# Hypercholesterolemia During Pregnancy is Caused by Increased Endogenous Cholesterol Synthesis: Let's Use it for Screening of Familial Hypercholesterolemias Too!

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**Abstract:** *Aims*: To demonstrate the origin and the diagnostic significance of non-cholesterol sterols (NCSs) in healthy pregnant women with gestational hypercholesterolemia.

*Patients* and Methods: Based on a total of 21,000 clinical biochemistry tests of healthy pregnant women with hypercholesterolemia observed during pregnancy, a group of 84 women with TC (total cholesterol) >7.0 mmol/L was recruited to analyze their NCSs using Gas Chromatography–Mass Spectrometry. The NCSs under examination comprised lathosterol (Lat) and desmosterol (Des) as markers of endogenous cholesterol synthesis, and campesterol (Cam) and sitosterol (Sit) as markers for intestinal absorption.

*Results*: In the total of 21,000 pregnant women, the median values were: TC 6.8 mol/l, LDL-C 4.6 mmol/L, and HDL-C 2.2 mmol/L. In the testing group of 84 women, the average values were: Lat 7.8+/-1.7 µmol/L, Des 4.7+/-0.9 µmol/L, Cam 9.8+/-2.6 µmol/L, and Sit 9.6 +/-3.8 µmol/L. Lat was found to correlate with TC (r = 0.53), LDL-C (r = 0.36), and non-HDL-C (r = 0.35). No such correlations were observed for Sit (r = 0.162) or Cam (r = 0.153).

*Conclusion*: Our findings show that the high incidence of hypercholesterolemia during pregnancy is caused by increased endogenous cholesterol synthesis via lathosterol. The enormous rise of TC levels during pregnancy can be effectively used to detect familial hypercholesterolemia in women.

**Keywords:** Hypercholesterolemia during pregnancy, familial hypercholesterolemia, non-cholesterol sterols, lathosterol, desmosterol, campesterol, sitosterol.

## 1. INTRODUCTION

Total cholesterol (TC) levels rise by 30 to 40 per cent during normal human pregnancy, peaking in the fifth to sixth gestational month. The increase is more marked in its low-density lipoprotein (LDL) fraction than in its high-density lipoprotein (HDL) fraction [1]. The rise in triglycerides (TG) is also present but independent from TC levels.

Experts regard the elevated TC during pregnancy to be harmless and even beneficial for mother and foetus, as it provides for the increased cholesterol consumption in the cell membrane synthesis of the foetus [2-5].

To emphasize the ultimate importance of cholesterol during gestation, it is also worth mentioning that there have been considerations about the higher offer and demand of the sonic hedgehog (SHH) protein during pregnancy. This molecular chaperone with its C-terminal cholesterol molecule attached to the embryonal tissue induces formation of the peptide bond, thus initiating development of limbs, parts of brain, etc. Therefore, the appropriate TC concentration is likely to ensure the appropriate SHH functioning in the foetal organogenesis [6, 7]. The other SHH terminal is occupied by the palmitate, whose significance in the biochemical induction during organogenesis still remains to be clarified.

Non-cholesterol sterols (NCSs) form a large group of cholesterol-like metabolites allowing an insight into its long and complex endogenous synthesis, as well as the intricate mechanism of the sterol absorption in the intestine. The structures of the NCSs under discussion in this paper are shown in Figure **1**.

In 'familial hypercholesterolemia' (FH), a hereditary cholesterol metabolism disorder with autosomal dominant mode of inheritance (OMIM 143890) caused by at least three so far known metabolic mechanisms (molecularly conditioned LDL receptor deficit, Apo B (FBD) deficit, and PKS9 deficit), the TC percentage increase is the same as in pregnancy. This practically

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E-ISSN: 1929-5634/17 

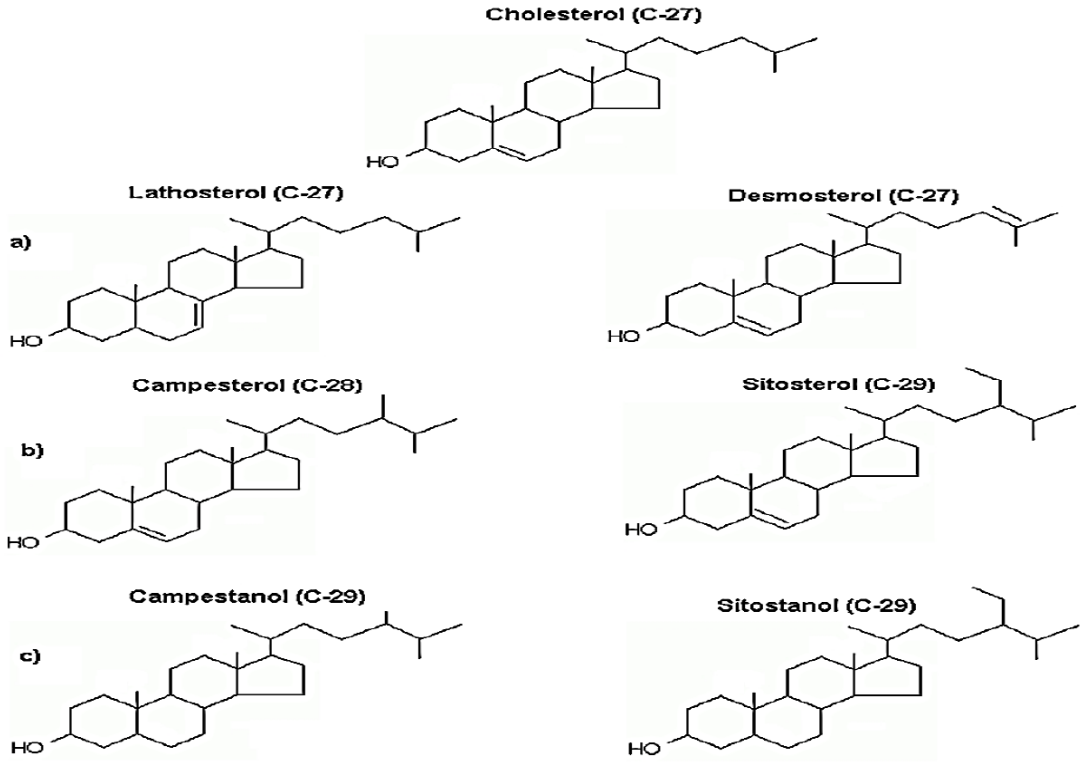


**Figure 1:** Most important sterols under discussion in paper: **a**) Lat and Des – markers of endogenous synthesis of cholesterol; **b**) Cam and Sit – markers of phytosterol absorption, **c**) saturated NCSs used for dietetic treatment of hypercholesterolemias.

means that TC easily reaches pathologic levels of up to 8–9 mmol/L. The incidence of heterozygotes of this most frequent lipid metabolism deviation is high (1 to 350–400). Textbooks have not been very informative about the relevance and consequences of this 'gestational familial hypercholesterolemia' as yet [1, 8]. Only case studies of the rare homozygote forms of FH have been published, where TC levels exceed 15 mmol/L and the high risk of cardiovascular complications requires careful monitoring and possible treatment by lipoprotein apheresis [9-11].

## 2. PATIENTS AND METHODS

On the population of 21,000 clinical biochemistry results of healthy pregnant women of different gestational age attending the Prague Institute for the Care of Mother and Child in 2014–2016, random sampling was performed among those with TC > 7.0 mmol/L to establish a testing group of 84 women for a detailed analysis of their NCS levels. Lathosterol (Lat) and desmosterol (Des) were examined as markers of cholesterol synthesis, whereas sitosterol (Sit) and campesterol (Cam) were used to mark the intestinal absorption of phytosterols. The routine biochemical, haematological and immunological analyses including the lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, non-HDL-cholesterol and triglycerides) were performed using Synchron Beckman Coulter LX20 and Advia 120 Bayer systems.

The plasma NCSs were determined using Gas Chromatography–Mass Spectrometry (GC/MS) via the Theunissen [12] modification by a Finnigan Mat 120b with epicholesterol applied as an internal standard, CV < 10%. The overall lipid extraction from the plasma samples was performed using the Folch method [13]. The lipid saponification was performed using the modified Thompson and Merola method [14]. The derivatization was performed using the modified Hušek method [15]. The complete methodology has been previously described in detail by Hyánek, Pehal, Dubská *et al*. [16].

The statistical assessment was performed using the R software. The basic statistical parameters included confidence intervals for the mean or median at a 95 percent confidence level, sample size, maximum and minimum values, and the respective standard deviations. The correlation analysis was based on Pearson's product-moment correlation coefficient.

The study was approved by the Na Homolce Hospital Ethics Committee under Project No NA 7452-3 supported by the Internal Grant Agency of the Czech

Ministry of Health. Informed consent for inclusion was obtained from all participants.

## 3. RESULTS

Table **1** shows the mean values of the NCS lipid fractions for the group of 84 hypercholesterolemic healthy pregnant women. The correlations of the individual lipid fractions and the individual NCSs are presented in Table **2**. Positive correlations were found for Lat with TC (r = 0.53), with LDL-C (r = 0.36), and with non-HDL-C (r = 0.35), as well as for Des with TC (r = 0.35). The correlations between phytosterols and the increased cholesterol synthesis were insignificant: Sit (r = 0.162) and Cam (r = 0.153). The HDL-C and TG values did not correlate with the NCS levels at all.

**Table 1: Mean Values of Standard Serum Lipid Parameters (mmol/L) and Non-Cholesterol Sterols (µmol/L) in the Group of 84 Healthy Pregnant Women with Hypercholesterolemia >7.0 mmol/L**

| | Mean value | Significant deviation |
|---|---|---|
| Lathosterol | 7.8[+)] | 1.7 |
| Desmosterol | 4.7 | 0.9 |
| Sitosterol | 9.6 | 3.8 |
| Campesterol | 9.8 | 2.6 |
| Total cholesterol | 7.2 | 1.2 |
| LDL-cholesterol | 4.1 | 1.7 |
| Non-HDL-cholesterol | 4.5 | 1.3 |
| HDL-cholesterol | 1.8 | 0.3 |
| Triacylglycerols | 2.5 | 1.0 |

[+)] $p<0.01$.

Our results prove that the increase of TC during pregnancy, which takes places largely in its LDL or non-HDL fractions, is due to the increased endogenous synthesis of cholesterol, as demonstrated by the raised Lat and Des levels. The compensatory decreased absorption of phytosterols, as shown by the Sit and Cam levels is insignificant (see Table **1**). So far we have not found a suitable explanation for the correlation between Lat and TG.

## 4. DISCUSSION

The elevated cholesterol levels in maternal plasma during pregnancy seem to be a logical response of human organism to the demands placed by the growing maternal organs as well as the organogenesis and intensive growth of the foetus [17, 18].

The increased synthesis of cholesterol provides the explanation for gestational hypercholesterolemia via the higher values of Lat and partially also Des.

It was not until we were evaluating the lipid profile outcomes and collecting the samples suitable for the NCS analyses that we came to realize the enormous potential of the increased cholesterol levels found during pregnancy in detecting the as yet undiagnosed cases of hereditary FH. It is for the first time that a large population study of 20,000 Slavic pregnant women has proved an unexpectedly high incidence of TC >7 mmol/L (1 to 132), a fact which definitely calls for our further preventive efforts.

We have not yet encountered comparable studies into other populations in literature globally. The larger and tentative US studies by Mudd and Palinski do not explain the etiology of TC, but only assess hypercholesterolemia as a possible factor of a preterm birth and conversely hypocholesterolemia as a reason for a higher incidence of IUGR [19, 20]. However, we have also found a contradictory opinion [21]. Edison has found preterm births in mothers with low TC [22].

In line with our 20-year experience, we recommend high TC during pregnancy as a suitable marker for early detection of as yet undiscovered FH [23]. Its differentiation, subsequent treatment and other

**Table 2: Correlation quotients of classical lipid parameters with noncholesterol sterols in the group of 84 hypercholesterolemic healthy pregnant women. Reference NCS values (µmol/L) for healthy non-pregnant women are: Lat 6.4+/-0.7, Des 4.2+/-0.4, Cam 9.7+/-1.0, and Sit 8.7+/-0.8**

| | Lathosterol | Desmosterol | Sitosterol | Campesterol |
|---|---|---|---|---|
| Total cholesterol | 0.53 | 0.35 | 0.26 | 0.02 |
| LDL-cholesterol | 0.36 | 0.4 | 0.36 | 0.02 |
| non-HDL-cholesterol | 0.35 | 0.12 | 0.29 | 0.01 |
| HDL-cholesterol | 0.02 | 0.01 | 0.02 | 0.01 |
| Triglycerides | 0.52 | 0.21 | 0.01 | 0.01 |

measures in the family are then possible following the end of pregnancy and lactation.

## 5. CONCLUSION

Our findings show that the high incidence of hypercholesterolemia during pregnancy is caused by increased endogenous cholesterol synthesis via lathosterol. The enormous rise of TC levels during pregnancy can be effectively used to detect familial hypercholesterolemia in women.

## CONFLICT OF INTERESTS

All the authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

## ABBREVIATIONS

Apo B = apolipoprotein B

Cam = campesterol

TC = total cholesterol

Des = desmosterol

FH = familial hypercholesterolemia

GC/MS = Gas Chromatography–Mass Spectrometry

HDL = high-density lipoprotein

HDL-C = high-density lipoprotein cholesterol

Lat = lathosterol

LDL = low-density lipoprotein

LDL-C = low-density lipoprotein cholesterol

non-HDL = non-high-density lipoprotein

NCS = non-cholesterol sterol

Sit = sitosterol

TG = triglycerides (triacylglycerols)

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Received on 16-10-2017 Accepted on 14-12-2017 Published on 04-01-2018

DOI: https://doi.org/10.6000/1929-5634.2017.06.04.1