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Maternal serum cholesterol levels are elevated from the 1st trimester of pregnancy: A cross-sectional study

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Cholesterol is monitored in the non-pregnant adult population, where normal values are established. Although reported to be elevated in pregnancy, cholesterol is neither routinely measured nor treated. We aimed to investigate cholesterol levels throughout pregnancy and to establish reference values for cholesterol in healthy pregnant women. This was a cross-sectional analysis of serum cholesterol in healthy women with an uncomplicated singleton pregnancy. Pregnant women attending for antenatal care were recruited and cholesterol levels assayed at 12, 20, 28 and 36 weeks' gestation and on day 1–3 postpartum. A total of 222 women were recruited. The majority (95%) were white Irish, with a median age of 31 years (range 16–46). Median BMI was 25.9 kg/m^2 (range 18–40) and 16% were smokers. Cholesterol levels were elevated in all trimesters of pregnancy, with median values from 1st trimester raised outside the non-pregnant adult range. High-density lipoprotein (HDL) levels ranged from 0.9 to 3.7 mmol/l and low-density lipoprotein (LDL) levels ranged from 1.3 to 6.1 mmol/l. Fasting, smoking and obesity did not have any significant effects on results. Total and LDL-cholesterol levels were raised throughout pregnancy. Levels were above non-pregnant adult ranges as early as the 1st trimester. The implications of this on fetus and mother are undetermined and deserve further investigation.

Keywords: Cholesterol, high-density lipoprotein (HDL), hypercholesterolaemia, low-density lipoprotein (LDL), pregnancy

Introduction

Cholesterol is an important part of the cell membrane, and decreases cell membrane fluidity while controlling permeability. Bile, essential for fat emulsification in the intestine, also contains cholesterol. Finally, cholesterol is a direct precursor of steroid hormones, some of which are produced in the placenta (Byanes and Dominiczak 2009). Human cholesterol levels are determined by synthesis in the liver and by dietary intake (Bartels and O'Donoghue 2011).

Lipoproteins are made up of a cholesterol layer around a triglyceride core. Cholesterol transported from the intestine as chylomicrons is metabolised to form very low-density lipoprotein (VLDL) in the liver. Once VLDL reaches peripheral tissues it is hydrolysed to form low-density lipoprotein (LDL), thereby facilitating the transfer of fatty acids from triglyceride in the LDL particle into the cells through LDL cell membrane receptors (Byanes and Dominiczak 2009). Human high-density lipoprotein (HDL) does the reverse and transports cholesterol from the peripheral tissue back to the liver for excretion in bile. Cholesterol reaches the fetus as HDL, after crossing the syncytiotrophoblast as LDL (Bartels and O'Donoghue 2011; Palinski 2009; Woollett 2005). One-fifth of cholesterol in the embryo comes from placental sources and possibly more in women with hypercholesterolaemia (Woollett 2005).

Hypercholesterolaemia is associated with atherosclerosis causing cardiovascular disease. Endothelial damage caused by hypertension, diabetes, smoking, as well as an excess of lipoproteins, allows their deposition in the intima of arterial walls. The resultant inflammatory reaction leads to formation of a fatty plaque, made-up of oxidised LDL particles (Byanes and Dominiczak 2009; Berliner et al. 1995; Palinski et al. 2007; Napoli et al. 1997). Elevated levels of LDL combined with reduced HDL levels increase the risk of development of atherosclerotic plaques.

Lipid parameters such as total cholesterol, LDL, HDL and triglycerides are elevated in pregnancy, particularly in the 2nd and 3rd trimester (Bartels and O'Donoghue 2011; Brizzi et al. 1999; Piechota and Staszewski 1992; Lippi et al. 2007; Husain et al. 2008). This is due to an increase in sex steroid hormones, as well as altered hepatic and adipose metabolism (Brizzi et al. 1999; Chiang et al. 1995; Butte 2000). Increased progesterone contributes to the rise in LDL levels and in return circulating LDL cholesterol is the chief substrate for placental progesterone synthesis (Chiang et al. 1995). Hepatic lipase activity also increases during pregnancy, which causes surges of triglyceride synthesis in the liver and is associated with raised LDL levels. Finally, elevated maternal oestrogen also causes an increase in total cholesterol, LDL cholesterol and triglycerides. It has also been reported that LDL in pregnancy becomes a smaller lipoprotein and is therefore more atherogenic (Brizzi et al. 1999).

There are no reference standards defined for lipid parameters during pregnancy; cholesterol is not usually measured and hypercholesterolaemia is not treated. HMG CoA-reductase inhibitors (statins) are contraindicated in pregnancy, although they are the most prescribed drugs for treatment of high cholesterol in the non-pregnant adult population (Bartels and O'Donoghue 2011; Kenis et al. 2005; Lockshin 2010; Pollack et al. 2005). Omega-3 fatty acids are not widely used in pregnancy, although are linked with increased HDL and reduced triglycerides in early pregnancy (Williams et al. 2006). The CARRDIP (Cardiovascular Risk

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Reduction Diet in Pregnancy) study found a cholesterol-lowering diet during pregnancy achieved a beneficial effect on maternal LDL cholesterol levels (Khoury et al. 2007; Khoury et al. 2005).

High cholesterol levels in pregnancy have been linked with an increased risk of delivering pre-term (Catov et al. 2007). Pregnancy-related hyperlipidaemia has also been linked to gestational diabetes mellitus (GDM) and pre-eclampsia (Gonzalez-Clemente et al. 2007). In the CARDIA (Coronary Artery Risk Development in Young Adults) study, low HDL levels were directly associated with GDM (Gunderson et al. 2010), while women with impaired glucose tolerance were found to have increased LDL levels months postpartum (Savvidou et al. 2010; Retnakaran et al. 2010). Three different studies showed that women with high LDL levels had increased risks of developing pre-eclampsia and that the abnormal lipid profile persisted for up to 3 years postpartum (Qui et al. 2006; Sanchez et al. 2005; Gratacós et al. 2003).

Pregnancy is speculated to lead to a rapid progression of atherosclerosis, postulated by some authors to be driven by changes in sex steroids, insulin resistance, inflammation, oxidative stress, as well as acute elevations in lipids. Independent of other risk factors, the reduction in HDL cholesterol levels and the progression of carotid intima-media thickness in women who gave birth over a 6-year time period in the 'Cardiovascular Risk in Young Finns' study provides some evidence to support this theory (Skilton et al. 2010). Atherosclerosis is also among the first conditions for which a role of developmental programming was described. The link between low birth weight and the increased risk of cardiovascular disease in adult life was described in 1986, and it is since clear that many other factors *in utero* can influence adult health (Bartels and O'Donoghue 2011). The FELIC (Fate of Early Lesions in Children) study showed that aortic fatty streak formation was more advanced in children of mothers with hypercholesterolaemia (Napoli et al. 1999). In addition, murine models of maternal hypercholesterolaemia changed hepatic cholesterol homeostasis in the offspring and promoted fetal atherosclerosis (Napoli et al. 1999; Goharkhay et al. 2008; Palinski et al. 2001).

Cholesterol is carefully monitored in non-pregnant adults, where the association with atherosclerosis and cardiovascular disease is well understood. It is known that cholesterol levels rise in pregnancy, though due to uncertainty as to this significance, cholesterol is not routinely measured or treated. Normal reference standards for cholesterol in pregnancy are not available for the clinician (Bartels and O'Donoghue 2011). In this study, we aimed to investigate total cholesterol, HDL, LDL and triglyceride levels in pregnant women and to examine changes in levels across different gestational ages.

Materials and methods

Study design

This was a cross-sectional analysis of serum cholesterol in healthy pregnant women with a singleton pregnancy, attending a tertiary-referral maternity hospital with around 9,000 deliveries annually. The study was approved by the Clinical Research Ethics Committee (ECM 4 (g) 07/07/09). Two researchers were responsible for collecting blood samples and recruiting the study population. Recruitment began in August 2009 and was completed in August 2010. Participants were divided into five groups according to gestation: 11–14 weeks; 19–23 weeks; 27–29 weeks; 35–37 weeks and 12–72 h postpartum. Each participant answered a short questionnaire and had blood drawn at the same occasion, often in conjunction with other clinically-indicated blood tests. Blood was analysed for total cholesterol, LDL, HDL and triglycerides. Each subject was sampled on only one occasion.

Study population

Women were considered for participation in the study if they had a singleton uncomplicated pregnancy. Inclusion criteria included a body mass index (BMI; kg/m^2) of <41 and >17. Weight and height measures were taken as recorded in antenatal charts at the booking visit (10–14 weeks). Amounts of alcohol consumed before and during pregnancy were recorded in international standard units, and individuals who consumed more than 15 units of alcohol per week before or during the pregnancy were excluded. Women with any history of liver disease, including cholecystectomy, were excluded from the study, and those with hyperlipidaemia or hypercholesterolaemia prior to pregnancy, including those with known familial hyperlipidaemia were also excluded. Once participants gave informed consent, information on age, parity, ethnicity, time since and type of last meal was collected, and a detailed medical and family history recorded.

Laboratory analysis

Analysis was performed on the same day of sampling. Tests were performed on the Olympus 5400 analyser, which automatically computes the cholesterol concentration of each sample. The Olympus cholesterol reagent uses an enzymatic method to measure total serum cholesterol. Cholesterol esters are hydrolysed by cholesterol esterase, and the free cholesterol produced is oxidised by cholesterol oxidase to cholestene-3-one producing hydrogen peroxide, which oxidatively binds with 4-aminoantipyrine and phenol in the presence of peroxidise to yield a chromophore. The red quinone imine dye formed is measured spectrophotometrically at 540/600 nm as an increase in absorbance (Allain et al. 1974). HDL-cholesterol is quantified by the presence of an enzyme chromogen system, measured at 600 nm. The test principle of the Olympus triglyceride procedure is based on a series of enzymatic reactions, where triglycerides are hydrolysed first to glycerol and fatty acids, and later to hydrogen peroxide (H2O2) to produce a chromophore, which is read at 660/800 nm. The increase in absorbance is proportional to the triglyceride content of the sample (Shephard and Whiting 1990).

LDL and VLDL are calculated by the Friedewald equation. VLDL is calculated as triglycerides multiplied by 0.456. LDL equals total cholesterol minus HDL cholesterol minus VLDL cholesterol. The Friedewald formula provides an accurate estimation of LDL for fasting patients but may be reduced in the non-fasting state (Friedwald et al. 1972). The calculation is not reliable for a triglyceride level above 4.52 mmol/l (Warnick et al. 1990; Schectmann et al. 1996; Bukan et al. 2012).

Results were compared with reference standards for cholesterol in the non-pregnant adult population, and with levels recommended by clinical advisory bodies. The reference ranges for fasting cholesterol levels in the healthy non-pregnant adult plasma used were: total cholesterol 3.5–5.0 mmol/l; HDL 0.9–2.2 mmol/l; LDL 2.1–3.0 mmol/l and triglycerides 0.3–1.7 mmol/l. The Irish Heart Foundation states recommended levels for healthy adults of total cholesterol of ≤ 5 mmol/l; HDL of >1 mmol/l; LDL of ≤ 3 mmol/l and triglycerides of ≤ 2 mmol/l.

Statistical analysis

For all measurements, separated into each gestational time-period, median and interquartile ranges were calculated, and a reference range was estimated based on the observed 5th and 95th centiles. The null hypothesis that the sample populations from each gestational period had equal median values was tested at the 5% significance level, using Kruskal–Wallis analysis (the non-parametric version of the classical one-way ANOVA), after rejecting at the 5% significance level, the null hypothesis of

Table I. Total cholesterol levels in pregnancy.

Gestation (weeks)	Number of women (*n*)	Median (μmol/l)	Range (μmol/l)	5th percentile	95th percentile
12 (11–14)	48	5.1	3.8–6.7	3.9	6.6
20 (19–23)	50	5.8	4.0–8.5	4.4	7.7
28 (27–29)	32	6.5	5.3–10.7	5.3	9.3
36 (35–37)	45	7.2	4.5–9.2	4.7	9.1
Postnatal (day 1–3)	47	6.0	3.8–8.5	4.0	8.0

sample normality at each time-point (Lilliefors test). In the box plot, two medians are significantly different at the 5% significance level if their intervals do not overlap. Interval endpoints are the extremes of the notches.

To evaluate the difference between fasting and non-fasting total cholesterol and triglyceride levels, for all gestations, a two-sample Mann–Whitney test was performed using a significance level of 5%. This analysis was repeated comparing cholesterol levels of smokers to non-smokers, and women with normal BMI (≤ 25) to elevated (> 25) BMI.

Results

A total of 222 pregnant women participated in the study. All had a singleton pregnancy and 94% (207/222) were White Irish. Participants were recruited into five groups: 11–14 weeks ($n = 48$); 19–23 weeks ($n = 50$); 27–29 weeks ($n = 32$); 35–37 weeks ($n = 45$) and 12–72 h postpartum ($n = 47$). The median maternal age was 31 years (range 16–46) and 42% women were nulliparous. Mean BMI (kg/m^2) was 25.9 (range 18–40). These demographics are similar to the general antenatal booking population.

One-third (32%; 72/222) had a 1st-degree relative with hypercholesterolaemia. One woman had a history of pre-eclampsia and four were diagnosed with gestational diabetes mellitus after recruitment. There were 36 (16%; 36/222) smokers, smoking between 2 and 20 cigarettes daily (mean, 8). Outside pregnancy, 39% (86/222) women denied alcohol intake, while the majority (135/222) consumed on average of 3.9 units per week. During pregnancy, 14 (6%; 14/222) women disclosed alcohol intake of 1–6 units per week (mean, 1.5 units).

All women were asked how many hours ago they had eaten and what they had consumed. In seven cases, this information was not recorded. The interval from food consumed to blood sampling ranged from 0 to 12 h (mean, 3.4 h). A total of 30 (14%) women had fasted overnight (> 9 h). Of the remainder the majority, 72% (133/185), had eaten a light breakfast, such as cereal, toast and/or fruit, as samples were collected mid-morning.

Total cholesterol for all gestations ranged from 3.8–10.7 mmol/l, with a mean of 6.0 mmol/l. Out of the 222 women, 174 (78%) had total cholesterol levels > 5 mmol/l (Table I). Figure 1 shows the distribution of total cholesterol levels around the median in each gestational group. Total cholesterol was significantly elevated from the 1st trimester, rose progressively throughout pregnancy and then significantly dropped postpartum, although the median remained higher than the normal non-pregnant range (Table I).

High-density lipoprotein ranged from 0.9–3.7 mmol/l across all gestations (median, 1.8 mmol/l), while low-density lipoprotein ranged from 1.3–6.1 mmol/l (median, 3.3 mmol/l). High-density lipoprotein remained within normal ranges at weeks 11–14, weeks 35–37 and postpartum. From weeks 19–29, HDL levels

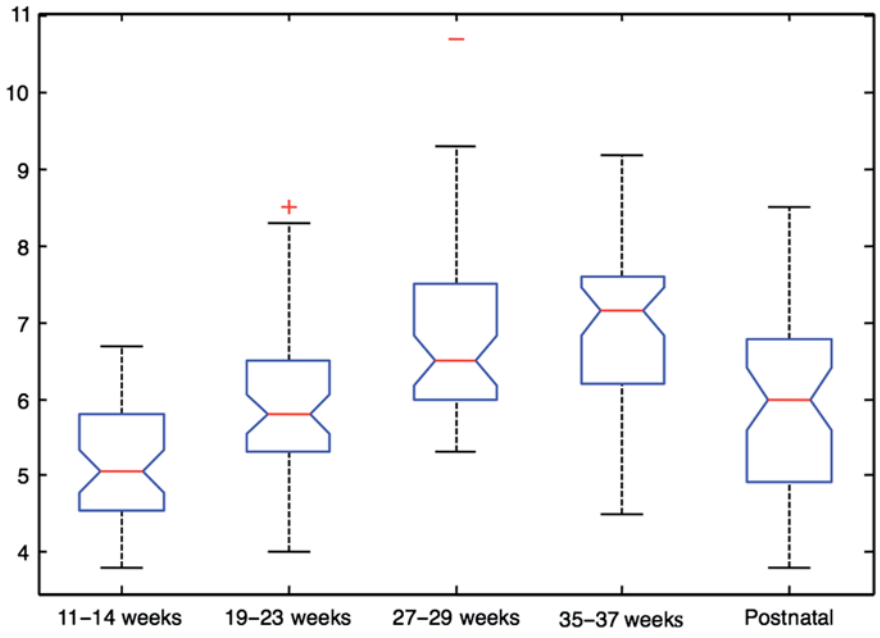

Figure 1. Total cholesterol (normal range 3.5–5 mmol/l). Data are presented as a box-whisker plot. In each figure, the tops and bottoms of each 'box' are the interquartile range of the samples. The line in the middle of each box is the sample median. Whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually. An 'outlier' is defined as a value that is more than 1.5 times the interquartile range (IQR) away from the top or bottom of the box.

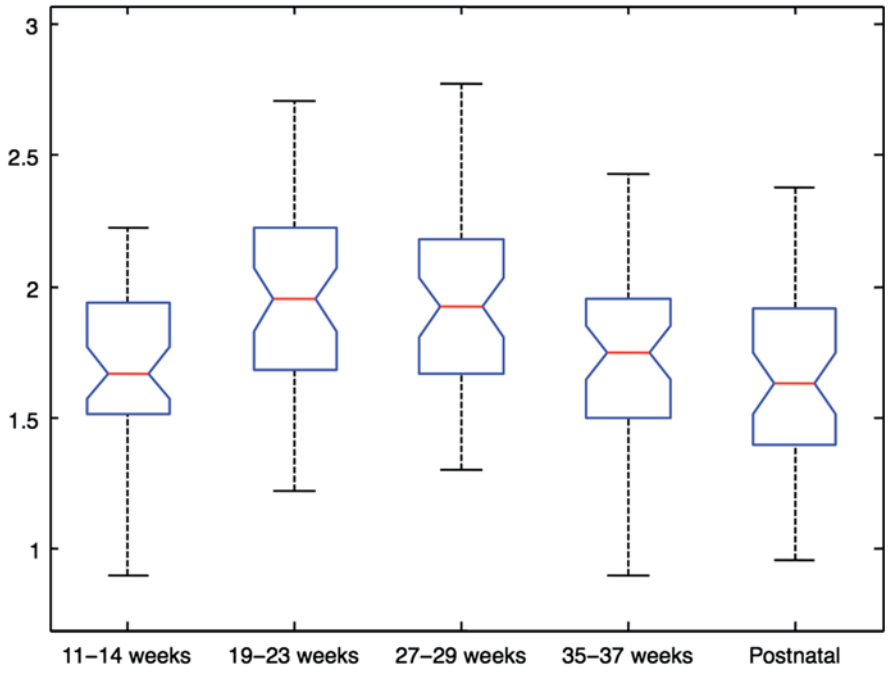

Figure 2. High-density lipoprotein (normal range 0.9–2.2 mmol/l).

were significantly increased. Seven women did not have HDL levels analysed. Low-density lipoprotein peaked at 27–29 weeks, although levels were elevated at all gestations. Figures 2 and 3 show the distribution of HDL and LDL results around the median in each gestational group.

Triglycerides ranged from 0.55–5.38 mmol/l in 213 women. The median triglyceride level was 1.9 mmol/l, and six women had a triglyceride above 4.52 mmol/l. Triglycerides rose progressively as gestation advanced, and did not significantly decrease in the postpartum period. The median triglyceride levels remained below 1.7 mmol/l until 27 weeks' gestation. Finally, liver function tests were performed in all women at the time of blood sampling and were within the normal ranges established for pregnancy.

The means of total cholesterol and triglycerides of women who had fasted for >9 h ($n=30$) were compared with those of women who had eaten within 6 h ($n=185$). There was no statistical significant difference between the fasting and non-fasting groups when total cholesterol ($p=0.69$) and triglyceride ($p=0.88$) levels were examined (Table II).

The effects of being overweight (BMI >25) and smoking on total cholesterol levels were also examined. There was no statistical significant difference between total cholesterol levels of

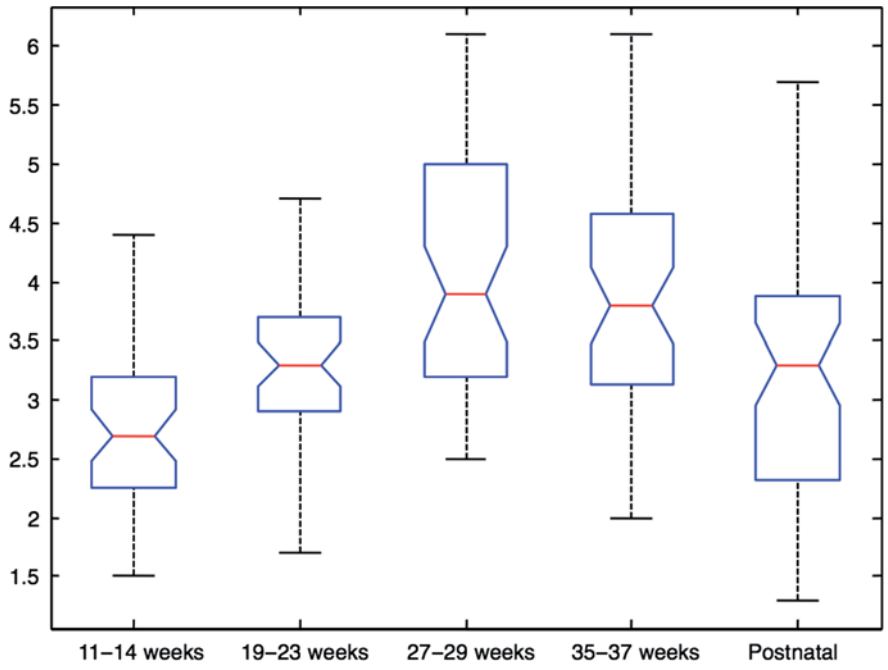

Figure 3. Low-density lipoprotein (normal range 0.9–2.2 mmol/l).

Table II. Total cholesterol and triglycerides – fasting levels.

	0–6 h				>9 h				
	n	(%)	Median	IQR	*n*	(%)	Median	IQR	*p* value
Total cholesterol	185	84	6.0	5.2–6.8	30	16	6.1	5.3–7.5	0.69
Triglycerides	178	85.6	1.9	1.3–2.6	30	14.4	1.72	1.4–2.4	0.88

IQR, interquartile range.

women who had a normal BMI compared with those with an elevated BMI ($p = 0.93$). Similarly, there was no statistical significant difference in total cholesterol levels between smokers and non-smokers ($p = 0.14$; Table III).

Discussion

Hypercholesterolaemia is not treated in pregnancy, partly due to the absence of normal parameters for pregnancy, as well as clinicians' uncertainty as to the significance of elevated levels for a limited time. The increased cholesterol levels found during pregnancy are speculated to affect pregnancy outcome and promote atherosclerosis in offspring. This study showed that cholesterol levels were significantly raised throughout pregnancy, with levels being above non-pregnant adult ranges as early as the 1st trimester.

Of the pregnant study participants, 78% had a serum cholesterol above the recommended level for non-pregnant adults. Cholesterol levels rose progressively from the 1st to the 3rd trimester, and began to fall in the early postpartum period, though still remaining outside non-pregnant ranges. There was a significant increase in total cholesterol levels after the 1st trimester, with peak values seen in the 3rd trimester. HDL values remained at recommended levels throughout pregnancy, with the highest levels found in the 2nd trimester. Triglyceride values increased with advancing gestation, although the mean triglyceride level remained below the recommended level for non-pregnant adults until 27 weeks. A total of 60% of pregnant women had an LDL level above 3.0 mmol/l and peak levels of LDL at 27–29 weeks were 30% above the recommended level for non-pregnant adults.

Lipid parameters, including total cholesterol, LDL, HDL and triglycerides have been shown in other published reports to be elevated in pregnancy (Bartels and O'Donoghue 2011; Piechota and Staszewski 1992; Lippi et al. 2007; Husain et al. 2008; Chiang et al. 1995). Previous studies have divided women into three groups by trimester of pregnancy and compared with non-pregnant women, the rise in lipid parameters occurred from the 2nd trimester in the majority of reports. In contrast, in this study, we found a progressive rise of total cholesterol and low-density lipoprotein from early pregnancy.

Table III. Body mass index (BMI) and smoking – total cholesterol.

	n	(%)	Median	IQR	*p* value
BMI					0.93
≤25	119	56.4	6	5.2–7.0	
>25	92	43.6	6	5.3–6.7	
Smoking					0.14
Smokers	36	16.4	5.8	5.3–6.3	
Non-smokers	184	83.6	6.1	5.3–7.1	

A total of 11 women had missing BMI data and two women had missing smoking data. These were excluded from the unpaired *t*-test. IQR, interquartile range.

Total cholesterol levels above 5.0 mmol/l are associated with a higher risk of cardiovascular disease, and the majority of pregnant women in this study had a total cholesterol level above this. A combination of elevated LDL and decreased HDL in the blood further enhances the atherosclerotic risk. In our population, while HDL levels were within normal adult ranges, LDL levels were elevated throughout pregnancy. This may put pregnant women at higher risk of developing atherosclerosis.

Even though the sample size in this study is reasonable, the numbers in each gestational group are small and can be criticised. This study was not longitudinal but cross-sectional in design, which may affect the differences in cholesterol levels between gestational groups. However, longitudinal studies require significant participant commitment and often suffer high dropout rates leading to smaller study numbers. Further, pre-pregnancy values for cholesterol for this study population were not known. Finally, only 14% of the study population had fasted for more than 9 h before testing. Generally, lipid profiles are measured after a fast of at least 12 h. If the Friedewald formula is used to calculate low-density lipoprotein, a 12-hour fast is necessary as the presence of any chylomicrons undermines the results. Lipoprotein fractionation is the alternative method; however it is not used in our biochemistry laboratory. A high triglyceride level (>4.52 mmol/l) also makes the LDL calculation unreliable (Friedewald et al. 1972; Warnick et al. 1990; Schectmann et al. 1996) but in our study only 3% (6/213) of women had triglycerides above this level. However, on further analysis, there was no significant difference found in total cholesterol and triglyceride levels between the patients who were fasting and those who were post-prandial.

Cholesterol is carefully monitored in the non-pregnant adult population and its association with cardiovascular disease is well understood (Berliner et al. 1995). At present, high cholesterol levels are not routinely measured or treated in pregnancy. However, a growing body of evidence from animal and human studies suggests adverse consequences of maternal hypercholesterolaemia (Bartels and O'Donoghue 2011; Palinski 2007; Chiang et al. 1995; Catov et al. 2007; Gonzalez-Clemente et al. 2007; Napoli et al. 1999; Goharkhay et al. 2008). With established pregnancy-specific reference values, extreme maternal hypercholesterolaemia could be recognised and monitored. Intervention with a cholesterol-lowering diet during pregnancy may be necessary and prove beneficial, and other lipid lowering interventions should be investigated. Women identified with high cholesterol levels during pregnancy may need follow-up postnatally as persistent hypercholesterolaemia substantially increases the risk of atherosclerosis.

This study supports previous published work on maternal hypercholesterolaemia and presents pregnancy-specific reference ranges for total cholesterol, HDL, LDL and triglycerides. Using these reference ranges, future studies can identify excessively high maternal cholesterol levels and relate them to pregnancy outcome as well as the risk of fetal atherosclerosis and maternal cardiovascular disease.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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