Does high density lipoprotein protect vascular function in healthy pregnancy?

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Abstract

The maternal adaptation to pregnancy includes hyperlipidaemia, oxidative stress and chronic inflammation. In non-pregnant individuals these processes are usually associated with poor vascular function. However maternal vascular function is enhanced in pregnancy. It is not understood how this is achieved in the face of the adverse metabolic and inflammatory environment. Research into cardiovascular disease demonstrates that plasma high density lipoprotein (HDL), by merit of its functionality rather than its plasma concentration, exerts protective effects on the vascular endothelium. HDL has vaso-dilatory, anti-oxidant, anti-thrombotic and anti-inflammatory effects and can protect against endothelial cell damage. In pregnancy, plasma HDL concentration starts to rise at 10 weeks' gestation, peaking at 20 weeks. The initial rise in plasma HDL occurs around the time of the establishment of the feto-placental circulation, a time when the trophoblast plugs in the maternal spiral arteries are released generating oxidative stress. Thus there is the intriguing possibility that new HDL of improved function is synthesised around the time of the establishment of the feto-placental circulation. In obese pregnancy and to a greater extent in preeclampsia, plasma HDL levels are significantly decreased and maternal vascular function is reduced. Wire myography studies have shown an association between the plasma content of apolipoprotein AI, the major protein constituent of HDL, and blood vessel relaxation. These observations lead us to hypothesise that HDL concentration, and function, increases in pregnancy in order to protect the maternal vascular endothelium and that in preeclampsia this fails to occur.

242 words

Introduction

There is a large body of research concerning the role of plasma HDL as a protective agent against cardiovascular disease. This has come from a wide variety of epidemiological studies, human clinical studies and, particularly with respect to the molecular function of HDL, from rodent animal models. However there is surprisingly little known about HDL in pregnancy. Research in humans remains limited to measurement of plasma concentration in healthy and complicated pregnancy whereas pregnant rodent models focus predominantly on metabolic programming of offspring rather than maternal adaptation to pregnancy. Here, the evidence for a role of HDL in maternal pregnancy in protecting vascular endothelium will be discussed incorporating the small amount of data available from human pregnancy and drawing parallels from the larger body of evidence, from humans and animals, in the wider cardiovascular field.

Metabolic changes in healthy pregnancy

The maternal adaptation to pregnancy involves significant metabolic change primarily to meet the demands of fetal growth and development (reviewed in[1]). In early pregnancy the mother becomes insulin sensitive in order to acquire adipose tissue required to supply fatty acids in later gestation. By the third trimester there is increased insulin resistance, hyperlipidaemia, increased inflammation and enhanced coagulation status. Oxidative stress increases with gestation as evidenced by an increase in markers of oxidative stress[2]. Insulin resistance in healthy pregnant women preserves the supply of glucose to support the growing fetus and supports lipolysis from maternal adipose tissue providing fatty acids for placental transport[1]. In later gestation, high fatty acid supply to the liver results in hypertriglyceridaemia as a consequence of increased very low density lipoprotein (VLDL) secretion. Plasma total cholesterol and low density lipoprotein (LDL) concentrations also increase

but to a lesser extent[1]. Plasma high density lipoprotein (HDL) levels increase and reach their peak at mid gestation before declining in the last trimester[3].

Vascular function in pregnancy

The metabolic and inflammatory changes described above would normally be associated with poor vascular function in non-pregnant individuals. High plasma lipid levels, in the presence of oxidative stress, leads to lipid peroxidation and production of reactive oxygen species (ROS) which are highly damaging to the vascular endothelium[4]. Gestational hypertriglyceridaemia also results in an increase in the production of highly atherogenic and easily oxidised small, dense LDL species[1]. However, pregnant women have enhanced vascular function. In healthy pregnancy, blood pressure progressively falls to reach a nadir at around 20 weeks mediated by a fall in maternal systemic vascular resistance due to vasodilation, followed by a gradual increase in blood pressure to reach pre-pregnancy levels. Studies looking at endothelium-dependent vascular function using flow-mediated dilatation (FMD) of the brachial artery showed that pregnant women had a significantly higher increase in flow-mediated diameter than non-pregnant women, mediated at least in part by the endothelium-dependent vasodilator nitric oxide (NO)[5]. Flow-mediated dilatation progressively increased throughout gestation reaching a peak in the last trimester[5]. A positive correlation between percentage FMD and plasma triglyceride concentration confirms that pregnancy-related enhanced vascular function exists despite gestational hyperlipidaemia[6]. Microvascular function, both endothelium-dependent and endothelium-independent, determined using non-invasive laser Doppler imaging was also improved during gestation[7]. Using wire myography, it has also been shown that small resistance arteries, isolated from healthy pregnant women, had better flow-mediated relaxation than arteries from non-pregnant women[8]. It is not understood how improved vascular function is maintained in the face of a barrage of metabolic and inflammatory mediators normally associated with vascular damage. While the gestational period of nine months might appear to be a short time over which to influence vascular function by changes in the metabolite composition of plasma, there is an abundance of evidence showing that that the post-prandial increase in lipid concentration impairs vascular function in healthy individuals over a number of hours[9].

One study has looked at maternal HDL and endothelium-dependent brachial artery flow-mediated dilatation (FMD) in healthy pregnancy[6]. A multivariate analysis found a positive association between %FMD and plasma triglyceride, but no association with plasma HDL when both were entered into the model together. The authors interpreted this as the plasma triglyceride level reflecting the degree of maternal gestational adaptation driven by plasma estradiol levels and concluded that endothelial function improvement in pregnancy was likely due to the increased concentrations of HDL. It is difficult to interpret these data in light of the metabolic link between plasma triglyceride and HDL and the fact that plasma HDL concentration may not reflect HDL function (detailed later). Clearly more experimental data looking directly at the effects of HDL on vascular function are required.

HDL has functions that protect the vascular endothelium

HDL is a protein-rich and cholesteryl-ester rich lipoprotein complex and epidemiological studies show an inverse relationship between its plasma concentration and the risk of cardiovascular disease[10]. Its major protein constituent is apolipoprotein AI (Apo AI). HDL was initially thought to exert all its anti-atherogenic effects via its key role in reverse cholesterol transport, whereby HDL removes cholesterol from peripheral tissues and delivers it to the liver for excretion. HDL mediates cholesterol efflux from cells involving several cellular mechanisms such as protein kinase C activation, increasing cAMP concentration in macrophages and retroendocytosis[11]. However recent

clinical trials of inhibitors of an enzyme intrinsic to HDL metabolism, cholesteryl ester transfer protein (CETP), have demonstrated that despite effective increases in HDL plasma concentration, the risk of cardiovascular disease was unaffected or even increased[12]. Numerous studies support the notion that CETP mediates reverse cholesterol transport[13]. By inhibiting CETP, reverse cholesterol transport may also be blocked demonstrating that increased HDL concentration may not be beneficial in every instance, especially if it reflects decreased turnover[14]. Such clinical data emphasise the importance of differentiating plasma HDL steady state concentration from HDL function. Recent research focus has shifted away from the benefits of high plasma concentrations of HDL towards the study of specific HDL functions mediated by the proteins and lipids associated with the HDL particle (Figure 1).

HDL enhances vasodilation of blood vessels at least in part by increasing the availability of NO. Endothelial NO is key for the regulation of vascular tone and structure. HDL regulates endothelial NO synthase (eNOS) expression[15] and activity, stimulating NO production and inducing vasodilation[16]. An eNOS knockout mouse model confirmed the role of eNOS when the vasodilatory effect of HDL on aorta was lost in this animal[17]. Another study in scavenger receptor class B member 1 (SR-B1, a plasma membrane receptor for HDL) knockout mice using isolated HDL from pre- and post-menopausal women demonstrated that HDL-associated estradiol stimulates eNOS activity via the SR-B1 receptor[18]. The sphingosine-1-phosphate (S1P) receptor also has a role in mediating eNOS activation by HDL[17]. These protective effects of HDL on endothelium dependent vasodilatation through NO production are lost in cardiovascular disease patients[19].

Other properties of HDL include antioxidant effects. HDL attenuates the oxidative stress produced by oxidised LDL and the smaller, denser subspecies of HDL have the most potent anti-oxidative effects[20]. HDL carries enzymes that act as antioxidants such as paraoxonase-1 (PON-1) and platelet activating factor acetyl hydrolase (PAF-AH)[21]. In addition, integral HDL proteins such as apo AI can be sacrificed to oxidation to protect other molecules[22]. Reactive oxygen species (ROS) disrupt the active eNOS dimer and HDL can prevent oxidised LDL's inhibitory effect on eNOS. HDL prevents LDL oxidation through inactivation of lipid hydroperoxides mediated by PON-1[23] and also via Apo AI which uses specific methionine residues to reduce peroxides into inactive lipid hydroxides[24]. HDL PON-1 content determines the capacity of HDL to stimulate NO production and protect the endothelium[25]. Plasma HDL concentrations are associated with a reduced risk of venous thrombosis[26]. SR-B1 knock out mice, which have high plasma HDL concentrations, have low platelet levels possibly due to an alteration in platelet structure resulting in increased clearance[27]. HDL can increase the anticoagulant activities of protein S and activated protein C, an effect significantly reduced by anti-apo AI antibodies[28]. There is an inverse association between clot lysis time and plasma levels of HDL and apo AI which supports an anti-thrombotic role for HDL[29].

HDL inhibits TNFα-induced endothelial expression of adhesion molecules (ICAM-1 and VCAM-1) in human umbilical endothelial cells[30]. HDL exerts its anti-inflammatory action through the SR-B1 receptor as siRNA against SR-B1 attenuated HDL's inhibitory effect on adhesion molecule expression[30]. Lipopolysaccharide (LPS)-induced pro-inflammatory cytokines, such as TNF-α, ICAM-1 and IL-6, were also reduced in THP-1 macrophages pre-treated with HDL[31]. Other anti-inflammatory effects of HDL and apo AI include inhibition of neutrophil activation, adhesion and infiltration[32]. HDL can protect against apoptosis of endothelial cells and damage the integrity of the endothelium monolayer[33]. HDL and apo AI can inhibit endothelial cell apoptosis mediated by oxidized LDL[34]. Isolated HDL from healthy subjects was able to inhibit the apoptosis of endothelial cells *in vitro* and in contrast, HDL isolated from coronary artery disease patients exerted a pro-apoptotic effect[35].

Sphingosine-1-phospate and HDL

Sphingosine-1-phosphate (S1P) is an HDL-associated lysophospholipid, anchored to the HDL particle via apolipoprotein M, whose levels correlate directly with HDL, apo AI and apo AII levels[36]. Smaller, denser HDL is found to carry approximately twice the amount of S1P than that carried by larger HDL species[37]. S1P is involved in many of HDL's biological effects including vasodilation, anti-oxidative actions and anti-inflammatory functions[38]. S1P is the major contributor to HDL-related effects on blood vessel relaxation, Akt activation and eNOS phosphorylation as these effects are totally abolished in S1P3 receptor deficient mice[17]. S1P also plays an important part in the anti-oxidative properties of HDL by protecting against the cytotoxic effects of oxidised LDL[39]. S1P's effects are complex and may act through different signaling pathways at different concentrations[30].

HDL and vascular function in pregnancy

Plasma HDL concentrations increase throughout pregnancy[3]. Data from our laboratory, compiled using many samples collected at different gestations of pregnancy, shows a detailed profile of plasma HDL and triglyceride concentrations throughout pregnancy (Figure 2). Plasma triglyceride levels begin to increase substantially around 20 weeks' gestation and continue to rise until delivery. After delivery there is a sharp fall to slightly below pre-pregnancy levels and a rebound to pre-pregnancy levels by about 20 weeks post-partum. Plasma HDL concentration shows a different pattern. After an early slight decline in concentration, a rise in plasma HDL concentration is initiated at 10 weeks' gestation, peaking at a maximum level of 42% increased concentration at 20 weeks' gestation and declining to a plateau only 7% above early pregnancy levels by 30 weeks' gestation. The post-partum decline in HDL concentration is delayed until about 10 weeks after delivery and eventually pre-pregnancy levels are reached by 20 weeks after delivery.

The co-existence of high plasma HDL concentration, in the face of high plasma triglyceride concentration, is contrary to our metabolic understanding of the links between triglyceride and HDL metabolism in non-pregnant individuals. High plasma triglyceride in the non-pregnant population causes HDL and LDL to become enriched with triglyceride via the substrate-driven action of CETP. CETP facilitates the transfer of cholesteryl ester from HDL and LDL in exchange for triglyceride from VLDL. The resultant triglyceride rich-HDL is acted on by hepatic lipase leading to particle shrinkage and increased plasma HDL turnover thus linking high plasma triglyceride concentration with low plasma HDL[40]. Therefore in healthy pregnancy, high HDL concentration in the presence of high triglyceride concentration is unusual and this suggests that HDL metabolism becomes decoupled from triglyceride metabolism perhaps due to the estrogenic drive increasing HDL synthesis[41]. In addition, the timing of the increase in plasma HDL is interesting as concentrations begin to rise around the time of the establishment of the feto-placental circulation at 10-13 weeks' gestation. At this time trophoblast plugs in the maternal spiral arteries which protect embryonic tissue from oxidative damage are released generating oxidative stress. Thus there is the intriguing possibility that new HDL of improved function is synthesised around the time of the establishment of the feto-placental circulation and prior to the increase in plasma triglyceride.

Maternal vascular function and plasma HDL in obese pregnancy and preeclampsia

While both non-obese and obese pregnant women have improved endothelium-dependent vascular function compared to the non-pregnant state, non-obese mothers have better endothelium-dependent microvascular function than obese mothers[7]. In addition, myometrial arteries isolated from pregnant women with high BMI (>36kg/m^2) showed decreased relaxation to bradykinin compared to vessels obtained from pregnant women with non-obese BMI (<30kg/m^2)[42]. Obese pregnancy is associated with metabolic syndrome in which plasma concentrations of triglyceride and VLDL are higher and concentrations of HDL lower than in non-obese pregnancy[1, 7]. It is possible that in obese pregnancy, HDL concentrations may be insufficient to fully protect the maternal vascular

endothelium and the obese pregnant woman is at higher risk of endothelial dysfunction increasing her risk for preeclampsia[43].

Preeclampsia, a multi-system disorder particular to pregnancy, is a leading cause of maternal and neonatal morbidity and mortality and is one of the most difficult challenges facing obstetric medicine. The disease is characterised by widespread endothelial dysfunction, resulting in hypertension due to vasoconstriction, proteinuria attributable to glomerular damage and oedema secondary to increased vascular permeability. It complicates 2-8% of pregnancies, eludes early detection and there is no effective intervention other than iatrogenic delivery. In preeclampsia the maternal metabolic adaptation to pregnancy is abnormal with development of metabolic syndrome features including hypertriglyceridaemia and reduced HDL[1]. Low pre-conception plasma HDL [odds ratio (OR) 1.61 (95%CI 1.29-2.01)] and high plasma triglycerides [OR 1.33 (95%CI 1.09-1.63)] were independently associated with preeclampsia and/or gestational diabetes mellitus after adjustment for maternal confounders[44]. Furthermore, there was a significant (P<0.001) interaction between low HDL and high triglycerides. A genetic predisposition to low plasma HDL is also associated with an increased risk of preeclampsia[45]. In preeclampsia there is increased lipid peroxidation, antioxidant levels are reduced[46] and plasma PON-1 levels are decreased [47]. Maternal obesity, increased insulin resistance and aberrant fatty acid metabolism are involved in the pathogenesis of at least some phenotypes of preeclampsia[48]. Vascular dysfunction in preeclampsia has been observed both at the physiological level by a variety of techniques[8, 49, 50] and in isolated blood vessels. In *ex vivo* wire myography studies, small resistance arteries derived from subcutaneous adipose tissue (representing systemic maternal vascular resistance) and myometrial biopsies (representing the vessels that supply the placenta) from preeclampsia pregnancy showed lower endothelium-dependent relaxation than vessels from healthy pregnancy[51, 52]. NO-, EDHF- and eicosanoid-mediated pathways were implicated. Preincubation of myometrial vessels from healthy pregnancy with plasma from women with preeclampsia, even if plasma was sampled at a gestation prior to the clinical manifestation of preeclampsia, inhibited endothelium-dependent relaxation[53]. Interestingly, an association between the concentration of the major protein constituent of HDL, apo AI, in plasma and the vaso-relaxation was observed[54]. These effects are specific to gestational adaptive functions of the endothelium as no effects on vaso-relaxation were seen in non-pregnant vessels[55].

Hypothesis

We hypothesise that establishment of the placental circulation triggers the formation of more and/or new, more effective, HDL as a pregnancy adaption that protects and enhances function of the vascular endothelium in the face of the oxidative, lipid and inflammatory stresses of healthy pregnancy. In healthy pregnancy, HDL is able to counter the oxidative and inflammatory stresses of the maternal adaptation to pregnancy. In preeclampsia, HDL fails to optimally adapt resulting in failure to protect the maternal endothelium resulting in vascular dysfunction.

Future experimental work

There are a number of questions regarding HDL in pregnancy that could be answered experimentally. *Ex-vivo* myography studies using vessels from pregnant animal models or in tissue from pregnant women can test whether HDL enhances vessel relaxation. Compositional studies including lipidomics and proteomics can test whether newly synthesised HDL in pregnancy differs from HDL in non-pregnant individuals. *In vitro* analysis of the vascular protective properties of HDL can demonstrate whether HDL from pregnant women has enhanced protective abilities as compared to HDL in the non-pregnant state. Comparison of HDL from women with preeclampsia with HDL from healthy pregnancies can test whether HDL gestational adaptive changes fail to take place in preeclampsia.

Clinical implications of a vascular protective role for HDL in pregnancy

An understanding of the role of HDL in preeclampsia could inform clinical understanding and shape future management of the disease. The well-recognised athero-protective effects of HDL have led to the development of therapeutic agents to raise HDL concentration or increase HDL function for the prevention of cardiovascular disease. These agents include HDL infusing agents, recombinant LCAT, apo AI transcriptional upregulators and apo AI mimetic peptides[56]. Administration of synthetic HDL improves endothelial function in patients with hypercholesterolaemia[57]. Apo AI/phospholipid complexes and apo AI mimetics in CVD are in pre-clinical development[58]. Other studies showed HDL infusion was able to reduce atherogenic events including inflammation, thrombosis as well as apoptosis[59]. If HDL in pregnancy exerts vascular protective effects, then trials of synthetic HDL and apo AI mimetics in preventing/managing preeclampsia could be indicated. In pregnancy it is difficult to recommend interventions other than diet or lifestyle, for safety reasons. The HDL mimetics have enormous potential utility in pregnancy as they would be safe to administer and would allow manipulation of HDL function as well as concentration.

2,812 words

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Figure 1. HDL protective actions at the endothelium. A number of the constituents of HDL may interact with the vascular endothelium. Production of nitric oxide (NO) induces blood vessel dilation. The main apolipoprotein on HDL, apoAI, may increase NO production via AMP-activated protein kinase (AMPK) signalling. Sphingosine-1-phosphate (S1P) is a lipid anchored to the HDL particle via the apolipoprotein apoM, and it can interact with $S1P_1$ and $S1P_3$ receptors on the vascular endothelial cell leading to increased endothelial nitric oxide synthase (eNOS) activity, partly via AMPK pathways. HDL also carries the antioxidant enzyme paraoxonase-1 (PON-1). This enzyme, possibly in addition to other anti-oxidative effects of HDL, inhibits the oxidation of LDL to form oxidised LDL (OxLDL). PON-1 also can inhibit OxLDL receptor (LOX-1) activity thus reducing the production of reactive oxygen species (ROS) via NAHPH oxidase, and preventing the inhibitory effects of ROS on eNOS.

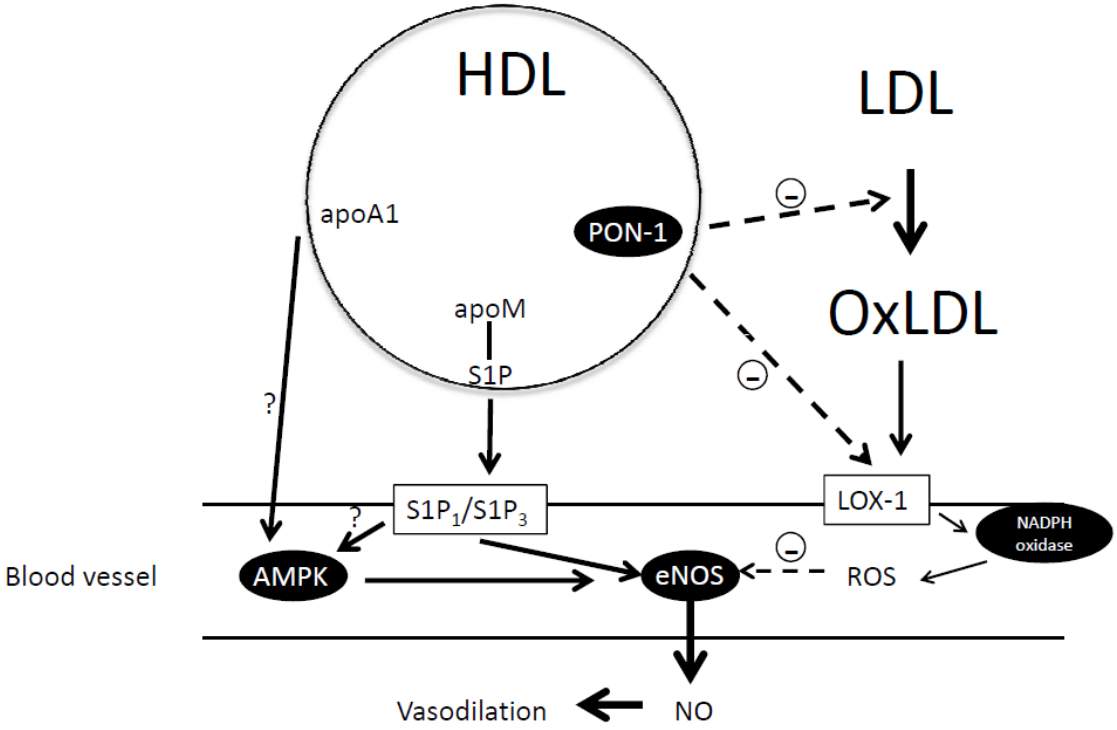

Figure 2 Maternal plasma HDL-cholesterol and plasma triglyceride concentrations during pregnancy and post partum. Unpublished data from a series of overlapping longitudinal and cross-sectional studies in our laboratory. Lipid measurements were carried out by Vascular Biochemistry, University of Glasgow a Centre for Disease Control (CDC) UK laboratory for the Lipid Standardisation Programme (cholesterol, triglyceride, HDL-C) and the CDC Reference Laboratory for cholesterol and HDL-C, thus lipid measurements are standardised over time.