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MATERNAL-FETAL MEDICINE

Reference intervals of serum lipids in the second and third trimesters of pregnancy in a Caucasian cohort: the LIFE Child study

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Abstract

Background The study aimed to establish reference intervals for serum lipids and apolipoproteins in pregnant women depending on trimester and parity, and to investigate the influence of various factors on lipid and apolipoprotein concentrations.

Materials and methods A total of 748 pregnant women ($n = 683$ in the second trimester, $n = 676$ in the third trimester) were included in the study and reference intervals for total cholesterol (TC), HDL, LDL, triglycerides (TG), apoA1 and apoB were determined as empirical quantiles. The measurement of serum lipids was performed using a validated specific homozygous enzymatic color test. Hierarchical models were used to investigate hypothesized relations.

Results Except for apoA1, all serum lipids levels showed a significant change from the second to the third trimester. This increase was most pronounced for TGs. Especially in the third trimester, the concentrations of serum lipids exceeded the currently accepted reference values for non-pregnant women by a factor of 2.5. Reference intervals of serum lipids at the second and third trimesters in healthy pregnant women were as following: TC 4.45–8.99 and 4.83–9.71 mmol/l, HDL 1.33–3.06 and 1.16–3.13 mmol/l, LDL 2.14–6.11 and 2.35–6.98 mmol/l, TG 0.92–3.0 and 1.37–4.76 mmol/l as well as apoB 0.69–1.93 and 0.85–2.21 g/l. Parity and nutrient intake were not significantly associated with changes in lipid concentration. Prematurity was associated with a significant decrease in TC and TG levels.

Conclusion Detailed reference values for serum lipids and apolipoproteins in pregnancy are now available for a Caucasian cohort. Further, long-term studies are still needed to assess the effect of the extensive concentration changes of serum lipids in pregnancy and their atherogenic risk definitively.

Keywords LIFE Child · Pregnancy · Reference data · Serum lipids · Caucasian cohort

Abbreviations

~	Approximately
ANOVA	Analysis of variance
apoA1	Apolipoprotein A1
apoB	Apolipoprotein B
Body mass index	BMI
DHEAS	Dehydroepiandrosteron sulfate
ECLIA	Electrochemiluminescence immunoassay
FFQ	Food frequency questionnaire
GDM	Gestational Diabetes Mellitus
HDL	High-density lipoprotein cholesterol

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LC–MS/MS	Liquid chromatography–mass spectrometry
LDL	Low-density lipoprotein cholesterol
PAH	Pregnancy associated hypertension
SHBG	Sex hormone binding globulin
TC	Total cholesterol
TG	Triglyceride

Introduction

Chronic diseases such as obesity, diabetes mellitus and cardiovascular diseases continue to increase [1, 2] and are still leading causes for premature death in adulthood in Germany [3]. Such civilization diseases occur in younger generations and women in reproductive age progressively. Investigations have shown a tripled increase in the prevalence of obesity in pregnant women, which is affecting fertility and the risk for pregnancy-related complications [4].

Serum lipids were influenced by many factors and were a frequently investigated object in relation to civilization diseases [5]. Conditioned by altered endogenous synthesis and transport mechanisms [6] as well as sex hormone concentrations [7, 8], the levels of serum lipids change (physiologically) during pregnancy [9, 10]. These shifts of concentration can be observed as early as the 12th week of pregnancy and progress with advancing gestational age [7]. Dyslipidaemias in pregnancy correlate with several diseases such as preeclampsia, gestational diabetes (GDM), preterm delivery [11] and gestational hypertension [12], and are associated with increased maternal and neonatal morbidity. A manifested hypertriglyceridemia in the first trimester precedes preeclampsia [13, 14]. Hyperlipidemias are also a major risk factor for atherosclerotic pathologies in pregnancy and their risk of developing a future cardiovascular disease [8, 15, 16]. Probably, this effect will be triggered by multiparity [17].

Serum lipids and the biosynthesis of steroid hormones are closely linked [18]. The physiological increase in androgen levels is essential for establishing and maintaining pregnancy as well as cervical remodeling processes and the onset of childbirth [19]. Nevertheless, the effects of these increases are not entirely understood. Only a few studies investigate the relation between serum lipids and androgen/steroid hormones levels of pregnant women. Further, reference values for serum lipids often do not include apolipoproteins and ignore possible influential factors like nutrition intake. Also, the effect of multiparity or twin gestation is neglected. Therefore, the study aimed to establish prospective values of serum lipids in a healthy, representative Caucasian cohort and to detect the relation to various factors.

Study population and design

The LIFE Child cohort is a longitudinal cohort, initiated in July 2011, by the Leipzig Medical Faculty, Department for Child and Adolescent Medicine. The aim of the LIFE Child study is the detection and analysis of environmental factors related to growth and development and health of newborns, children and adolescents. Subjects are examined in the second trimester (24th–26th week of pregnancy) and the third trimester (34th–36th week of gestation) to investigate later the influence of prenatal factors [20]. In addition, follow-up examinations of mothers and children are carried out postpartum. The participation on the LIFE Child study is voluntary. Between July 2011 and August 2018, a total of 748 Caucasian pregnant women were recruited ($n_{\text{2nd trimester}} = 683$, $n_{\text{3rd trimester}} = 676$). All women with at least one valid blood sample in one of the two trimesters were included in the investigation. Ten women had a twin gestation. The mean maternal age of the total cohort was 30.8 years (± 4.4 years). The average age of primipara was 29.9 years, of multi-para was 32.3 years. Thus, the ages were slightly above the statistical average values of the Federal Republic of Germany [21, 22]. Only healthy pregnant women were included to establish reference values. Subjects with gestational diabetes (GDM), gestational hypertension (PAH), placental insufficiency, preeclampsia and premature labor were excluded, respective considered as a separate group. Patients on lipid-lowering medication were excluded from analyses, but no one fulfilled this criterion.

The study was approved by the Ethical Committee of the University of Leipzig (reference number: Reg. No. 264-10-19042010). LIFE Child is registered by the trial number: NCT02550236.

Mutterpass (maternity notes)

Since 1968, the *Mutterpass* (maternity notes) has been introduced in Germany [23]. It is used for standardized documentation of the course of pregnancy from conception to delivery. Complications such as GDM, PAH or preterm birth were taken prenatal or postpartum from this document. In the maternity notes, the diagnosis preeclampsia is not listed clearly. Therefore, all subjects with the criteria of proteinuria and hypertension (> 140/90 mmHg) or placental insufficiency and hypertension and/or proteinuria are summarized below. The gain of the body mass index (BMI) was calculated as difference of BMI at the beginning of the pregnancy and time of delivery.

Federal food key (bundeslebensmittelschlüssel—BLMS)

Listing and calculation of nutrients based on the federal food key (BLMS), a German database for the nutrient content of food. The data were collected by a food frequency questionnaire (FFQ). This FFQ is part of the GINI PLUS study [24, 25] and was originally developed to assess the intake of fatty acids and antioxidants in school age and the development of allergic diseases [26]. It was assumed that the data are documented in detail and truthfully especially during pregnancy.

Lipid and hormones measurements

Venous blood was taken from the fasting subjects of the LIFE study. The predetermined fasting times were 12 h in the second and at least 4 h in the third trimester, because prolonged fasting periods did not seem acceptable. Fasting times less than 9–12 h may affect the level of triglycerides especially. Non-compliance was not an exclusion criterion, but most of the subjects adhered to the predetermination. Non-compliance was observed only in very few cases of the second trimester. The measurement of laboratory parameters was carried out in the Institute for Laboratory Medicine of the University Hospital. The measurement of serum lipids was performed on a "Cobas 8000 Clinical Chemistry Analyzer" with test kits from Roche Diagnostics GmbH. The determination of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides was performed using a validated specific homozygous enzymatic color test. LDL cholesterol was also measured and not calculated according to Friedewald. ApoA1 and apoB were determined by an immunological turbidity testing. The concentrations for DHEAS, testosterone, estradiol and cortisol were measured by liquid chromatography–mass spectrometry (LC–MS/MS). The measurement of SHBG was performed by ECLIA on a "Cobas Clinical Chemistry Analyzer" by Roche. Comparative values of non-pregnant, young females were based on the reference values recommended by the Institute of Laboratory Medicine of the University Hospital Leipzig [27].

Statistical analysis

Preprocessing and analysis of data were done using the statistics program R [28]. Plausibility for all values was tested and ascertained, but no outliers were eliminated. Missing values were handled as missing completely at random. The reference intervals were estimated as the 2.5th–97.5th percentiles of the measurements. Confidence intervals on quantiles were estimated based on binomial testing. Differences between trimesters and pregnancy-related complications were investigated and tested on significance by analysis of variance (ANOVA) for repeated measurements. To account for multiple (dependent) measurements per person (second and third trimesters), linear mixed models/hierarchical models were used. The distribution of serum lipid levels was visualized as boxplots.

Results

The study included a total of 748 subjects ($n_{\text{2nd trimester}} = 683$, $n_{\text{3rd trimester}} = 676$). Four hundred and fifty-two primipara and 296 multi-para were included. Thirty-six subjects were calculated separately because they fulfilled one or more of the following conditions: GDM ($n = 20$, 2.7%), PAH ($n = 5$, 0.7%), placental insufficiency ($n = 1$) or premature labor ($n = 10$). Prematurity defined as a delivery $\leq 37 + 0$ weeks of gestation occurred in 48 cases (6.4%). There was no case of preeclampsia.

The 95% reference intervals (2.5th–97.5th percentiles) and the associated confidence intervals are listed for all serum lipid levels (Table 1). The upper reference limit of TC exceeded the currently accepted medical cut-off of 5.2 mmol/l for non-pregnant women by a factor of 1.8 (Table 1). The mean increase in the concentration of TC from the second to the third trimester was 0.73 mmol/l ($p < 0.001$) (Table 2). For parity (primipara, multiple gestation) or a twin gestation, no significant differences in lipid concentrations (TC, HDL, LDL, TG, apoA1, apoB) could be shown. For HDL cholesterol, we found a reference range of 1.33–3.06 mmol/l in the second trimester and a slightly wider reference interval in the third trimester (1.16–3.13 mmol/l) (Tables 1, 2), while the mean HDL level drops by 0.15 mmol/l ($p < 0.001$). Thus, the reference range is comparable to the cut-off of non-pregnant women (Table 1). The reference interval of LDL cholesterol was 2.14–6.11 mmol/l in the second trimester and 2.35–6.98 mmol/l in the third trimester (Table 1). Thus, the upper reference value exceeded the medical limit value for non-pregnant women (less than 4.2 mmol/l) by factor 1.6. A significant increase in 0.62 mmol/l in the concentration of LDL cholesterol is observed from second to third trimester (Table 2). The LDL/HDL ratio in the second trimester was 1.61–1.99 and 2.03–2.23 in the third trimester. Triglycerides showed the strongest change in concentration; there was a significant mean increase of 0.93 mmol/l ($p < 0.001$) from the second to the third trimester (Table 2). The upper reference limits of 3.0 mmol/l and 4.8 mmol/l for the second respective the third trimester exceeded the conventional cut-off by the factor of 1.8 respective 2.8. Even the mean values exceeded the upper reference limit of 1.7 mmol/l clearly. The

Table 1 Reference values for total cholesterol (TC) (mmol/l), HDL cholesterol (HDL) (mmol/l), LDL cholesterol (LDL) (mmol/l), triglycerides (TG) (mmol/l), apolipoprotein A1 (apoA1) (g/l) and apolipoprotein B (apoB) (g/l) depending on trimesters of the healthy LIFE Child cohort ($n = 712$)

	P2.5	CI		P50	CI		P97.5	CI		Ref. data[a]
TC (mmol/l)										
Total	4.61	4.47	4.70	6.52	6.41	6.61	9.61	9.33	9.74	< 5.2
Second trim.	4.45	4.32	4.54	6.13	6.02	6.24	8.99	8.64	9.61	
Third trim.	4.83	4.68	4.98	6.94	6.84	7.03	9.71	9.60	10.22	
HDL (mmol/l)										
Total	1.21	1.15	1.28	2.05	2.01	2.09	3.09	3.01	3.23	> 1.03
Second trim.	1.33	1.21	1.38	2.14	2.11	2.17	3.06	2.98	3.29	
Third trim.	1.16	1.08	1.21	1.97	1.93	2.01	3.13	2.98	3.31	
LDL (mmol/l)										
Total	2.20	2.05	2.28	4.03	3.92	4.12	6.60	6.40	7.11	< 4.2
Second trim.	2.14	1.91	2.24	3.66	3.56	3.77	6.11	5.91	6.47	
Third trim.	2.35	2.07	2.58	4.34	4.23	4.45	6.98	6.61	8.08	
LDL/HDL ratio										
Total	1.81						2.14			< 2.5[b]
Second trim.	1.61						1.99			
Third trim.	2.03						2.23			
TG (mmol/l)										
Total	0.97	0.92	1.00	2.02	1.94	2.08	4.20	4.04	4.71	< 1.7
Second trim.	0.92	0.87	0.94	1.59	1.53	1.63	3.00	2.87	3.38	
Third trim.	1.37	1.21	1.44	2.47	2.38	2.56	4.76	4.43	5.29	
apoA1 (g/l)										
Total	1.56	1.48	1.60	2.15	2.13	2.19	2.81	2.75	2.91	1.04–2.25
Second trim.	1.55	1.40	1.60	2.15	2.11	2.20	2.75	2.69	2.91	
Third trim.	1.58	1.45	1.63	2.17	2.12	2.21	2.86	2.80	3.10	
apoB (g/l)										
Total	0.72	0.67	0.78	1.30	1.28	1.33	2.11	2.02	2.29	0.6–1.17
Second trim.	0.69	0.65	0.76	1.18	1.14	1.23	1.93	1.86	2.15	
Third trim.	0.85	0.65	0.93	1.43	1.38	1.48	2.21	2.10	2.69	

The table shows the 2.5th (P2.5), 50th (P50, median) and 97.5th percentile (P97.5) and their associated 95% confidence intervals (CI)

[a]Medical limit values/cut offs of non-pregnant, healthy women recommended by the Institute for Laboratory Medicine of the University Hospital Leipzig

[b]Cut-off recommendation for LDL/HDL ratio [32]

reference interval of TG estimated for the second trimester was 0.92–3.0 mmol/l and 1.37–4.76 mmol/l for the third trimester (Table 1). There was no relevant increase in apolipoprotein A1 from the second to the third trimester (Table 2). The upper reference limit in pregnant subjects exceeded the upper limit in non-pregnant women by 0.5 g/l. In apolipoprotein B, a significant increase of 0.23 g/l was observed between the trimesters (Table 2). The reference interval estimated during the second trimester was 0.69–1.93 g/l and 0.85–2.21 g/l during the third trimester (Table 1). Thus, the reference value exceeded the reference value of non-pregnant women by factor 1.8.

No significant increases in the concentration of serum lipids could be shown for the gestational pathologies such as GDM, PAH, premature labor or placental insufficiency (data not shown). Only prematurity was significantly associated with lower levels in total cholesterol and triglycerides (Table 2, Fig. 1a, b). Surprisingly, after combining all pregnancy-related complications (GDM, PAH, prematurity, preterm labor, placental insufficiency), significantly lower lipid concentrations were found for total cholesterol as well as significantly higher levels for HDL cholesterol and apoA1 compared to inconspicuous pregnancies (Table 2, Fig. 1c, d). The analysis of the nutrition intake according to the FFQ showed the following mean values: fats 600 g/week (~ 90 g/day), total carbohydrates 2000 g/week (~ 300 g/day) and fructose 100 g/week (~ 15 g/day). The mean energy input was ~ 17000 kcal/week (~ 2400 kcal/day). There was no significant association between the nutrient intakes and any of the lipid subtypes (Table 3). Also, no significant correlations between the serum lipids and the hormones testosterone

Table 2 Variance analyses of the individual serum lipids (TC, HDL, LDL, TG, apoA1, apoB)

	Mean diff.[a]	CI[b]	*p**
TC (mmol/l)			
Third versus second trimester	0.73	[0.59; 0.86]	< 0.001*
Prematurity versus mature born	− 0.44	[− 0.81; − 0.08]	0.02*
Pregnancy complications versus "healthy pregnancy"	− 0.27	[− 0.53; − 0.01]	0.04*
HDL (mmol/l)			
Third versus second trimester	− 0.15	[− 0.20; − 0.10]	< 0.001*
Prematurity versus mature born	0.11	[− 0.03; 0,25]	0.12
Pregnancy complications versus "healthy pregnancy"	0.1	[0.002; 0.20]	0.04
LDL (mmol/l)			
Third versus second trimester	0.62	[0.47; 0.79]	< 0.001*
Prematurity versus mature born	− 0.36	[− 0.81; 0.1]	0.12
Pregnancy complications versus "healthy pregnancy"	− 0.25	[− 0.54; 0.04]	0.09
TG (mmol/l)			
Third versus second trimester	0.93	[0.84; 1.01]	< 0.001*
Prematurity versus mature born	− 0.39	[− 0.65; − 0.13]	0.004*
Pregnancy complications versus "healthy pregnancy"	− 0.13	[− 0.31; 0.05]	0.16
apoA1 (g/l)			
Third versus second trimester	0.04	[− 0.006; 0.09]	0.09
Prematurity versus mature born	0.06	[− 0.08; 0.20]	0.36
Pregnancy complications versus "healthy pregnancy"	0.09	[0.007; 0.18]	0.03
apoB (g/l)			
Third versus second trimester	0.23	[0.18; 0.28]	< 0.001*
Prematurity versus mature born	− 0.13	[− 0.28; 0.02]	0.08
Pregnancy complications versus "healthy pregnancy"	− 0.07	[− 0.17; 0.02]	0.13

Shown are the mean differences between the target variables, the 95% confidence interval of the difference and the *p* values. With the exception of apoA1, significant differences in concentration (*) between the second and third trimesters could be shown. No significances were observed for parity and twin gestation. All other variance analyses for individual lipid markers and pregnancy-related complications such as GDM, PAH and premature contractions showed no significance

[a]Mean difference between the indicated groups (mmol/l)

[b]95%—confidence interval (CI) for difference between the indicated groups

[c]Significance $p < 0.05$

and SHBG could be detected (Table 3). However, the protective lipid types HDL cholesterol and apoA1 showed a significant positive relation to the hormone DHEAS. Also, cortisol and estradiol showed a significantly positive relation to HDL cholesterol and apolipoprotein A1. Furthermore, estradiol was significantly positively associated with TC (Table 3). The overall mean systolic blood pressure was 115 mmHg, and the mean diastolic was 70.5 mmHg. There was a significant increase of around 0.1 mmol/l in triglycerides per increase of 10 mm Hg. Interestingly, an increasing BMI gain was associated with significantly higher HDL levels. Increasing maternal age was significantly related to lower cholesterol and apoB concentrations. The respective association with LDL cholesterol was also inverse but did not reach the level of significance (Table 3).

Discussion

Serum lipids are influenced multifactorial, e.g., by ethnic and geographical factors [29, 30]. Moreover, there is a significant change in concentrations of serum lipids during pregnancy resulting by complex processes that have not been fully clarified yet. The atherogenic risk is discussed controversially [15, 17, 31]. Therefore, it is necessary to establish nationally conclusive reference levels for serum lipids in a pregnant Caucasian cohort.

A comparative Polish study designed by Piechota et al. (1992) with similar numbers of subjects showed a similar pattern for the distribution of the serum lipids in pregnancy. The values for LDL cholesterol, triglycerides, apoA1 and apoB were measured approximately

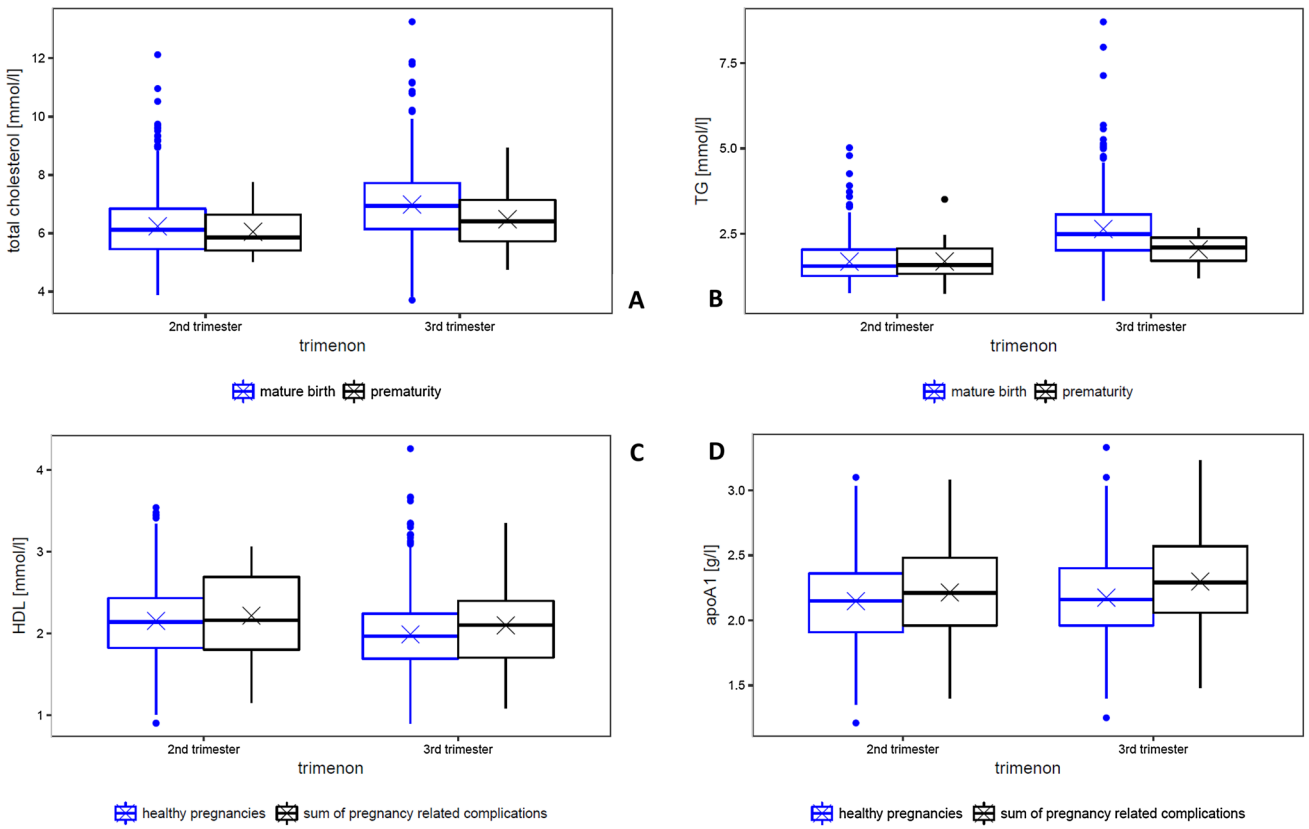

Fig. 1 Significant changes in concentration between the second and third trimesters via boxplots. Figures **a** and **b** show the significantly lower concentrations for total cholesterol ($n=676$) **a** and triglycerides **b** in prematurity ($\leq 37+0$ weeks of pregnancy, $n=48$). Figures **c** and **d** show the significantly higher concentrations for HDL **c** as well as apoA1 **d** in pregnancy-related complications ($n=84$)

0.1–0.5 mmol/l and g/l lower than our results. This difference may results in the divergent definition of the upper reference limits (95th vs. 97.5th percentile). However, the concentration of HDL cholesterol was higher approximately 0.3–0.4 mmol/l in the LIFE Child study, also after alignment of the reference levels. The 95th percentile for TC in the third trimester by *Piechota* was 9.83 mmol/l. Therefore, the value measured higher than the 97.5th reference level of 9.71 mmol/l in the LIFE Child study [31]. In contrast, *Piechota* observed an increase in apoA1 levels between the trimesters, which was not detected in our study [31].

The LDL/HDL ratio measured in the second and third trimesters was less than the recommended cut-off of 2.5 [32, 33]. The reported Chinese ratio in pregnant women was significantly higher. In addition, the investigations by Ying et al. [34] showed lower apoA1 concentrations and a higher upper standard value for triglycerides over twice. Based on the low ratio in our cohort, it can be speculated that there is no evidence for an increased risk of cardiovascular events in Caucasian pregnant women. Furthermore, lipid levels return after delivery to baseline, encouraging the hypothesis of low atherogenic risk [10]. But reliable conclusions on atherosclerotic risk driven by data from the non-pregnant state cannot be assigned to pregnancy. Therefore, further long-term studies, also in the later life of the subjects, are necessary to give a reliable statement about the atherogenic risk.

In general, the observed changes in lipids and lipoproteins during pregnancy are consistent with most literature data [31, 34, 35]; they do not bring really new information, unfortunately. But they confirm among other long past investigations.

The exact biochemical processes predisposing for the change in lipid levels during pregnancy are not fully understood. The altered androgen concentrations as well as altered transport and oxidation processes seem to be responsible [6, 7, 31]. Since the 1960s, androgens have been thought to act as substrates for estrogen formation in the placenta during pregnancy [19]. Physiologically, there is an increase in bioavailable testosterone in the third trimester, which is associated with significantly higher LDL and low HDL cholesterol levels [8]. Estradiol, as a cleavage product of testosterone, is also rising strongly. Circulating levels of DHEAS decrease over 50%, while SHBG increases dramatically during pregnancy [19]. The concentrations in serum lipids

Table 3 Elevation of relation (β) between serum lipids and various independent variables as well as their p values

	TC	HDL	LDL	TG	apoA1	apoB
BMI gain						
β	0.034	*0.021*	0.010	0.015	0.015	0.002
p	0.29	*0.04*	0.95	0.53	0.16	0.99
Systolic blood pressure (per 10 mmHg)						
β	0.006	< −0.001	0.001	*0.010*	0.003	0.003
p	0.34	0.99	0.98	*0.002*	0.20	0.29
Diastolic blood pressure (per 10 mmHg)						
β	0.008	< −0.001	0.003	*0.014*	0.003	0.003
p	0.39	0.99	0.92	*< 0.001*	0.37	0.37
Maternal age						
β	*−0.026*	−0.005	−0.018	0.002	< 0.001	*−0.009*
p	*0.03*	0.47	0.24	0.95	0.97	*0.04*
DHEAS						
β	0.009	*0.070*	−0.047	−0.042	*0.049*	−0.010
p	0.98	*< 0.001*	0.29	0.14	*< 0.001*	0.55
SHBG						
β	< −0.001	< −0.001	< 0.001	< 0.001	< −0.001	< 0.001
p	0.99	0.28	0.97	0.77	0.15	0.78
Testosterone						
β	−0.016	−0.004	−0.032	−0.010	0.001	−0.002
p	0.93	0.98	0.58	0.94	0.99	0.99
Cortisol						
β	< 0.001	*< 0.001*	< 0.001	< −0.001	*< 0.001*	< 0.001
p	0.30	*0.02*	0.81	0.73	*0.002*	0.91
Estradiol						
β	*< 0.001*	*< 0.001*	< 0.001	< 0.001	*< 0.001*	< 0.001
p	*< 0.001*	*< 0.001*	0.09	0.20	*< 0.001*	0.19
Total fat intake						
β	< −0.001	< 0.001	< −0.001	< −0.001	< 0.001	< −0.001
p	0.73	0.99	0.55	0.99	0.59	0.41
Total carbohydrate intake						
β	< −0.001	< −0.001	< −0.001	< 0.001	< 0.001	< −0.001
p	0.24	0.53	0.19	0.79	0.99	0.33
Total sugar intake						
β	< −0.001	< −0.001	< 0.001	< 0.001	< 0.001	< −0.001
p	0.23	0.56	0.84	0.84	0.99	0.34
Total energy intake						
β	< −0.001	< −0.001	< −0.001	< 0.001	< 0.001	< −0.001
p	0.28	0.86	0.18	0.99	0.88	0.23

Significant relations are highlighted in italicized values

Significance $p < 0.05$

and apolipoproteins rise during pregnancy, conditioned by increasing synthesis and secretion of a triglyceride-rich apoB. However, in this investigation, no relation between the androgen/steroid hormones and the triglycerides, LDL and apoB could be established. Only estradiol was positively correlated with TC. According to *Piechota,* HDL cholesterol is not correlated with any of the hormones. However, our investigations showed such positive relation to DHEAS, cortisol and estradiol. Similar correlations could be demonstrated for apoA1.

In agreement with the Polish study, no significant influence on serum lipids was found for parity. The lower levels for apoB and cholesterol, which are correlated to the maternal age and the summarized pregnancy pathologies, may be related to increased oxidation processes and atherosclerotic changes within the placenta [36].

The recommended balanced nutritional intake during pregnancy is given as 300–400 g/day carbohydrates, 70–80 g/day fats and 2200 kcal/day energy intake in the second trimester and 2500 kcal/day in the third trimester [37]. The evaluation of the FFQ showed an increased intake of fats by almost 10 g/day, but observed no effect on the lipid concentrations. The intake of carbohydrates and total energy corresponded to the recommended values in pregnant women. In total, the levels of serum lipids were not influenced by the nutrition intake.

The relation between triglyceride levels and prematurity is discussed controversially. In line with our results, Emet et al. [38] showed significantly lower TG levels at premature birth. In addition, the LIFE Child study showed a lower concentration in total cholesterol of 0.44 mmol/l in the cohort of prematurity. However, this relation is described inversely by other authors [11, 39]. *Ghodke* et al. observed significantly higher triglyceride levels in preterm delivery in their Indian cohort.

The strength of the study was the large number of subjects and the variety of collected variables. In addition, there have been only a few studies for reference levels of apolipoproteins in pregnancy in a Caucasian cohort. The study is limited due to the small number of pregnant women with pregnancy-related diseases and twin gestations. Therefore, the associations between preeclampsia, GDM, PAH and dyslipidemia, which have been frequently reported in the literature [11, 40], were not observed. Also no significant effect of a twin gestation could be shown, probably due to the low number of subjects ($n = 10$). Thus, it is necessary to establish a large-scale study to investigate the effect of twin gestations on the concentration of serum lipids and apolipoproteins. Another limitation of the study is the fasting time of only 4 h in the third trimester and its possible effect on the level of triglycerides. According to the National Cholesterol Education Program guidelines from 2001, measurements of serum lipids are recommended after fasting periods of 9–12 h to minimize individual variations in lipid levels [41]. Nevertheless, the presented reference values constitute a realistic representation of the distribution of triglycerides, because also in clinical practice an absolutely sobriety in pregnant women cannot be guaranteed.

Conclusion

The changes in concentration of serum lipids in pregnancy are caused by multiple factors. Actually, the exact biochemical processes are not understood completely. However, this process seems to be affected less by hormones and nutrient intake than previously thought. In general, the data underline that the metabolic state and lipid profile during pregnancy differs from the non-pregnant state. The observed changes in lipids and lipoproteins during pregnancy in this Caucasian cohort are consistent with the most international literature data. However, additional case–control studies are needed to evaluate the relation between a preterm birth and total cholesterol or triglycerides as well as the influence of a twin gestation on the expression of lipid concentrations. Based on these data, it can be only speculated that the extreme changes in the concentration of serum lipids are not associated with an increased atherogenic risk. Therefore, further long-term investigations beyond pregnancy are needed to support this claim. The determined reference levels of serum lipids and apolipoproteins in the second and third trimester may help to detect women with disadvantageous lipid profiles to protect them.

Author contributions ADS was involved in protocol development, data analysis and writing manuscript. MV was involved in data management and analysis. AJ was involved in protocol development. JT was involved in data collection. WK was involved in project development, data collection and manuscript editing. HS was involved in manuscript editing.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Informed consent Informed consent was obtained from all individual participants involved in the study. All subjects gave their written consent for study participation. For participants under the age of 12 years, parents had to give their written consent.

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Ethical approval All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975 (in its most recently amended version). The study was approved by the Ethical Committee of the University of Leipzig (reference number: Reg. No. 264-10-19042010). LIFE Child is registered by the trial number: NCT02550236.

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