.8	48.5 ± 3.2	64.2 ± 3.1	60.3 ± 3.8	70.1 ± 4.2	62.0 ± 4.9	0.376	70.5 ± 3.9	61.6 ± 6.6	60.1 ± 4.7	59.8 ± 4.2	56.7 ± 3.8	52.2 ± 3.8

Values are expressed as mean ± SE.
[a] GLM, repeated measures, trimesters I–III, adjusted for BMI and age, GDM versus controls. All P values postpartum were nonsignificant.
[b] GLM, univariate analysis, adjusted for BMI and age, $P < 0.05$, GDM versus controls at the respective time-point.

Fig. 1. The ratios of serum squalene and noncholesterol sterols to cholesterol ($100 \times \mu mol/mmol$ of cholesterol) in I, II, and III trimester of pregnancy, and 6 weeks, 6 months, and 12 months postpartum (p.p.) in control subjects (open circles) and subjects with GDM (filled circles). Values are mean ± SE. *$P < 0.05$, controls versus GDM. GLM adjusted for BMI.

concentration differed during pregnancy between GDM and control groups ($P < 0.05$, GLM repeated measures). The concentration of serum squalene, however, increased ($P < 0.01$) only in the GDM group, and differed from the controls during pregnancy (GLM repeated measures, $P < 0.05$). In the second trimester, serum squalene concentration was higher in the GDM group than in controls ($P < 0.05$).

As the cholesterol levels also increased substantially, only the ratios of desmosterol and cholestanol to cholesterol increased significantly ($P < 0.0001$ in both groups), while that of $\Delta 8$-cholestenol decreased in both groups ($P < 0.01$ in GDM; $P < 0.05$ in controls) (Fig. 1). This decrease was somewhat greater in subjects with GDM than in the controls ($P < 0.05$), but the ratio of $\Delta 8$-cholestenol to cholesterol was higher in the GDM group already in the first trimester [22.3 ± 1.8 vs. 18.2 ± 1.1 (100 × μmol/mmol of cholesterol), GDM vs. controls, $P < 0.05$]. The ratio of lathosterol to cholesterol decreased during pregnancy only in the GDM group ($P < 0.05$). Although the ratios of sitosterol (Fig. 1), as well as those of campesterol and avenasterol to cholesterol (data not shown), tended to be lower in the GDM group than in the control group, the differences were not statistically significant. However, during pregnancy, the ratio of squalene to cholesterol differed between the GDM and control group ($P < 0.05$, GLM repeated measures). In GDM pregnancies, the serum ratio of squalene to cholesterol (100 × μmol/mmol of cholesterol) was significantly higher in the second (11.5 ± 0.5 vs. 9.1 ± 0.5, $P < 0.01$) and third (12.1 ± 0.8 vs. 10.0 ± 0.7, $P < 0.05$) trimester than in the controls.

Pregnancy-related change in BMI or weight did not correlate significantly to the change in squalene or noncholesterol sterols during pregnancy in either of the groups.

Squalene and noncholesterol sterols postpartum

Six weeks after delivery, in both groups, the concentration of serum cholestanol reduced to half of that of the third trimester, and serum cholesterol and lathosterol concentrations decreased by 20–30% (Table 2). The most marked increase at 6 weeks postpartum was seen in the ratio of desmosterol to cholesterol by 25–30% ($P < 0.01$) in both groups (**Fig. 2**). The ratios of squalene and $\Delta 8$-cholestenol to cholesterol also increased similarly, in both groups, 6 weeks after the delivery ($P < 0.05$). At 12 months postpartum, the ratio of $\Delta 8$-cholestenol was slightly higher in GDM subjects than in the controls ($P < 0.05$). Plant sterol ratios to cholesterol did not markedly change after delivery.

At 6 weeks postpartum, 84% of mothers were breastfeeding, at 6 months 48%, and at 12 months 21%, similarly in both groups.

Correlations

In the first trimester, the ratios of desmosterol and lathosterol to cholesterol correlated negatively to the ratios of campesterol, sitosterol, and cholestanol to cholesterol in both groups, suggesting that cholesterol absorption and synthesis were in homeostasis at this point. However, this homeostasis was partly lost in the third trimester of the pregnancy in the GDM group (**Table 3**). At that time, in the control group, the ratios of both desmosterol and lathosterol to cholesterol correlated negatively to the ratio of cholestanol to cholesterol, and the ratio of desmosterol to cholesterol correlated negatively to ratios of campesterol and sitosterol to cholesterol, but in the GDM group, only the ratio of desmosterol to cholesterol correlated to the ratio of cholestanol to cholesterol.
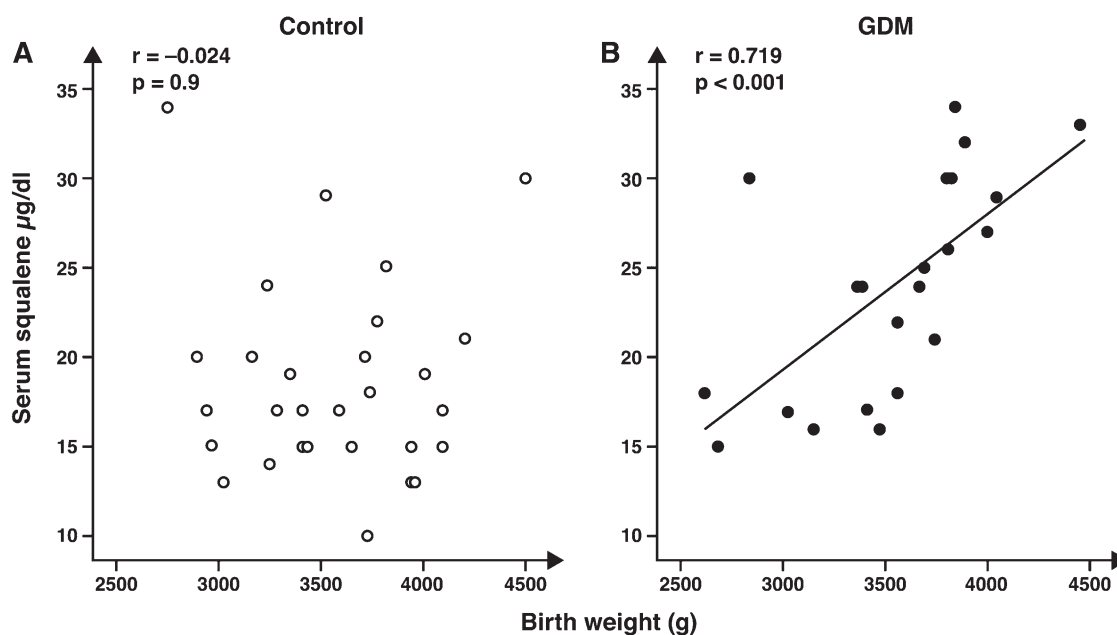
Fig. 2. Correlation between neonatal birth weight (g) and maternal serum squalene concentration (μg/dl) in the second trimester of control pregnancies (n = 30) (A) and pregnancies affected by GDM (n = 22) (B).

TABLE 3. Spearman correlation coefficients of the serum ratios of squalene and noncholesterol sterols to cholesterol (100 × μmol/mmol of cholesterol) in the controls and subjects with GDM in the third trimester of pregnancy

	Squalene	Lanosterol	Δ8-Cholestenol	Lathosterol	Desmosterol	Sitosterol	Campesterol
Control (n = 27)							
Lanosterol	0.562^b	—	—	—	—	—	—
Δ8-Cholestenol	0.297	0.317	—	—	—	—	—
Lathosterol	0.078	0.076	0.753^c	—	—	—	—
Desmosterol	0.190	0.011	0.488^a	0.446^a	—	—	—
Sitosterol	0.216	0.267	−0.133	−0.140	−0.358	—	—
Campesterol	0.162	0.203	−0.206	−0.230	$−0.429^a$	0.950^c	—
Cholestanol	0.121	0.226	−0.242	$−0.407^a$	$−0.536^b$	0.563^b	0.592^b
GDM (n = 21)							
Lanosterol	0.621^b	—	—	—	—	—	—
Δ8-Cholestenol	0.454^a	0.622^b	—	—	—	—	—
Lathosterol	0.370	0.370	0.788^c	—	—	—	—
Desmosterol	0.009	−0.056	−0.090	0.094	—	—	—
Sitosterol	0.433	0.338	0.169	0.134	−0.290	—	—
Campesterol	0.484^a	0.217	0.149	0.221	−0.345	0.899^c	—
Cholestanol	0.146	0.088	0.074	−0.074	$−0.500^a$	0.561^b	0.474^a

$^a P < 0.05.$
$^b P < 0.01.$
$^c P < 0.001.$

In the first and second trimester, plasma glucose concentration did not significantly correlate with serum squalene or noncholesterol sterols, with the exception of a negative correlation to serum cholestanol concentration in the controls in the first trimester. Serum insulin correlated negatively with the ratio of cholestanol to cholesterol ($r = −0.495$, $P < 0.05$) in the first trimester and positively with the ratio of desmosterol to cholesterol in the first ($r = 0.542$, $P < 0.05$) and second trimester ($r = 0.618$, $P < 0.01$) in the control group only.

In the third trimester, plasma glucose and serum insulin concentrations correlated with the serum ratio of squalene to cholesterol in control subjects ($r = 0.445$, $P < 0.05$ and $r = 0.572$, $P < 0.01$, respectively). Also, the change in serum insulin levels during pregnancy correlated with the change in squalene (both serum concentration and ratio to cholesterol, $r = 0.560$ and $r = 0.500$, $P < 0.05$, respectively) in the control subjects, but not in the GDM group.

Plasma ALAT levels did not correlate with serum squalene or noncholesterol sterol concentrations or ratios to cholesterol.

Neonatal birth weight and maternal lipids

We first looked at potential correlations between third trimester maternal serum lipids and birth weight of the newborns. There were no correlations between maternal serum triglyceride or total, LDL, or HDL cholesterol concentrations and birth weight, but in the control group, serum phospholipids correlated with birth weight and with relative birth weight ($r = 0.462$ and $r = 0.450$, $P < 0.05$, respectively). Analysis of serum squalene and noncholesterol sterols revealed a correlation between birth weight and both maternal serum cholestanol concentration ($r = 0.437$, $P < 0.05$) and the ratio of cholestanol to cholesterol ($r = 0.452$, $P < 0.05$) in the controls. On the other hand, in the GDM subjects, maternal serum desmosterol concentrations correlated with birth weight ($r = 0.528$, $P = 0.01$). However, no significant correlations were found between

maternal plasma glucose or insulin levels with birth weight in either of the groups.

We next investigated the possibility that the change in maternal lipids or noncholesterol sterols during pregnancy (i.e., between the first and third trimester), rather than a single measurement in the third trimester, would associate with birth weight. No correlations were observed between changes in serum total, LDL, or HDL cholesterol levels, or serum triglycerides or phospholipids and birth weight. Examination of correlations of the change in serum squalene and noncholesterol sterols during pregnancy with birth weight revealed that only the change in the ratio of cholestanol to cholesterol correlated with birth weight both in the controls ($r = 0.519$, $P < 0.01$) and in the GDM group ($r = 0.454$, $P < 0.05$).

Because the main difference between GDM and control pregnancies was the finding of higher squalene levels already in the second trimester, we next examined whether serum squalene correlated with birth weight at that point. There was a strong correlation between second trimester maternal serum squalene concentration ($r = 0.760$, $P < 0.001$) (Fig. 2) and the ratio of squalene to cholesterol with neonatal birth weight ($r = 0.614$, $P = 0.002$) and with relative birth weight ($r = 0.658$, $P = 0.001$) in GDM subjects. Analysis of other sterols during the second trimester revealed that serum lathosterol and desmosterol ($r = 0.504$, $P < 0.05$, $r = 0.582$, $P < 0.01$, respectively) and Δ8-cholestenol ($r = 0.505$, $P < 0.05$) concentrations also showed significant, although less profound, correlations with birth weight and also with relative birth weight. Thus, it appeared that second trimester maternal cholesterol synthesis markers correlated with fetal weight in subjects with GDM. No correlation was detected in control subjects.

To further analyze the relative strength of maternal parameters associating with neonatal birth weight, we performed stepwise multiple regression analysis separately at each trimester. When maternal prepregnancy BMI, weight

gain, age, and trimester-specific plasma glucose and serum insulin levels, serum total, LDL, and HDL cholesterol, serum triglycerides and squalene and noncholesterol sterols were included as independent variables in the analysis, no significant associations were detected in the first trimester. In the second trimester, only maternal serum squalene (concentration and ratio to cholesterol) (R^2 of the model: 0.425, $P < 0.01$) and in the third trimester only serum desmosterol concentration (R^2 of the model: 0.295 and $P < 0.05$) associated with neonatal birth weight in the GDM group. In the control group using the same independent variables, only the third trimester serum cholestanol (concentration and ratio to cholesterol) associated with birth weight (R^2 of the model: 0.191, $P < 0.05$).

DISCUSSION

The present study explores, for the first time, serum squalene and noncholesterol sterols in pregnancies affected by obesity and GDM, and further follows these variables 12 months postpartum. We proposed that impaired glucose metabolism during pregnancy could be associated with changes in cholesterol metabolism, detectable by analyzing serum squalene and noncholesterol sterols. As maternal lipids, along with glucose levels, have been shown to be important determinants of fetal growth, we also explored the potential associations between maternal squalene and noncholesterol sterols and neonatal birth weight. The main new findings in our study were that, in obesity, GDM associated with higher cholesterol synthesis markers in the first trimester, and elevated serum squalene levels in the second and third trimester of pregnancy, and that maternal serum squalene and cholesterol synthesis markers correlated with birth weight.

Effect of obesity, serum insulin, and plasma glucose

Because we enrolled only pregnant women who were at increased risk for developing GDM, all our study subjects were obese. Previously, we have shown that obesity and weight change influence cholesterol metabolism (14, 28, 29). Therefore, it was somewhat surprising that the change in weight did not associate with the change of any of the measured sterols, though it did associate with serum insulin and plasma glucose concentrations. It may be that pregnancy itself is metabolically such a demanding state requiring increased cholesterol synthesis (18), that minor potential changes induced by weight change in greatly obese subjects could be unmasked.

Although GDM mimics T2D, it also differs greatly regarding diagnostic criteria, i.e., diagnosis is based on 2 h OGTT and the diagnostic thresholds are much lower. This is based on findings in the HAPO study showing continuous association of glucose levels to pregnancy complications, even with glucose levels below the diagnostic values for GDM (1). By current definition, all the subjects in our present study group had GDM, based on OGTT, and their plasma glucose values were higher than in the control subjects, but they were in good glycemic control and had normal nonpregnant plasma glucose values. Although we have previously shown that insulin regulates cholesterol metabolism to a greater extent than obesity (30), and that cholesterol metabolism was altered already in subjects with impaired fasting glucose in the METSIM study (31), the plasma glucose values in our previous studies have been higher. The tight glycemic control in GDM subjects might have attenuated the impact of glycemic variation on cholesterol metabolism.

Lipids and lipoproteins in pregnancy

Pregnancy hyperlipidemia, as previously described, consisted of the increase of serum total cholesterol and triglycerides. Furthermore, all measured lipids, i.e., LDL and HDL cholesterol and serum phopholipids were higher in pregnancy than postpartum. The controversy observed in previous studies exploring lipid levels in GDM is not completely clear, but might be due to varying diagnostic criteria used for GDM over the years, use of insulin, and differences in glycemic control and steroid hormone levels during pregnancy (32). We did not observe any significant differences in serum total, LDL, or HDL cholesterol, triglycerides, or phospholipids in our GDM subjects with good glycemic control, as compared with the controls. It thus seems that noncholesterol sterols are more sensitive markers than serum and lipoprotein lipids to show subtle changes occurring in cholesterol metabolism in GDM.

Cholesterol homeostasis

In many conditions, but not always (33), cholesterol synthesis and absorption are in homeostasis, i.e., increased cholesterol synthesis associates with decreased cholesterol absorption [reviewed in (17)]. This is reflected by negative correlation between synthesis markers and absorption markers. In the present study, cholesterol synthesis and absorption were in homeostasis in the beginning of pregnancy, but in the third trimester in pregnancies affected by GDM, only desmosterol and cholestanol were interrelated, suggesting that homeostasis was partially lost in the GDM group.

Squalene

The observations that the serum Δ8-cholestenol concentration and ratio to cholesterol, as well as the serum lathosterol/sitosterol ratio, were higher in the first trimester in the GDM group than in the controls, suggest that cholesterol synthesis was somewhat elevated in pregnancies affected by GDM, which is in accordance with our previous studies regarding T2D (13, 29). In the second or third trimester of pregnancy, the groups did not differ in serum concentrations or ratios of noncholesterol sterols, cholestanol, or plant sterols. However, the finding that the ratio of squalene to cholesterol in the second and third trimester was higher in subjects with GDM than in the controls is of interest.

Squalene is a nonsteroidal precursor of cholesterol, and it is both absorbed from the intestine and synthesized in the adipose tissue, liver, and skin. Adipose tissue synthesizes and stores high amounts of squalene, but releases

it extremely slowly, if at all (34). Squalene can be obtained from the diet, but it is unlikely that dietary causes would account for the higher serum squalene levels in the GDM group, because subjects with GDM did not receive any dietary counseling favoring consumption of squalene-rich foods (e.g., olive oil). Squalene, in contrast to the noncholesterol sterols, is mainly carried in triglyceride-rich lipoproteins (35), but serum triglyceride levels did not explain the observed difference, as serum triglycerides did not differ between the groups. Serum squalene concentration has been shown to associate with visceral fat, but not with BMI, in subjects with normal glucose tolerance (36). In the present study, BMI did not correlate with serum squalene level, nor is it likely to explain the differences between the groups, as the results were adjusted for BMI. However, in the controls, serum insulin correlated with serum squalene concentration in the third trimester. Whether this correlation was lost in GDM subjects, or whether the lack of correlation simply reflects insensitivity of single serum insulin measures, requires further investigation and larger study material.

What do the elevated serum squalene levels then indicate in GDM pregnancy? In many conditions, serum squalene reflects cholesterol synthesis, but less frequently than serum Δ8-cholestenol, or desmosterol and lathosterol [reviewed in (17)]. Serum squalene has been high in obesity, metabolic syndrome, and T2D, i.e., in situations with increased cholesterol synthesis from redundancy in the substrate pool. In the second trimester of pregnancy, serum squalene concentration correlated positively to serum lanosterol, Δ8-cholestenol, and desmosterol concentrations, and most likely simply reflects increased cholesterol synthesis. These results suggest that serum squalene might be the most sensitive marker to detect changes in cholesterol metabolism induced by mild disturbances in glucose metabolism in obese GDM subjects.

Noncholesterol sterols

During pregnancy, only the ratios of desmosterol and cholestanol to cholesterol increased significantly in both groups. It has previously been shown that serum cholestanol increases more in cholestatic pregnancies than in noncholestatic ones, but even in noncholestatic pregnancies, the rise in serum cholestanol is substantial, while the amounts of serum plant sterols do not change (18), a situation resembling our present findings in both groups. The increase of serum cholestanol may indicate either increased cholestanol synthesis from cholesterol or decreased elimination via biliary excretion, i.e., mild cholestasis, frequently present in pregnancy, or both (18). The role of maternal serum cholestanol and its association to birth weight in control pregnancies is ambiguous, especially if cholestanol reflects mild cholestasis. In the cord blood of fetuses, serum cholestanol has been shown to be high, and to drastically reduce during the first year of life (37). Further studies are required to examine whether associations exist between maternal and cord-blood cholestanol, and whether increased serum cholestanol simply reflects cholestasis in pregnancy. In this respect, it is of

interest that maternal cholestasis during pregnancy has been shown to program metabolic disease and obesity in the offspring (38, 39).

The observation that the ratio of Δ8-cholestenol decreased during pregnancy is in accordance with the previous study (18). It might be that during pregnancy the activity of the enzyme converting Δ8-cholestenol to lathosterol is enhanced more than the other enzymes in the Kandutsch-Russell cholesterol synthesis chain. The finding that in GDM subjects both the ratios of Δ8-cholestenol and lathosterol decreased during pregnancy, while that of desmosterol increased, suggests that the cholesterol synthesis pathway through the desmosterol, i.e., the Bloch route, might have been preferred instead of the Kandutsch-Russell route. There are many situations in which either of the pathways are preferred [reviewed in (40)], but the importance of that is not yet known. The marked rise in the serum ratio of desmosterol in both groups after delivery, again apparently reflects increased cholesterol synthesis through the Bloch pathway, required in lactation for secretion of cholesterol to human milk, which is very rich in desmosterol (41). Taken together, it may be that, in pregnancy, cholesterol synthesis via the Bloch pathway is preferred, especially if the pregnancy is affected by GDM.

Neonatal weight and maternal cholesterol

Previous studies have shown variable associations between maternal serum lipids/lipoproteins and neonatal weight. Many, but not all (42), studies are limited to only one varying time-point measurement during pregnancy. Neonatal birth weight has been shown to correlate with maternal serum triglycerides in pregnancies with normal glucose tolerance (43–45) or GDM (3, 46), and negatively with serum HDL cholesterol concentration (6), but only in cases with obesity (42). In our study, only third trimester maternal serum phospholipids correlated with birth weight in controls, but not in pregnancies affected by GDM. In diabetic pregnancy, altered lipoproteins in the maternal side could potentially affect the quantity and quality of lipids available for the fetal needs. Phospholipid transfer protein is expressed in placental endothelium and enhances cholesterol efflux by interaction with ABCA1 (47). HDL cholesterol concentration peaked already in second trimester, as reported previously and also in other studies, and HDL cholesterol and phospholipids might be important for fetal development in the early pregnancy.

Cholesterol and fetal cholesterol synthesis are crucial for embryonic development. This is demonstrated by rare genetic disorders caused by defects in cholesterol biosynthesis, for example, Smith-Lemli-Opitz syndrome, in which inactivating mutations in the gene coding for 7-dehydrocholesterol reductase results in accumulation of 7-dehydrocholesterol, lack of cholesterol, and severe congenital abnormalities (48, 49). However, even in newborns with 7-dehydrocholesterol reductase-null mutations, there is a detectable amount of cholesterol in serum, suggesting the presence of an exogenous cholesterol source.

Furthermore, in cord blood of healthy fetuses, plant sterol concentrations were 40–50% of the maternal levels, indicating active maternal-fetal sterol transport (37).

There is growing evidence that the fetus depends on the mother's cholesterol metabolism mostly in the beginning of pregnancy, before fetal endogeneous cholesterol synthesis begins [reviewed in (50)]. Interestingly, already at that time, cholesterol synthesis appears to be slightly upregulated in subjects who were later diagnosed for GDM. Recent data analyzing cholesterol sterols in amniotic fluids at different stages of pregnancy showed that the ratios of lanosterol and lathosterol to cholesterol increases after 19 weeks of gestation, reflecting the beginning of fetal cholesterol synthesis (51, 52). Thus, the most sensitive period regarding fetal cholesterol requirements for the mother, is the time up to 19 weeks of pregnancy. It is of note, that apparently at that time maternal serum squalene levels were higher in GDM pregnancies and correlated significantly to neonatal birth weight. Also, in multiple stepwise regression analysis, only second trimester serum squalene associated with birth weight of the infants born to GDM mothers. It is conceivable that serum squalene reflects increased maternal cholesterol synthesis and increased fuel supply for the growing fetus. This assumption is supported also by the association of another cholesterol synthesis marker, i.e., maternal serum desmosterol in the third trimester, with birth weight. Taken together, these findings suggest that in this fairly small group of obese GDM subjects, activity of maternal cholesterol metabolism might have contributed to neonatal weight. At least partly due to their good glycemic control, GDM subjects did not have any large-for-date newborns. Thus, further studies and larger study populations are required to investigate whether maternal cholesterol synthesis could play any clinical role in fetal macrosomia, often complicating GDM pregnancies with poor glycemic control. Also, it remains to be seen if the differences observed in GDM pregnancies in the present study apply also in normal weight pregnancies.

CONCLUSION

We have shown that, in obesity, GDM associated with elevated serum markers of cholesterol synthesis in the first trimester and with higher levels of serum squalene in the second and third trimester of pregnancy. In pregnancies affected by GDM, maternal serum squalene in the second trimester and desmosterol in the third trimester associated with birth weight, suggesting the potential contribution of maternal cholesterol synthesis to neonatal birth weight.

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