DISSERTATION

Investigating the Impact of Obesity and Gestational Hypertension on HDL Metabolism and Function in Women and their Offspring

submitted by

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STATUTORY DECLARATION

I hereby declare that this thesis is my original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the “Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz”.

Julia Stadler

Graz, June 2023

DISCLOSURES

This cumulative dissertation is based on the following publications:

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I further contributed to the following publications during my PhD studies:

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TABLE OF CONTENTS

ABBREVIATIONS

A	ABCA1	ATP-binding cassette subfamily A member 1
	ABCG1	ATP-binding cassette subfamily G member 1
	Apo	Apolipoprotein
B	BMI	Body mass index
C	CETP	Cholesteryl-ester transfer protein
	CVD	Cardiovascular disease
G	GDM	Gestational diabetes mellitus
	GHTN	Gestational hypertension
H	HDL	High-density lipoprotein
	HDL-C	High-density lipoprotein cholesterol
	HL	Hepatic lipase
L	LCAT	Lecithin-cholesterol acyltransferase
	LDL	Low-density lipoprotein
	LDL-C	Low-density lipoprotein cholesterol
	LDL-R	Low-density lipoprotein receptor
	LPL	Lipoprotein lipase
	$LpPLA_2$	Lipoprotein-associated phospholipase A2
N	Non-HDL-C	Non-high-density lipoprotein cholesterol
P	PE	Preeclampsia
	Plgf	Placental growth factor
	PON1	Paraoxonase-1
S	SAA	Serum amyloid A
	sFlt-1	Soluble Fms-like tyrosine kinase-1
	SR-BI	Scavenger Receptor B1
	S1P	Sphingosine-1-phosphate
T	TGRL	Triglyceride-rich lipoproteins
V	VLDL	Very-low-density lipoprotein

ZUSAMMENFASSUNG

Fettleibigkeit ist ein weltweit wachsendes Gesundheitsproblem. Sie erhöht das Risiko für verschiedene Krankheiten wie Herz-Kreislauf-Erkrankungen, Typ-2-Diabetes, nichtalkoholische Fettlebererkrankungen, bestimmte Krebsarten und schwangerschaftsbedingte Erkrankungen. Trotz dieser Risiken nimmt die Prävalenz von Fettleibigkeit weltweit weiter zu. Niedrige High-Density Lipoprotein (HDL)-Cholesterin Spiegel wurden lange Zeit mit einem erhöhten kardiovaskulären Risiko in Verbindung gebracht. Jedoch ist die Vielfalt der schützenden Funktionen von HDL von größerer Bedeutung als der Cholesteringehalt. Es wird angenommen, dass die funktionelle Vielfalt der HDL-Partikel, die weitgehend durch ihre Zusammensetzung bestimmt ist, eine entscheidende Rolle bei ihrer Schutzwirkung gegen Entzündungen und Herz-Kreislauf-Erkrankungen spielt.

Zu Beginn meiner Dissertation habe ich eine Übersichtsarbeit zum aktuellen Stand der vorhandenen Literatur zu fettlebigkeitsbedingten Veränderungen des HDL-Stoffwechsels und der HDL Funktion verfasst. Ein interessanter Aspekt dieser Arbeit war, dass bariatrische Chirurgie momentan als die effektivste Behandlungsmethode gilt um bei fettleibigen Personen die HDL-Cholesterin Spiegel zu erhöhen und die HDL-Funktionen zu verbessern.

In meinem ersten Forschungsprojekt habe ich in einer Studienkohorte untersucht, ob Adipositas den Metabolismus, die Zusammensetzung und die Subklassen Verteilung von HDL beeinflusst. Die Untersuchungen wurden an Proben einer Studienkohorte von gesunden jungen, jedoch übergewichtigen oder fettleibigen Frauen durchgeführt. Die Aktivitäten von Enzymen, wie Lecithin-Cholesterin Acyltransferase und des Cholesterinester-Transferproteins waren in fettleibigen Frauen, im Vergleich zur normalgewichtigen Kontrollgruppe, stark erhöht. Dies ging mit einer Verschiebung in Richtung kleiner HDL Subklassen einher. Darüber hinaus war die Zusammensetzung der HDL-Partikel verändert. HDL adipöser Frauen war mit dem akute Phase Protein Serum Amyloid A und Triglyzeriden angereichtert, während der Anteil an enthaltenem Cholesterin und Apolipoprotein A-I geringer war. Interessanterweise wurden diese Änderungen nur bei fettleibigen Frauen mit einem Body-Mass-Index (BMI) von über 30 kg/m^2, aber nicht bei übergewichtigen Frauen mit einem BMI zwischen 25 und 29.9 kg/m^2 beobachtet.

Im zweiten Teil meiner Dissertation habe ich mich weiter auf Adipositas, HDL-Metabolismus und Funktion fokussiert. Allerdings untersuchte ich in diesem Teil nun die Auswirkungen in Müttern und deren Neugeborenen. Dazu wurden Serum-Proben der europäischen DALI-Studienkohorte

verwendet, welche übergewichtige und fettleibige schwangere Frauen (BMI ≥ 29 kg/m^2) inkludierte. Zusätzlich wurden post-partum Nabelschnurblutproben gesammelt. Als Kontrollgruppe dienten schwangere Frauen mit normalem Gewicht (BMI vor der Schwangerschaft < 25 kg/m^2). Im Zuge dieser Studie wurde herausgefunden, dass ein hoher BMI Veränderungen im Serum-Lipidprofil und im Metabolismus und der Funktion von HDL bei Müttern hervorruft und dies auch Auswirkungen auf deren Neugeborene hat. Die HDL-Funktionalitäten zwischen Müttern und Neugeborenen waren stark verlinkt, während HDL-C Levels nur schwach korrelierten. Wir konnten außerdem zeigen, dass die anti-oxidative Kapazität des Serums bei adipösen Müttern und deren Neugeborenen verringert war. Zudem haben wir untersucht, ob Schwangerschaftsdiabetes einen Einfluss auf die gemessenen Parameter hat, wobei wir keine signifikanten Veränderungen feststellen konnten. Dies legt nahe, dass Adipositas selbst einen Einfluss auf die HDL-Funktion bei Müttern und ihren ungeborenen Kindern hat, während zusätzlich auftretender Schwangerschaftsdiabetes scheinbar keinen weiteren negativen Effekt zeigt.

Im weiteren Verlauf habe ich im dritten Teil meiner Dissertation Proben der DALI Studienkohorte verwendet um die Veränderungen der HDL Funktion bei Schwangerschafts-induziertem Bluthochdruck zu untersuchen. Dieser tritt gehäuft bei adipösen Frauen in der Schwangerschaft auf. Die Gestationshypertonie (GHTN) erhöht das kardiovaskuläre Risiko für Mütter und deren Nachkommen im späteren Leben enorm. Da HDL, durch die Förderung der Cholesterinaufnahme von Makrophagen und der Suppression von Endothelzellaktivierung anti-atherogen agiert, könnte eine funktionelle Verringerung der HDL-Funktionalitäten zur Entwicklung von kardiovaskulären Krankheiten beziehungsweise einem erhöhten Langzeitrisiko beitragen. Während bei Müttern mit GHTN keine Veränderungen der HDL-Funktion auftraten, waren Cholesterineffluxkapazität und Paraoxonase-1 Aktivität im Nabelschnurblut beeinträchtigt, während die anti-oxidative Kapazität des Serums erhöht war. Eine erhöhte Aktivität der Paraoxonase-1 und der Apolipoprotein-M-Spiegel in der Frühschwangerschaft, war mit erhöhtem Risiko für die Entwicklung von GHTN assoziiert.

Aufgrund der beobachteten GHTN-induzierten Veränderungen untersuchten wir im vierten Teil meiner Dissertation den Lipidstoffwechsel bei Präeklampsie (PE), einer schweren Form von Bluthochdruck während der Schwangerschaft im Vergleich mit normotensiven Schwangeren. Dabei unterschieden wir zwischen früh und spät einsetzender PE. PE war bei den Müttern mit atherogener Dyslipidämie assoziiert, welche durch hohe Triglyzerid- und niedrige HDL-C Spiegel gekennzeichnet war. Bei Frauen mit früh einsetzender PE wurde eine Verschiebung von großen

zu kleineren HDL-Subklassen beobachtet, was mit einer erhöhten anti-oxidativen Kapazität des Plasmas bei Müttern zusammenhing. PE war außerdem mit erhöhten HDL-assoziierten Apolipoprotein C-II Spiegeln bei Müttern verbunden. Bei Neugeborenen von Müttern mit früh einsetzender PE waren die Gesamtcholesterinspiegel erhöht, während bei Neugeborenen von Müttern mit spät einsetzender PE die HDL-assoziierte Cholesterineffluxkapazität stark reduziert war.

Nachdem ich mich während meiner Dissertation intensiv mit Veränderungen des Metabolismus, der Zusammensetzung und der Funktion von HDL während der Schwangerschaft und bei Neugeborenen befasst habe, schrieb ich zu diesem Thema eine Übersichtsarbeit um den aktuellen Wissensstand bezüglich fetalem HDL und den bekannten Veränderungen des HDL bei Schwangerschaftskomplikationen zusammenzufassen.

Zusammenfassend konnte ich in meiner Dissertation nachweisen, dass Frauen mit Adipositas eine veränderte HDL-Funktion aufweisen, die mit Veränderungen in der Zusammensetzung der HDL-Partikel und der Verteilung der Subklassen einhergeht. Besonders bei schwangeren Frauen und ihren Neugeborenen stellt Fettleibigkeit ein erhebliches Gesundheitsrisiko dar, da ein hoher BMI mit atherogener Dyslipidämie und funktionellen Veränderungen des HDL assoziiert ist. Darüber hinaus hatte mütterliche Adipositas einen deutlichen Einfluss auf den HDL-Stoffwechsel und die funktionalen Parameter bei den Neugeborenen. Schwangerschafts-induzierter Bluthochdruck und PE sind ebenfalls mit Veränderungen im Lipidstoffwechsel und der HDL-Funktion verbunden, was das Risiko für kardiovaskuläre Ereignisse mutmaßlich erhöhen könnte. Diese Ergebnisse tragen zu einem tieferen Verständnis der komplexen Beziehung zwischen Adipositas, Bluthochdruck, HDL und Schwangerschaftskomplikationen bei. Diese komplexen Zusammenhänge müssen genauer verstanden werden, um präventive und therapeutische Strategien zur Verbesserung des Schwangerschaftsverlaufs bei Frauen mit Adipositas und PE zu entwickeln.

ABSTRACT

Obesity is a global health concern that is on the rise. It is strongly linked to an increased risk of numerous diseases, including cardiovascular disease, type 2 diabetes, non-alcoholic fatty liver disease, certain cancers, and pregnancy-related disorders. Despite the well-established health risks, the prevalence of obesity continues to increase worldwide. The association between low levels of high-density lipoprotein (HDL)- cholesterol (HDL-C) and cardiovascular risk has long been established, but the protective functions of HDL extend far beyond just cholesterol content, as the cholesterol carried by HDL has no protective role. The functional diversity of HDL particles, which is largely determined by their composition, is thought to play a crucial role in their protective effects against inflammation and cardiovascular disease.

As part of my dissertation, I started with summarizing the existing literature on obesity-related changes in HDL metabolism and function and wrote a comprehensive review article. One of the most intriguing findings in recent literature is that bariatric surgery is currently recognized as the most effective treatment for increasing HDL-C levels and improving HDL function in obese individuals.

In the first research part of my thesis, I investigated whether obesity affects metabolism, composition and subclass distribution of HDL particles and analyzed them in a study cohort of young and healthy but overweight or obese women. Interestingly, the activities of enzymes such as lecithin-cholesterol acyltransferase and cholesteryl-ester transfer protein were profoundly increased in obese women, which was associated with a shift towards the small HDL subclasses. The composition of HDL particles was also altered, with enrichment in acute phase protein serum amyloid A and triglycerides, and lower content of cholesterol and apolipoprotein A-I. Interestingly, these changes were only observed in obese women (BMI ≥ 30 kg/m^2) and not in overweight women (BMI 25-29.9 kg/m^2).

In the second part of my dissertation, I focused on obesity-related effects on HDL metabolism and function in mothers and their newborns. For this study, I used serum samples from the European study cohort DALI, which included overweight and obese pregnant women (BMI ≥ 29 kg/m^2). In addition, cord blood samples were available and samples from a control group of pregnant women of normal weight were collected. In this study, we found that a high BMI caused changes in serum lipid profile and HDL metabolism and function in mothers and also had effects on their offspring. Interestingly, HDL functionalities were strongly linked between mothers and children, while HDL-

C levels were only weakly correlated. We also showed that serum anti-oxidative capacity was decreased in obese mothers and their offspring. Furthermore, we investigated the potential influence of gestational diabetes on the measured parameters. However, our findings did not reveal any significant changes, indicating that the main factor affecting maternal and fetal HDL function is obesity, rather than gestational diabetes.

In addition, we investigated the changes in HDL function in gestational hypertension (GHTN), which is more prevalent in obese pregnant women in the DALI study cohort. GHTN significantly increases the risk of cardiovascular complications for mothers and their offspring later in life. Since HDL exhibits anti-atherogenic properties by promoting cholesterol uptake from macrophages and suppressing activation of endothelial cells, a reduction in HDL functionalities could potentially contribute to the disease or long-term risk of cardiovascular events. While no alterations in HDL function were observed in GHTN-affected mothers, impaired HDL-cholesterol efflux capacity and activity of paraoxonase-1 in cord blood were observed in newborns, while the antioxidant capacity in serum was increased. Elevated anti-inflammatory paraoxonase-1 activity and higher levels of apolipoprotein M early in pregnancy were linked to a higher risk of developing GHTN.

Based on the observed changes in gestational hypertension (GHTN), the fourth part of my dissertation focused on studying lipid metabolism in pre-eclampsia (PE), a severe form of hypertension during pregnancy, and comparing it with pregnant women with normal blood pressure. Based on the onset of the disorder, we distinguished between early-onset and late-onset PE. Mothers with PE exhibited atherogenic dyslipidaemia, characterized by elevated triglyceride and low HDL-C levels. In women with early-onset PE, a shift from large to smaller HDL subclasses was observed, which was related to increased plasma antioxidant capacity in mothers. PE was also associated with elevated maternal HDL-associated apolipoprotein C-II levels. Neonates from early-onset PE pregnancies exhibited elevated total cholesterol levels, whereas HDL cholesterol efflux capacity was reduced in neonates of late-onset PE group.

Finally, as part of my dissertation, I conducted a comprehensive review on the current understanding of HDL metabolism, composition, and function of fetal HDL, with a particular emphasis on the alterations observed in pregnancy complications.

In summary, my dissertation findings demonstrate that obesity in women is linked to alterations in HDL metabolism, particle composition, and subclass distribution. This is particularly significant in the context of pregnancy, as maternal obesity poses significant health risks to both mothers and

newborns. High BMI is associated with atherogenic dyslipidemia and functional changes in HDL. Moreover, maternal obesity exerts a significant influence on HDL metabolism and functional parameters in the fetus. Additionally, I observed that GHTN and PE were associated with alterations in lipid metabolism and HDL function. These changes could potentially further increase the risk of cardiovascular events. Our findings enhance our comprehension of the intricate interplay between obesity, HDL, and pregnancy complications, emphasizing the need for further in-depth investigation of these factors.

1. INTRODUCTION

1.1. Obesity: A major global public health problem

The prevalence of overweight and obesity is rapidly increasing and has become a major public health problem worldwide. Obesity is characterized by the accumulation of excess body fat to an extent that adversely affects health and usually defined as a body mass index (BMI) of 30 kg/m^2 or greater (7). Obesity is a significant risk factor for various clinical conditions, such as type 2 diabetes, cardiovascular disease (CVD), metabolic syndrome, gastrointestinal disorders, liver diseases, cancer, kidney diseases, and pregnancy-related disorders, as well as an increased risk of mortality (8–13). According to a report in 2019, more than half of the population in Europe was estimated to be overweight or obese, highlighting the gravity of this public health issue (14). Obesity is linked to a substantial rise in mortality, resulting in a reduction in life expectancy by 5 to 10 years (15–17). The transition from a lean to obese state is accompanied by significant changes in adipose tissue and the appearance of chronic low-grade inflammation (8,18–20). This inflammatory state is characterized by elevated levels of circulating free fatty acids, pro-inflammatory molecules, such as interleukin-1β, interleukine-6 and tumor necrosis factor α, as well as the activation and infiltration of immune cells into inflamed areas (20–22). Additionally, obesity often coincides with a distinct dyslipidemic profile known as atherogenic dyslipidemia (23,24).

1.1.1. Dyslipidemia in obesity

Dyslipidemia is a term that refers to an abnormal lipid profile in the blood, including elevated levels of triglycerides, very-low-density lipoprotein (VLDL), apolipoprotein (apo) B, and non-high-density lipoprotein cholesterol (non-HDL-C) (25–28). Conversely, levels of high-density lipoprotein (HDL)-cholesterol (HDL-C) and apoA-I are typically low (23,25–28). Levels of low-density lipoprotein (LDL)-cholesterol (LDL-C) levels are frequently in the normal range, but an increase in small dense LDL is often seen, resulting in an increased number of LDL particles (23,25,28,29) (Figure 1). These small, dense LDL particles are considered to be more pro-atherogenic than large LDL particles for several reasons (30). The affinity of small dense LDL particles for the LDL receptor is reduced, leading to an extended duration of their presence in the bloodstream (31,32). Moreover, these small particles enter the arterial wall more easily than large particles and are more prone to oxidation, making them more susceptible to uptake by macrophages (32,33). Moreover,

postprandial triglyceride levels are also increased in subjects with obesity leading to the presence of pro-atherogenic chylomicron remnants (24,34). The greater the increase in BMI the greater the abnormalities in lipid levels (35,36). Approximately 60-70% of patients who are obese exhibit dyslipidemia, while the occurrence is relatively lower in overweight patients, ranging from 50-60% (28). The increased risk for cardiovascular disease in patients with obesity is partially accounted for by this dyslipidemia (33).

Patients with obesity experience various abnormalities contributing to dyslipidemia (24,28,33,37,38). These abnormalities stem from increased delivery of free fatty acids to the liver due to greater adiposity, insulin resistance, and inflammation induced by macrophages infiltrating adipose tissue (24,33). Key abnormalities include the overproduction of VLDL particles by the liver, leading to elevated serum triglyceride levels (24,28,33,37,38). The rate of VLDL secretion is influenced by some factors including the increased flux of fatty acids from adipose tissue, particularly from visceral fat deposits, to the liver (33,37,38). Furthermore, insulin resistance in obesity diminishes the ability of insulin to inhibit the breakdown of triglycerides into free fatty acids in adipose tissue (39). As a consequence, there is an elevation in triglyceride lipolysis and an augmented supply of fatty acids to the liver (28,33,38). Additionally, numerous studies have shown that fatty acid synthesis is elevated in the liver of obese individuals, potentially influenced by hyperinsulinemia associated with insulin resistance. Insulin stimulates the activity of sterol regulatory element-binding protein-1c, a transcription factor that plays a role in enhancing the expression of enzymes involved in fatty acid synthesis (33,40).

Another factor which contributes to the increased serum triglyceride levels in obesity is reduced clearance of triglyceride-rich particles (24,41). The overproduction of VLDL impairs the breakdown of chylomicrons by competing with lipoprotein lipase (LPL) and leads to increased transport of remnant triglycerides to the liver (24,42). In obesity, lipolysis is further impaired due to decreased messenger-RNA expression of LPL in adipose tissue and decreased LPL activity in skeletal muscle (42,43). Furthermore, obese individuals have been found to have increased levels of apoC-III, which acts as an inhibitor of LPL (44). Hypertriglyceridemia also promotes the exchange of cholesteryl-ester and triglycerides between VLDL, HDL, and LDL through cholesteryl-ester transfer protein (CETP). This results in decreased levels of HDL-C and reduced triglyceride content within LDL and VLDL (45). Additionally, hepatic lipase (HL) plays a role in removing triglycerides and phospholipids from LDL, resulting in the formation of small, dense LDL (24,46,47). The small and dense LDL particles exhibit a prolonged residence time and are more

susceptible for oxidation, contributing to their increased atherogenicity (24,48,49). In individuals with obesity and insulin resistance, triglyceride-rich HDL becomes enhanced hydrolyzed through increased activity of HL (50–52) (Figure 1). This process results in the formation of smaller HDL3 particles, which are more prone to rapid degradation (53).

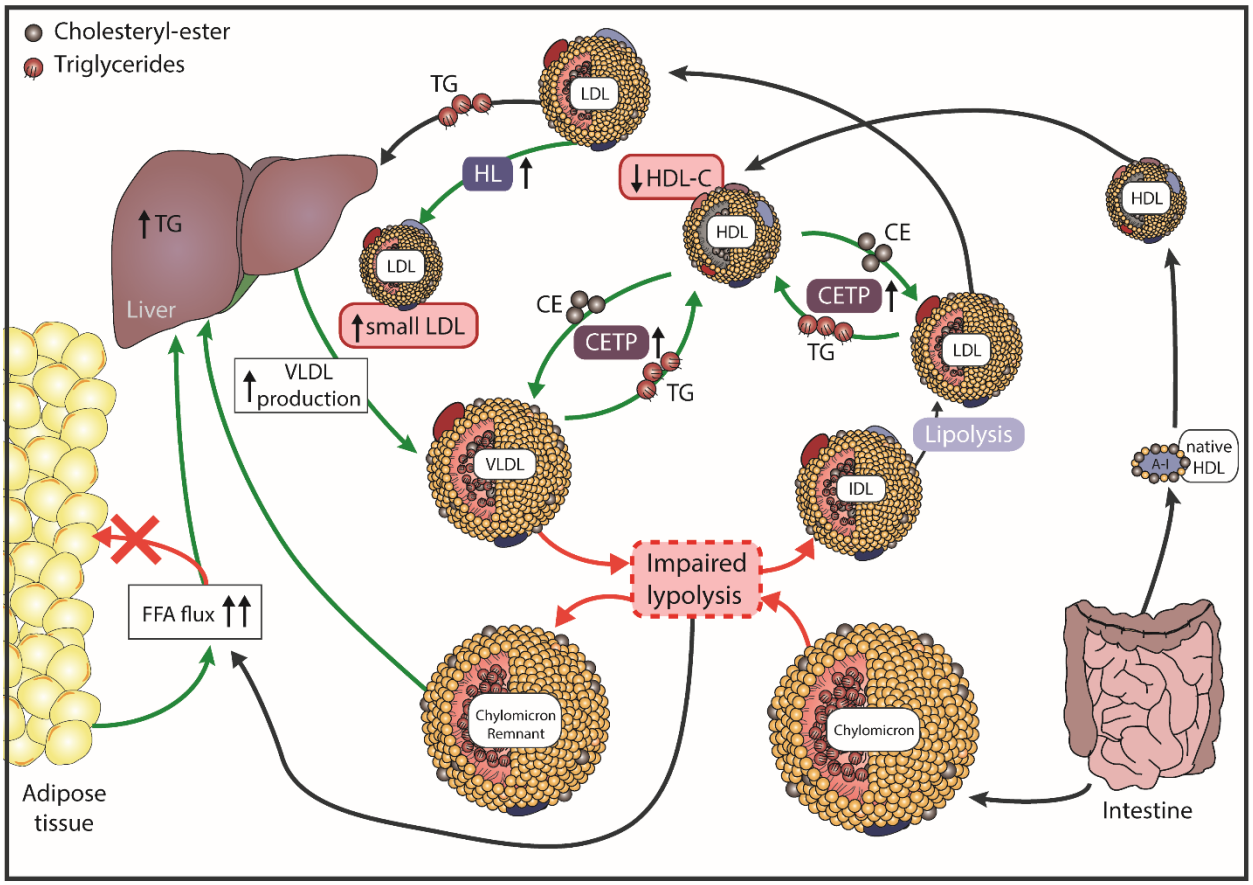

Figure 1. Obesity-related dyslipidemia. Dyslipidemia in obesity is characterized by hypertriglyceridemia, primarily attributed to increased free fatty acid (FFA) fluxes to the liver, resulting in hepatic triglyceride (TG) accumulation. This leads to elevated synthesis of large VLDL by the liver, which hinders the lipolysis of chylomicrons due to competition with lipoprotein lipase. Consequently, increased remnant TG is transported to the liver. Hypertriglyceridemia also promotes the exchange of cholesteryl-esters (CE) and TG between VLDL, HDL and LDL through cholesteryl-ester transfer protein (CETP). This process results in reduced concentrations of HDL-cholesterol (HDL-C) and a decrease in TG content within LDL. Additionally, hepatic lipase (HL) removes TG and phospholipids from LDL, resulting in the generation of small dense LDL, depleted in TG. Metabolic processes that are increased in obesity are indicated by green arrows, while reductions are indicated by red arrows. Image reproduced from Klop et al. (24) and Stadler et al. (54) under Creative Commons Attributions License.

1.1.2. Obesity: Effects on Pregnancy and Maternal Health

Obesity is a significant public health problem affecting millions of people worldwide. The rising prevalence of obesity is also evident in the population of pregnant women. Obesity in pregnancy, also known as maternal obesity, is linked to a wide range of adverse outcomes for both the mother and the child. Maternal obesity poses an increased risk for various pregnancy complications, such as gestational hypertension (GHTN), preeclampsia (PE) and gestational diabetes mellitus (GDM) (10,55–57). It has been estimated, that approximately 25% of pregnancy complications can be attributed to maternal obesity or overweight (58). Moreover, excessive weight gain during pregnancy and the retention of pregnancy weight postpartum are significant contributors to future obesity in women (59). Additionally, the health of the mother can significantly influence the intrauterine environment, impacting fetal development and the long-term health of the child (60,61).

In addition to the adverse outcomes for the mother and the child, obesity in pregnancy is associated with an increased risk of maternal morbidity and mortality (62–66). Obese women are more likely to have complicated deliveries, such as prolonged labor, cesarean section, and postpartum hemorrhage (67,68). They are also more likely to develop infections, such as endometritis and wound infections, and to require hospitalization after delivery (56,69).

The mechanisms by which obesity in pregnancy leads to these adverse outcomes are not fully understood. Excessive adipose tissue serves as an active endocrine organ, disrupting metabolic, vascular, and inflammatory pathways in various organ systems during pregnancy, thereby impacting obstetric outcomes (70,71). For example, obesity-related insulin resistance and abnormalities in inflammatory pathways can impair placental growth and function (72), contributing to the development of preeclampsia (73,74). The increased prevalence of obesity-related pregnancy complications with higher degrees of obesity further strengthens the role of obesity in the development of these adverse outcomes (75).

Maternal obesity potentially has long-term implications for offspring outcomes due to epigenetic changes triggered by fetal exposure to elevated levels of glucose, insulin, lipids, and inflammatory cytokines during development. These in utero effects can lead to persistent or temporary modifications in metabolic programming, resulting in unfavorable health outcomes in adulthood (known as the fetal origins of adult disease theory or Barker hypothesis) (76–78). However, studying the potential programming effects of maternal overnutrition is challenging due to the

intricate interplay between the maternal metabolic environment, fetal development, and the influence of postnatal factors such as lifestyle and environment (79).

1.2. High-density lipoproteins (HDL)

Lipoproteins are thought to be mainly involved in transporting hydrophobic dietary lipids within the aqueous environment of the circulatory system, however, their function extends beyond this traditionally recognized role (80–83). In particular, lipoproteins, including HDL, have a significant role in the immune system by providing protection against viral, bacterial, and parasitic infections (84–86). HDL, characterized by their high protein content, are the smallest and most dense class of lipoproteins. They are a highly heterogeneous group of particles, differing in size, density, electrophoretic properties, and in lipid and protein content. Moreover, HDL particles possess a wide range of advantageous properties (84–86).

1.2.1. Metabolism of HDL

The initiation of HDL formation involves the secretion of lipid-free apoA-I, primarily synthesized from the liver, but also from the intestine (87) (Figure 2). Shortly after secretion, lipid-poor apoA-I interacts with the ATP-binding cassette transporter A1 (ABCA1), which mediates the transfer of phospholipids and cholesterol from cellular cell membranes to apoA-I, resulting in the formation of a spherical particle form named pre-β HDL (51,88). Poorly lipidated apoA-I is partially catabolized in the kidneys and reabsorbed with the involvement of the proteins megalin and cubulin (89).Through intermediate steps, HDL acquires more lipids, but also proteins from the hydrolysis of triglyceride-rich lipoproteins by LPL, and nascent HDL is formed. The further efflux of cholesterol from the peripheral cells leads to progressive enlargement of the HDL particles and enrichment with cholesterol. The lipid transporter ABCA1 interacts preferentially with lipid-poor apoA-I and small HDL3, while the ATP-binding cassette transporter G1 (ABCG1) has a preference for HDL2 (90,91). Lecithin-cholesterol acyltransferase (LCAT) facilitates the esterification of free cholesterol on the surface of HDL, leading to the generation of cholesteryl-esters, which consitute the inner core of mature HDL (92,93). The formed cholesteryl-ester can be transferred to triglyceride-rich lipoproteins such as VLDL and LDL in exchange for triglycerides, which is mediated by CETP. HDL particles enriched with triglycerides are more susceptible to lipolysis by

hepatic and endothelial lipase, resulting in the formation of smaller HDL particles that are prone to faster catabolism (94). This pathway provides one of the possible routes for HDL cholesteryl-ester clearance. Another metabolic pathway of HDL clearance is the direct uptake of cholesteryl-ester by scavenger receptor B1 (SR-B1) by the liver or by steroidogenic tissues (92).

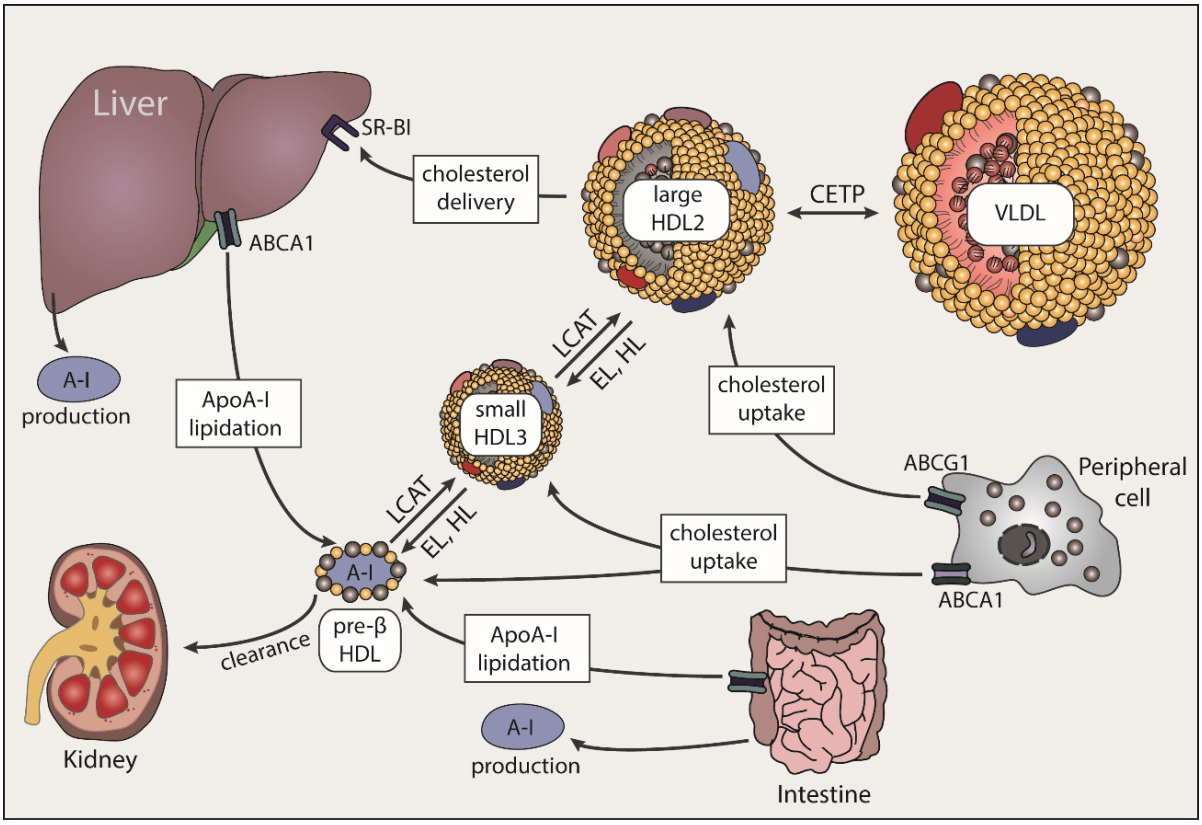

Figure 2. HDL biosynthesis and maturation. The synthesis of HDL initiates with the production and release of apolipoprotein A-I (apoA-I) by the liver and intestine. Lipid-poor apoA-I interacts with ATP-binding cassette A1 (ABCA1), leading to the acquisition of lipids and the formation of pre-β-HDL particles. Lecithin-cholesterol-acyltransferase (LCAT) plays a crucial role by esterifying free cholesterol on the surface of HDL, resulting in the generation of larger particles. ABCA1 exhibits a preference for pre-β-HDL or small HDL3 particles, while ATP-binding cassette G1 (ABCG1) facilitates the transfer of cholesterol to larger HDL2 particles. Cholesterol can be transported to the liver through the scavenger receptor BI (SR-BI) or transferred to very low-density lipoproteins (VLDL) via cholesteryl-ester transfer protein (CETP). HDL-associated triglycerides and phospholipids are primarily hydrolyzed by endothelial lipase (EL) and hepatic lipase (HL). Image reproduced from Stadler et al. (54) under Creative Commons Attributions License.

1.2.2. Major HDL-associated protein components

The structure and composition of HDL particles is highly heterogenous, and their functionality is determined by the relative distribution of lipids, proteins and enzymes associated with or within the HDL particle. With approximately 90% of total protein mass, the majority of the HDL proteome is composed of apoA-I and apoA-II, while other important proteins including apoE, apoA-IV, apoC-

II, apoC-III, apoD, apoM and serum amyloid A (SAA) are less abundant (95,96). In total, over 500 different proteins have been identified in HDL (95). However, it is important to emphasize that only a small number of these proteins have shown consistency across different studies, and the specific proteins identified can also vary depending on the isolation strategy employed (95,97). Importantly, not all different proteins are found on every single HDL particle, as most proteins are only associated with a small proportion of HDL particles (96).

The main structural but also functional protein constituent of HDL is apoA-I, which accounts for approximately 70% of total HDL protein content (98). Of particular interest, it is assumed that almost all HDL particles contain apoA-I (99,100). This protein plays a crucial role in HDL formation and is essential for promoting cholesterol efflux by interacting with cellular receptors (101). Moreover, apoA-I functions as an activator for LCAT, which serves as a key regulator in HDL maturation. Additionally, apoA-I is responsible for a range of other beneficial properties exhibited by HDL (99,102). Based on the amphipathic structure, apoA-I eagerly binds to lipids and shows detergent-like properties (102).

ApoA-II accounts for approximately 15-20% of total HDL protein content, making it the second major protein constituent of HDL, and it is estimated that about half of HDL particles contain apoA-II (103). This apolipoprotein is mainly synthesized in the liver, but also in the intestine (104), has a more hydrophobic structure than apoA-I and also poses a crucial role in HDL particle synthesis and function (105,106). ApoA-II circulates as a dimer and also interacts with other apolipoproteins such as apoA-I or apoE (106–108).

Proteins of the apoC family are mainly synthesized in the liver and are involved in the regulation of several enzymes activities (109). ApoC-II and ApoC-III represent the most abundant of these small exchangeable apolipoproteins and are present on HDL but also on triglyceride-rich lipoproteins (TGRL) (102). ApoC-II plays an important role in lipid metabolism, as it acts as a cofactor for LPL, an enzyme that hydrolyzes triglycerides on TGRL (110). ApoC-III in contrast, serves as a potent inhibitor of LPL, but is also present on HDL and TGRL (102). The involved mechanisms are not completely understood, but appear to involve reduced binding of TGRL to capillary endothelium, where lipoprotein lipase is present as well as displacement of apoC-II from TRGL surface (109,111,112).

Another protein, which has a key function on HDL is apoE. It is synthesized in several tissues and cell types, such as the liver, the central nervous system, endocrine tissues, and macrophages

(102). ApoE plays a critical role in the interaction of lipoproteins to cell-surface receptor, such as those of the LDL-receptor family and is therefore important in mediating remnant clearance (102,113,114). Endocytosis of apoE-containing lipoprotein particles decreases cholesterol levels in plasma and thus the risk of cardiovascular disease (115).

ApoM is a protein, which is mainly associated to HDL, but also to VLDL, LDL and chylomicrons (116,117). It is a lipocalin with a hydrophobic binding-pocket that enables binding of small hydrophobic molecules such as sphingosine-1-phosphate (S1P) or retinol (118,119). ApoM is synthesized in the liver and kidney and to a minor extend in adipose tissue (120,121). While apoM is found on only about 5% of circulating HDL, HDL particles containing apoM have been demonstrated to exhibit superior efficacy in promoting cholesterol efflux (122). Furthermore, these apoM-containing HDL particles possess a higher anti-oxidative capacity and display endothelium-protective activities (117,119,121–123).

Serum amyloid A (SAA) is a family of acute phase proteins and hepatic expression is induced in the acute phase of an inflammatory reaction, which results in increased circulating levels by approximately 1,000-fold (102,124). Of this family, SAA1 represents the main protein, which is predominantly associated to HDL (125). During inflammation, SAA is able to displace apoA-I, which leads to an SAA-enriched HDL particle with loss of HDL’s atheroprotective and anti-inflammatory properties (126,127).

1.2.3. Enzymes and lipid transfer proteins

The lecithin-cholesterol-acyltransferase (LCAT) is an important enzyme in HDL maturation (128). LCAT catalyzes cholesterol esterification mainly in HDL, but also in other lipoproteins. Around 75% of LCAT activity in plasma is associated to HDL (102).

Paraoxonase-1 (PON1) is another enzyme, which is predominantly associated with HDL particles (102). This enzyme is primarily expressed in the liver, but to a lesser extent also in kidneys and colon (129). The enzymes of the PON family are named after their originally observed function to detoxify organophosphates, such as paraoxone. On the other hand, PON1 has been found to have a broad spectrum of substrates, including homocysteine thiolactones and oxidized lipids in LDL. Additionally, PON1 has been identified as a contributor to HDL's anti-oxidative properties (130,131).

The cholesteryl-ester transferprotein (CETP) plays a significant role in modulating plasma lipoprotein composition. CETP facilitates the bidirectional transfer of triglycerides and cholesteryl-esters between TGRL and HDL (132). This transferprotein is expressed in the liver and adipose tissue and switches between HDL and TGRL while circulating (102,133).

1.2.4. HDL-associated lipids

In addition to HDL's complex apolipoprotein composition, researchers have identified more than 200 different lipid species, providing insights into the complexity of the HDL-lipidome (134–136). The location of these lipids within the particle is a significant factor in determining their structure and dynamic properties (137). The surface of HDL particles is composed of phospholipids, lyso-phospholipids, sphingolipids, including S1P, and free cholesterol, while the hydrophobic core contains triglycerides and cholesteryl-esters (136,138–140).

Phospholipids with approximately 35-50 wt% of total HDL lipids make up the major component of the HDL lipidome (136). Among phospholipids, phosphatidylcholine (33-45 wt% of total HDL lipids) is the most abundant molecular class in HDL. Additionally, significant amounts (≥ 1 wt% of total HDL lipids) of lysophosphatidylcholine , phosphatidylethanolamine, phosphatidylinositol, and plasmalogens are present in HDL (134–136,141). Minor phospholipids in HDL (< 1 wt% of total HDL lipids) include phosphatidylglycerol, phosphatidylserine, phosphatidic acid, and cardiolipin (139,142,143).

Sphingolipids make up 5-10 wt% of total lipids in circulating HDL particles, with sphingomyelin being the most abundant sphingolipid (136). Most of the sphingomyelin found in HDL is obtained from TGRL, whereas its contribution from nascent HDL is minimal (144). Sphingomyelin plays a crucial role in influencing the surface pressure of lipid membranes and lipoproteins, including HDL. It contributes to increased rigidity, which can influence the activity of embedded protein (145,146)

S1P is a highly active lysosphingolipid metabolite, which plays an important role in the regulation of several cellular processes (147). S1P acts as a ligand for G-protein-coupled S1P receptors found in endothelial and smooth muscle cells, regulating important cellular processes such as proliferation, angiogenesis, apoptosis, wound healing, and immune response (147–150). In plasma, around 65-80% of S1P is associated with HDL through binding to apoM (119). HDL-bound S1P demonstrates enhanced stability in comparison to S1P bound to albumin or free S1P (151).

This underscores the crucial role of HDL in the uptake, systemic function, and cellular degradation of S1P (151).

HDL triglycerides account for 5-12 wt% of the lipid content and originate from TGRL that contain apoB, primarily from VLDL (136). These triglycerides are transferred to HDL through CETP-mediated exchange with cholesteryl-esters derived from HDL (152). Triglycerides, like cholesteryl-esters, are hydrophobic and predominantly situated in the lipid core of HDL. However, in comparison to cholesteryl-esters, triglycerides contribute to a more fluid phase within HDL particles (136).The composition of HDL triglycerides is predominantly composed of species containing palmitic, oleic, and linoleic acid moieties (135). Recent findings have reported a significant molecular diversity of plasma triglycerides (6). This observation holds relevance not only for HDL but also for all plasma lipoproteins (136,153,154).

HDL steroids are dominated by cholesterol, in its free (unesterified) form, prominently present on the surface of the HDL lipid monolayer, comprising 5-10 wt% of the total HDL lipids (136). This abundance of cholesterol is significant for maintaining the fluidity of HDL particles, which is vital for their role in transporting cholesterol throughout the body (102). Additionally, HDL particles contain several other sterols, although in lesser quantities, such as oxysterols, estrogens and phytosterols (136).

Cholesteryl-esters constitute a significant portion (30-40 wt%) of the lipid content of HDL (136). These cholesteryl-esters, together with triglycerides, reside in the hydrophobic core of HDL particles. The majority of these cholesteryl-esters (up to 80%) are formed within plasma HDL through a trans-esterification reaction between phospholipids and cholesterol, catalyzed by LCAT (135). This enzymatic process causes the displacement of the cholesterol moiety from the surface lipid monolayer of HDL into its lipid core. Subsequently, cholesteryl-esters can be exchanged with triglycerides in apoB-containing lipoproteins through the action of CETP (136).

1.2.5. HDL subclasses

Depending on the maturation state, composition and site of origin, a wide range of HDL subclasses exist, which have also been shown to possess different functionality (155,156). Pre-β HDL represents the smallest plasma HDL species, composed of one or two apoA-I molecules, a layer of phospholipids and a trace amount of unesterified cholesterol (157). Unlike mature HDL with a

spherical shape, pre-β HDL is discoidal in shape and weighs approximately 67 kDa (157). Pre-β HDL plays a crucial role in lipid uptake by interacting with the ABCA1 transporter, which results formation of nascent HDL particles (92). Its efficient capacity to absorb cholesterol and phospholipids has led to the suggestion that pre-β HDL represents an important mediator in preventing the development of atherosclerotic plaques (158). Nascent HDL acquires more lipids, which derive from peripheral cells or from the hydrolysis of TGRL. Thereby, also apolipoproteins associate with HDL. The formed small HDL3 particles are denser and with approximately 60% have a high protein component. In contrast, the large HDL2 have less protein (43%), but a higher amount of associated lipids (159). Depending on the size of the HDL particles, various proteins are also more abundant, or exclusively present. On the smaller HDL3 subclass, apolipoproteins such as apoJ, apoF, but also enzymes including PON1 and PLTP have been shown to be exclusively abundant, while apoD, SAA1+2 and apoM are preferentially associated (96,156). On the contrary, apoE, apoC-II and apoC-III show higher abundance on the large HDL2 subclass (96). Therefore, the heterogeneity of the HDL subclasses also reflects the variation in their functionalities.

1.2.6. HDL and their multiple functions

HDL particles are multifaceted molecules that exhibits various functions, depending on their size and compositional constituents (Figure 3). These functions play a significant role in contributing to the anti-atherogenic properties of HDL (81,82). Among these functions are the capacity to remove excessive cholesterol from cells, anti-inflammatory, anti-oxidative, anti-thrombotic, and vasodilatory activities, but also anti-infectious activities.

Cholesterol efflux capacity

While circulating HDL-C concentrations are commonly used to assess cardiovascular risk, they do not offer a comprehensive understanding of the various beneficial activities of HDL in the body. The most well-studied function of HDL is its ability to promote reverse cholesterol transport, which involves the uptake of excessive cholesterol from peripheral cells and its transportation to the liver for excretion (160). However, the routine clinical measurement of HDL-C levels has limitations attributed to the heterogeneity of HDL particles. According to recent studies, the cholesterol efflux capacity of HDL appears to be a more accurate indicator of the risk of CVD than HDL-C levels (161,162). The reverse cholesterol transport process involves the release of lipid-poor apoA-I

from the liver and intestine, which then circulates to peripheral cells and forms nascent HDL. ABCG1 and SR-BI stimulate the cholesterol efflux to larger HDL subclasses (90,91), while ABCA1 preferentially lapidates lipid-poor apoA-I (163). Cholesterol can be actively transported by a variety of transporters and receptors, such as SR-BI, ABCA1, and ABCG1, as well as through passive diffusion (164–166).

In recent years, there has been significant research interest in the process of HDL-mediated cholesterol efflux. Various cell-based assays have been devised to assess HDL's capacity to facilitate cholesterol efflux, which is the initial step in the reverse cholesterol transport pathway (158). The most commonly used assay involves the use of a mouse macrophage cell line (J774), which is enriched with radioactive or fluorescently labeled cholesterol and cyclic adenosine monophosphate to increase the expression of ABCA1 (167). In these assays, apoB-depleted serum or isolated HDL from patients is added to the cell media, and the proportion of labeled cholesterol in the supernatant and in the cells is subsequently determined. Evaluation of this functionality in patients from several studies has revealed an inverse association between cholesterol efflux capacity and the occurrence of cardiovascular events (168–170).

HDL anti-inflammatory and endothelium-protective activities

Apart from its vital role in promoting reverse cholesterol transport, HDL also exhibits several anti-inflammatory and endothelial protective properties. Research studies have indicated that HDL is able to decrease the expression of adhesion molecules, such as intercellular cell adhesion molecule, vascular cell adhesion molecule, and E-selectin (171–173). This reduction in adhesion molecules limits the recruitment of monocytes, lymphocytes, and basophils to the vascular endothelium, leading to a deceleration of downstream inflammatory responses. The anti-inflammatory capacity of HDL, which can be assessed using cell-based assays, demonstrates an inverse association with the incidence of cardiovascular events in the general population (174). Furthermore, HDL can modulate nuclear factor kappa B and peroxisome proliferator-activated receptor gamma, resulting in a decrease in the production of chemokines and chemokine receptors both in vivo and in vitro (175). This involvement in the regulation of the immune system further contributes to the anti-inflammatory properties of HDL. ApoA-I plays a crucial role in mediating numerous anti-inflammatory activities of HDL, together with phospholipids saturated lyso-phosphatidylcholines and S1P (176–178).

HDL anti-oxidative and anti-thrombotic capacities

HDL, additionally to its various anti-inflammatory effects, further exhibits remarkable anti-oxidative properties. HDL is known to protect LDL and other lipoproteins from oxidative damages, thereby reducing their atherogenicity. ApoA-I is a key player in the antioxidative capacity of HDL, as it reduces lipid hydroperoxides through their methionine residues (179,180). Additionally, the HDL-associated enzyme PON1 contributes to the antioxidative activity of HDL, by reducing lipid peroxidation of LDL and HDL via specific cysteine residue (131). Other components of HDL, including lipoprotein associated phospholipase A2, LCAT, apoA-II, apoE, and apoJ also play a role in its antioxidant properties (156,181,182). Furthermore, HDL has the ability to suppress the generation of reactive oxygen species and alleviate intracellular oxidative stress (183–185).

HDL exhibits additional protective activities, including its antithrombotic effects mediated by various mechanisms. One of these mechanisms involves reducing the susceptibility of platelets to aggregation and inhibiting the activation of the coagulation cascade (186). HDL achieves this by upregulating the synthesis of endothelial nitric oxide and prostacyclin, which in turn prevents platelet activation (187). Moreover, HDL downregulates the synthesis of thromboxane A2 and the release of platelet activating factor, further contributing to its antiplatelet effects (188).

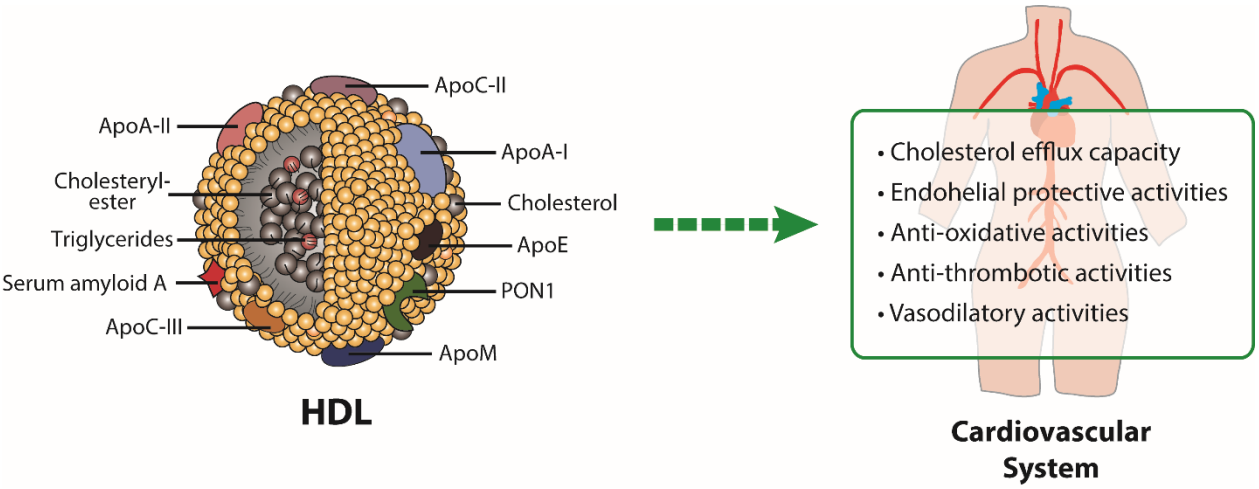

Figure 3. The composition of HDL and its protective mechanisms in cardiovascular disease are closely linked. Apolipoproteins (Apo) and paraoxonase 1 (PON1) are key components involved in these mechanisms. Image reproduced from Stadler et al. (54) under Creative Commons Attributions License.

1.3. Roles and Functions of Lipoproteins in Maternal and Fetal Circulation

During pregnancy, significant changes occur in glucose, protein, and especially lipid metabolism (189,190). Although there has been extensive research on maternal lipoproteins during human pregnancies (191–196), our understanding of the distinctions between maternal and fetal lipoproteins in late pregnancy, as well as their potential interrelationships remains limited. During the third trimester of pregnancy, the lipid profile of most women tends to display a pattern that would be regarded as highly atherogenic in a non-pregnant condition (192,197). This profile is characterized by an approximately three-fold increase in triglyceride levels, a 25-50% increase in total cholesterol, elevated levels of HDL-C and LDL-C, and an increased abundance of small dense LDL particles (192,198,199). Maternal hyperlipidaemia during pregnancy is attributed to increased insulin resistance and estrogen stimulation (200,201). Maternal fat accumulation, primarily occurring in the first and second trimester of gestation, is influenced by increased food intake (hyperphagia) and intensified lipid synthesis (192,202,203). However, during the last trimester, fat storage declines due to enhanced breakdown of fats (lipolytic activity) and reduced activity of LPL in adipose tissue (192,204,205). While these changes are temporary for most women and return to normal after delivery, the long-term implications of variations in the lipid profile remain uncertain. Moreover, alterations in maternal lipid metabolism and cholesterol supply may impact fetal outcomes, potentially leading to consequences later in life (206–209). Generally, fetal cord blood and neonatal plasma display significantly lower levels of lipids compared to adults (210–213). Moreover, there is a relatively higher proportion of cholesterol carried by HDL particles in the fetal circulation (210–213). Of particular interest, both the concentration, but also the composition of fetal HDL differ from those in adults (214–216). While LDL is the predominant lipoprotein in maternal serum, fetal HDL carries up to 50% of the total cholesterol. In fetal circulation, LDL and VLDL are present, but at relatively low concentrations (214,216–218). Further, the HDL proteome shows a substantially different composition, with higher abundance of apoE and lower concentrations of apoA-I, apoD, apoC-II and apoC-III (214,219,220). ApoA-I is a critical component of HDL, involved in various important functions including interacting with cellular receptors, activating LCAT, and exerting anti-atherogenic activities (101,221–224). Additionally, it contributes to HDL's anti-oxidative capacity, and the lower levels of apoA-I observed in fetal HDL may suggest a diminished anti-oxidant function (214,219,225). In contrast, apoE is more abundant in fetal HDL, compared to adult HDL, which also exerts several important functions including its

role in cholesterol transport and facilitating HDL binding to receptors of the LDL-receptor family. Further apoE has been identified as a physiological activator of LCAT, similar to apoA-I (226,227). Hence, it appears that fetal HDL serves a role in the transport of cholesterol to tissues, similar to LDL in adults.

1.4. Cholesterol transport across the human placenta

Cholesterol plays a crucial role in fetal development, acting as a structural component of cell membranes and serving as a precursor for oxysterols (228,229). While the fetus mainly synthesizes cholesterol de novo in various organs like the adrenals and liver, intriguingly, fetuses with genetic disorders affecting cholesterol biosynthesis (Smith-Lemli-Opitz syndrome) still exhibit measurable cholesterol levels (230,231). Fetuses affected by this congenital condition can still reach full term, indicating the essential need for maternal cholesterol to be transported across the placenta to meet the fetal requirements (231,232).

The human placenta, a remarkable organ, consists of various specialized cell types and facilitates numerous metabolic exchanges between the mother and the fetus. In order to meet the needs of the growing fetus, oxygen and nutrients pass through different cell layers, directly crossing from the maternal to the fetal circulation. The syncytiotrophoblast, which is a layer of multinucleated trophoblasts located between the microvillous and basal membranes facing the maternal and fetal sides respectively, forms the initial physical barrier that regulates nutrient transfer across the placenta (233,234).

The initial step in the process of cholesterol transport from the mother to the fetus occurs at the apical (maternal) side. Within human placental trophoblasts, various lipoprotein receptors, including LDL-R, SR-BI, and LDL receptor-related protein 1 are expressed (Figure 4) (235–238). The receptors facilitate the uptake of cholesteryl-esters and cholesterol from lipoproteins of maternal origin (239,240). Following receptor-mediated endocytosis, lipoprotein-associated cholesteryl-esters undergo intracellular hydrolysis (235). Subsequently, free cholesterol is transported across the cell to membranes or metabolically active compartments via the involvement of Niemann-Pick C1 and potentially other less extensively investigated cholesterol transporter proteins (235,241). The selective uptake of HDL-associated cholesteryl-esters is primarily facilitated by SR-BI (235). Cytosolic cholesterol esterases hydrolyze these cholesteryl-

esters, and potential carrier proteins transport them to the basal membrane (235). However, the precise mechanism of transcellular cholesterol transport is not yet fully understood, although multiple transporters, including sterol carrier protein-x/2, Niemann-Pick C1, Niemann-Pick C1-like protein 1, and ABCA2 have been shown to be expressed in the human placenta (242). For placenta cholesterol to enter the fetal circulation, it must traverse the endothelium at the fetoplacental vasculature. A study utilizing isolated endothelial cells from human term placentas observed the efflux/secretion of exogenous cholesterol through ABCG1 and ABCA1 (243). In cord blood, poorly lipidated apoA-I, apoE, and HDL serve as acceptors of cholesterol, with apoE-enriched HDL being particularly efficient (242,243).

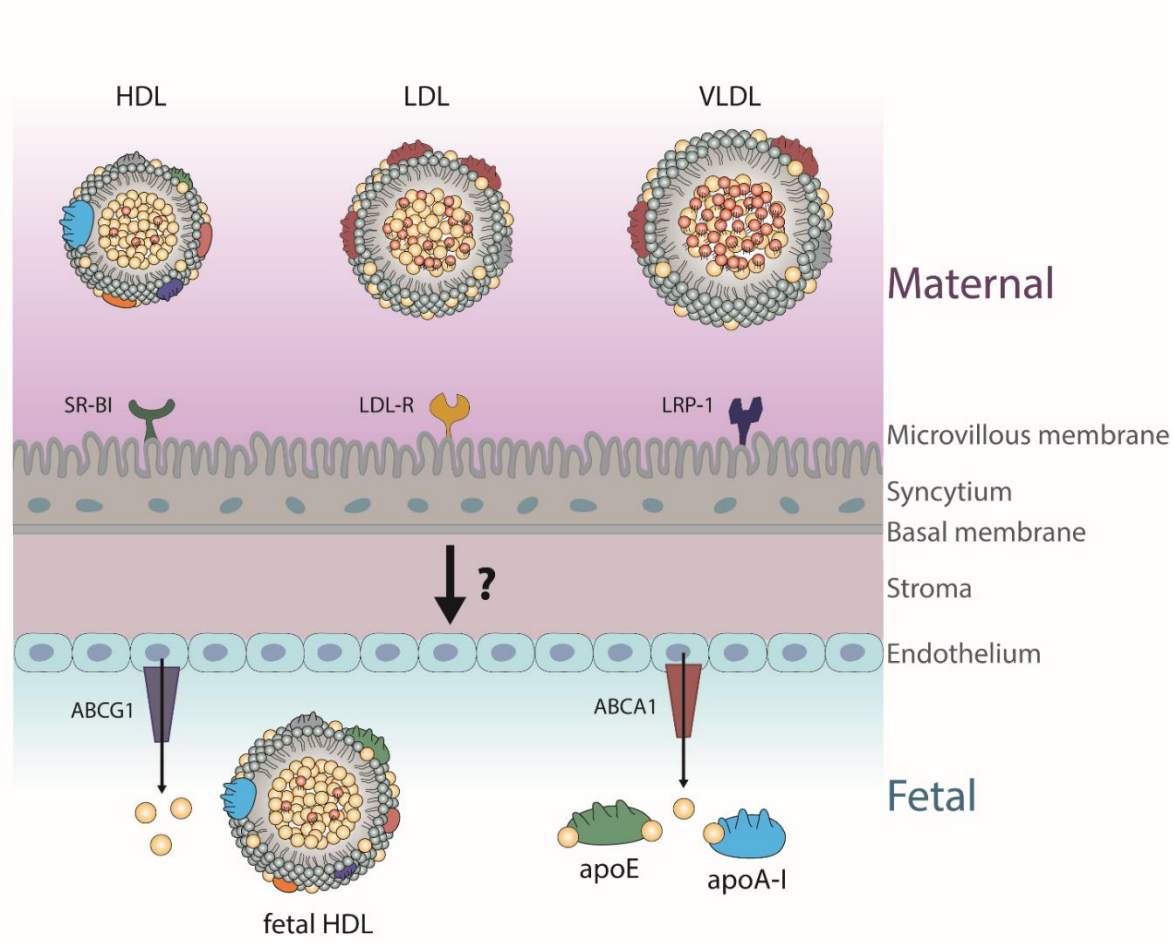

Figure 4: Transport pathways of maternal cholesterol across the human placenta. Initially, maternal lipoproteins interact with specific receptors located on the microvillous membrane of the syncytium. Cholesterol is then taken up by the syncytium and subsequently secreted or effluxed to fetal high-density lipoprotein (HDL) and lipid-poor acceptor apolipoproteins. However, the mechanism by which cholesterol is transferred from the stroma to the fetoplacental endothelium remains unclear. Key players in this process include HDL, scavenger receptor BI (SR-BI), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), low-density lipoprotein receptor (LDL-R), LDL receptor-related protein 1 (LRP-1), ATP-binding cassette A1 (ABCA1), and ATP-binding cassette G1 (ABCG1). Image reproduced from Stadler et al. (6) under Creative Commons Attributions License.

Cholesterol provided by the mother plays a significant role in fetal growth (229). While there is no direct connection of lipoprotein metabolism between mother and fetus, serum cholesterol levels of mothers during pregnancy are closely associated with the weight of the infant at birth (244,245). Low levels of maternal serum cholesterol during pregnancy have been linked to an increased risk of microencephaly, while elevated maternal cholesterol levels contribute to early development of atherogenicity (232,246). Moreover, several studies have shown a relationship between very high levels of cholesterol in mothers with complications such as prematurity and impaired fetal growth (247–249). During pregnancy, disruptions of maternal cholesterol homeostasis have also been linked with conditions like preeclampsia and pregnancy-induced hypertension (247,250–252).

1.5. Hypertensive disorders during pregnancy

Obesity poses a major risk factor for development of the hypertensive pregnancy-related disorders gestational hypertension (GHTN) and preeclampsia (PE) (253–255). These disorders are a major cause of maternal and fetal morbidity and mortality and also increase the risk of long-term cardiovascular complications (256–259). While GHTN is defined as maternal new-onset of blood pressure ≥ 140/90 mmHg, PE is combined with proteinuria (≥ 300 mg/24 hours) or one of the following diagnoses: thrombocytopenia, impaired liver function, renal insufficiency, cerebral or visual problems or pulmonary oedema (260,261). Hypertensive disorders during pregnancy are associated with a range of pathological changes, including endothelial dysfunction, oxidative stress, and hyperlipidemia (262,263).

During pregnancy, various physiological changes take place, such as increased insulin resistance and alterations in lipid profiles, including increased total cholesterol (LDL, VLDL and HDL) and triglycerides. These changes are particularly noticeable in women who develop PE (252,264,265). Moreover, in PE, hypercoagulability, a normal feature of pregnancy, may be exacerbated, resulting in increased thrombin generation, elevated fibrinogen levels, activated protein C resistance, and decreased levels of protein S, as well as impaired fibrinolysis (266).

During normal pregnancy, the diameter of uterine spiral arteries increases significantly due to remodeling of the endothelium and vascular smooth muscle. This remodeling process is initiated by protease release from endovascular trophoblast and uterine natural killer cells (267,268). In contrast, failure of spiral artery remodeling, which is characterized by the retention of smooth

muscle, is a hallmark of PE (269,270). This retention leads to decreased uteroplacental perfusion, which can be observed through noninvasive blood flow and perfusion studies using Doppler ultrasound or magnetic resonance imaging (269).

Abnormal placentation occurring in early pregnancy is recognized to result in alterations in angiogenic factors (268). Elevated levels of circulating soluble fms-like tyrosine kinase 1 (sFlt-1), an antiangiogenic factor originating from the placenta, results in neutralization and reduction of proangiogenic factors including placental growth factor (plgf) and vascular endothelial growth factor (268). These alterations contribute to the development of hypertension and glomerulopathy, which are characteristics of the syndrome (269). Innovative therapeutic trials for preeclampsia prevention have integrated measurements of angiogenic biomarkers into risk stratification (271–273). Recent research has shown that an elevated ratio of sFlt-1 to plgf may be particularly noticeable in women with severe preeclampsia before 34 weeks of gestation, a condition that some refer to as placental preeclampsia (274). This is due to the link between placental ischemia and adverse fetal outcomes, especially fetal growth restriction (268,275). On the other hand, maternal preeclampsia typically occurs later in pregnancy and is linked to more significant pre-pregnancy maternal vascular dysfunction, such as hypertension, diabetes, or obesity (275). Maternal preeclampsia demonstrates less pronounced placental pathology and is associated with fewer fetal complications (275). In the context of maternal preeclampsia, pregnancy serves as a physiological stress, worsening preexisting endothelial dysfunction (276). This highlights the heterogeneity of hypertensive disorders of pregnancy, where the various clinical subtypes may have distinct underlying mechanisms (275,277).

Following a preeclamptic pregnancy, both the mother and the offspring face an elevated risk of long-term cardiovascular complications (257,258,278–280). A systematic review and meta-analysis revealed that women with a history of PE face a higher relative risk of developing certain health conditions in the long term. Specifically, they have a 3.7-fold increased relative risk of hypertension, 2.2-fold increased relative risk of ischaemic heart disease, 1.8-fold increased relative risk of stroke and 1.8-fold increased relative risk of venous thromboembolism. These increased risks persist over a period of 5 to 15 years after delivery (256). The risk is even higher for women who experience more severe PE, including recurrent disease, early onset and neonatal morbidity (281–284).

2. RESULTS

The results section will shortly summarize the following publications:

1. Stadler, JT; Marsche, G. **Obesity-Related Changes in High-Density Lipoprotein Metabolism and Function**. Int J Mol Sci. 2020;21(23): https://doi.org/10.3390/ijms21238985

2. Stadler, JT; Lackner, S; Mörkl, S; Trakaki, A; Scharnagl, H; Borenich, A; Wonisch, W; Mangge, H; Zelzer, S; Meier-Allard, N; Holasek, SJ; Marsche, G. **Obesity Affects HDL Metabolism, Composition and Subclass Distribution**. Biomedicines. 2021; 9(3): https://doi.org/10.3390/biomedicines9030242

3. Stadler, J.T.; van Poppel, M.N.M.; Wadsack, C.; Holzer, M.; Pammer, A.; Simmons, D.; Hill, D.; Desoye, G.; Marsche, G.; DALI Core Investigator Group. **Obesity Affects Maternal and Neonatal HDL Metabolism and Function**. *Antioxidants* 2023, *12*, 199. https://doi.org/10.3390/antiox12010199

4. Stadler, J.T.; van Poppel, M.N.M.; Christoffersen, C.; Hill, D.; Wadsack, C.; Simmons, D.; Desoye, G.; Marsche, G.; DALI Core Investigator Group. **Gestational Hypertension and High-Density Lipoprotein Function: An Explorative Study in Overweight/Obese Women of the DALI Cohort.** Antioxidants 2023, *12*, 68. https://doi.org/10.3390/antiox12010068

5. Stadler, J.T., Scharnagl H., Wadsack C., Marsche G. **Preeclampsia affects lipid metabolism and HDL function in mothers and their offspring.** Antioxidants 2023, 12 (4), 795; https://doi.org/10.3390/antiox12040795

6. Stadler, JT; Wadsack, C; Marsche, G. **Fetal High-Density Lipoproteins: Current Knowledge on Particle Metabolism, Composition and Function in Health and Disease**. Biomedicines. 2021; 9(4): https://doi.org/10.3390/biomedicines9040349

Ad 1.) The first objective of my thesis was to conduct a systematic review and evaluation of existing literature regarding the impact of obesity on HDL metabolism and functionality (1). Most studies have shown that in obesity, not only the cholesterol content of HDL is reduced, but also HDL particle distribution appears to shift from large to smaller subclasses, which is accompanied by changes in HDL composition (2,285,286). We comprehensively summarized obesity-related changes in HDL metabolism and further discussed the impact of gastric bypass surgery and several different fasting strategies.

Ad 2.) A primary objective of this study was to examine the impact of obesity on HDL metabolism, composition, and subclass distribution within a cohort consisting of 26 normal weight (BMI 18.5-24.9 kg/m^2), 22 overweight (BMI 25.0-29.9 kg/m^2), and 20 obese (BMI ≥ 30.0 kg/m^2) young women (2). We assessed HDL-associated lipids and apolipoproteins, analyzed HDL subclass distribution using native gradient gel electrophoresis, and examined differences in enzyme activities involved in HDL metabolism. A shift towards smaller HDL subclasses was observed in obese women, which was linked to a reduction in serum anti-oxidative capacity. Additionally, obesity led to a significant decrease in apoA-I, cholesterol, and phospholipids and an increase in triglycerides and the pro-inflammatory acute-phase protein SAA. We further observed noticeable changes in LCAT and CETP enzyme activities. Interestingly, HDL subclass distribution, and levels of HDL-C were associated with soluble leptin receptor and adiponectin, while LCAT activity was linked to liver enzyme activities. Notably, most of these changes were only observed in obese, but not in overweight women. In summary (Figure 5), we have shown that obesity has a significant impact on HDL metabolism, composition, and subclass distribution, which are closely associated with changes in liver and adipose tissue. The presence of dysfunctional HDL may contribute to an elevated risk of cardiovascular complications associated with obesity (2).

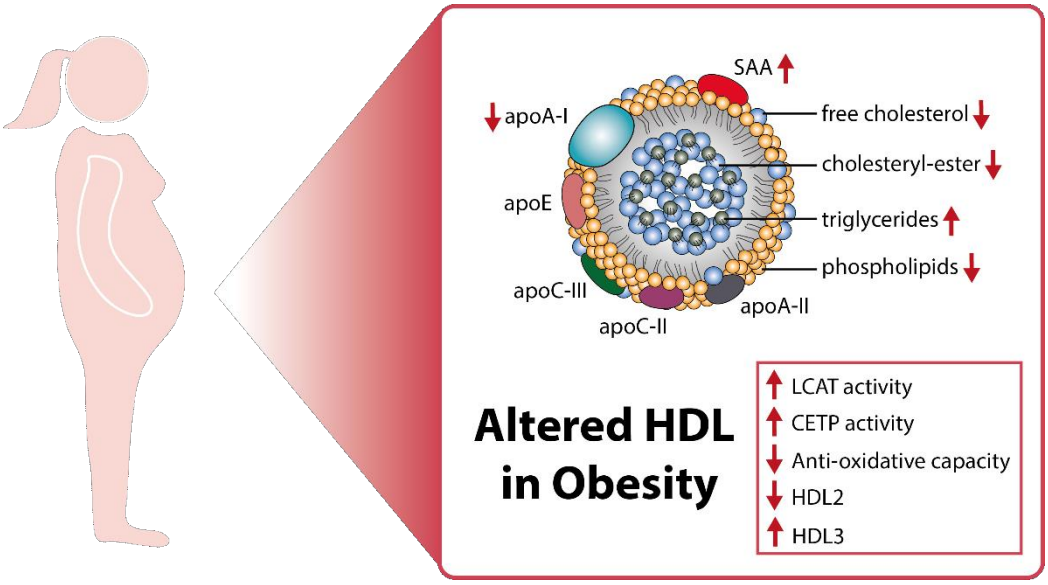

Figure 5: Graphical Abstract. Obesity affects HDL metabolism, composition and subclass distribution. The changes observed in obese compared to normal-weight women are summarized and indicated with red errors. Apo, apolipoproteins; SAA, serum amyloid A; LCAT, lecithin cholesterol-acyltransferase; CETP, cholesteryl-ester transferprotein. Image reproduced from Stadler et al. (2) under Creative Commons Attributions License.

Ad 3.) In the second phase of my dissertation, we directed our attention to examining the effects of obesity on HDL metabolism and functionality in pregnant women and their offspring (3). To achieve this, we obtained serum samples from matched maternal and cord blood samples from participants of the DALI (Vitamin D and lifestyle intervention for GDM prevention) trial. This trial took place between 2012 and 2015 across 11 study sites in Europe (287,288). In this study cohort, pregnant women with a pre-pregnancy BMI ≥ 29 kg/m^2 were recruited early in pregnancy, were followed until birth and the onset of pregnancy complications was recorded. For our analyses, maternal blood samples at term and paired umbilical cord blood of the neonate was used. Based on the results of our previous studies, we were interested in elucidating the effect of pre-gravid obesity on HDL metabolism and function. Therefore, we also collected serum samples of normal-weight women (pre-pregnancy BMI < 25 kg/m^2) and their offspring, which were used as controls. We observed that pre-pregnancy obesity was associated with changes in serum lipid levels, with an increase of triglycerides and a reduction of HDL-C in the mothers. Of particular interest, in new-borns of obese mothers, serum triglyceride levels were profoundly increased. Moreover, serum anti-oxidative capacity was impaired in obese mothers as well as in their offspring. Further, we observed changes in HDL metabolism and function in mothers with reduced activity of LCAT, while

HDL's cholesterol efflux capacity was increased. Another interesting finding was that some HDL functionalities, including PON1 activity and cholesterol efflux capacity, as well as serum anti-oxidative capacity correlated between mothers and children (Figure 6). Here we provided novel data on the effect of pre-pregnancy obesity on maternal and neonatal HDL. Moreover, our results may help to better understand the link between maternal and cord blood parameters (3).

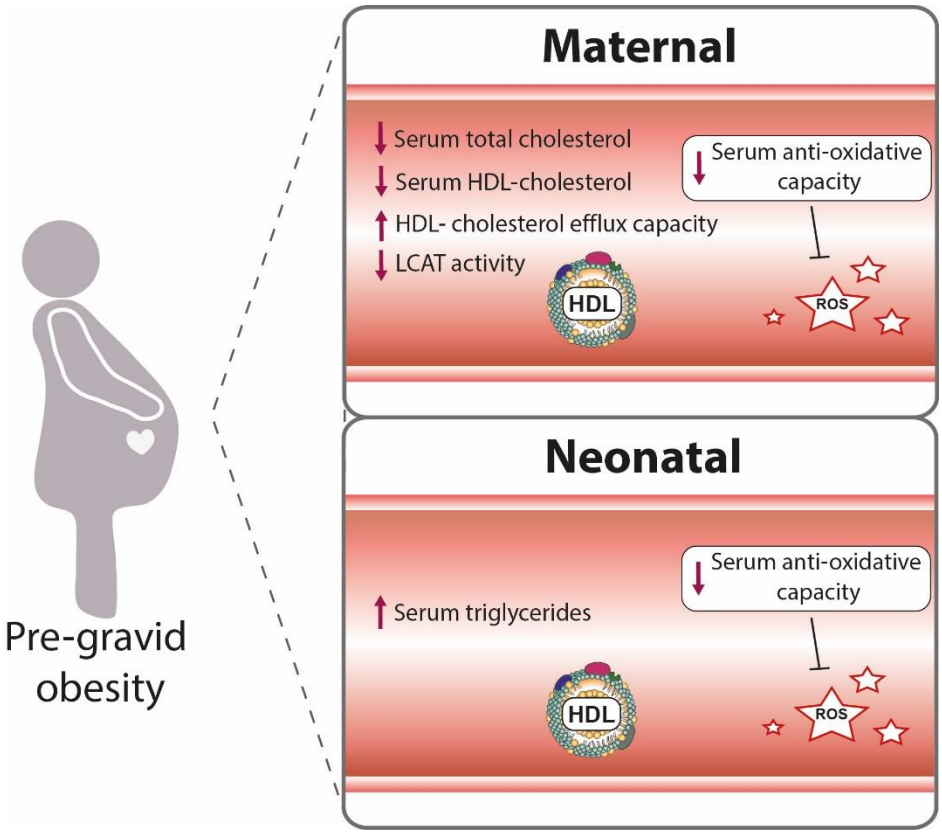

Figure 6: Graphical Abstract: Obesity affects maternal and neonatal HDL metabolism. LCAT, lecithin cholesterol-acyltransferase. Image reproduced from Stadler et al. (3) under Creative Commons Attributions License.

Ad 4.) In a subsequent study, we employed samples from the DALI cohort to evaluate the effects of gestational hypertension (GHTN) on HDL metabolism and functionality (4). Hypertension during pregnancy is associated with an increased risk for cardiovascular events later in life, in both mothers and offspring (258,289–292). Based on the multitude of beneficial anti-atherogenic properties of HDL, we hypothesized that HDL functionality may be affected during this disease and could also potentially serve as potential prognostic markers for the development of

hypertension during pregnancy. In the DALI study, maternal serum was taken at three time points during pregnancy, as well as cord blood after delivery, and patients were monitored over the course of the pregnancy. The occurrence of GHTN was detected in thirteen percent of the included women. Interestingly, we observed that HDL's cholesterol efflux capacity and serum levels of apolipoprotein M increased from early to late pregnancy, while activity of HDL-associated enzyme PON1 decreased (Figure 7). In cord blood, levels of HDL-C, cholesterol efflux capacity, PON1 activity and apoM levels were lower, compared to mothers, however, serum anti-oxidative capacity was higher. Furthermore, our findings indicated that GHTN had no significant impact on HDL-related functional parameters in obese mothers. However, it profoundly affected the HDL composition and functionality in cord blood samples. Follow-up studies are needed to determine whether these changes persist and potentially affect long-term cardiovascular risk.

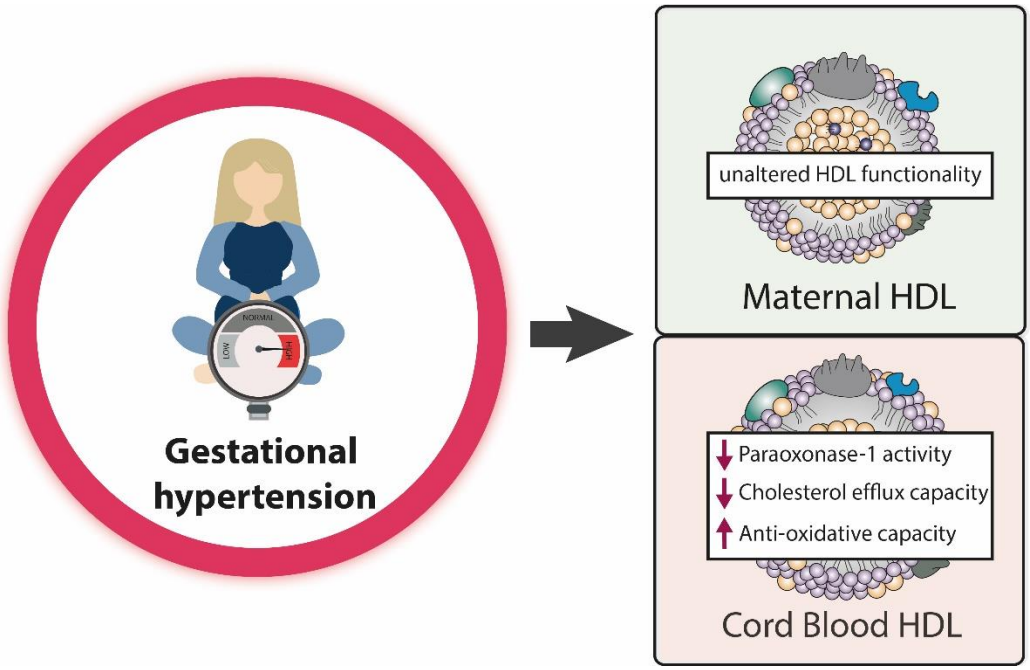

Figure 7: Graphical Abstract: Gestational hypertension and high-density lipoprotein function: An explorative study in overweight/obese women of the DALI cohort. Image reproduced from Stadler et al. (4) under Creative Commons Attributions License.

Ad 5.) In my final original research project, building upon our previous findings, I aimed to determine whether maternal or neonatal HDL exhibited similar alterations in patients with PE, a more severe form of hypertension during pregnancy. We collected paired samples of maternal serum at term and corresponding umbilical cord blood of 18 early-onset PE and 14 late-onset PE

pregnancies. As controls, we collected samples of normotensive pregnancies, without having any pregnancy complications. Maternal early-onset and late-onset PE was linked to atherogenic dyslipidemia, as characterized by elevated plasma triglycerides and decreased levels of HDL-C. Notably, we observed a shift from larger to smaller HDL subclasses in early-onset PE (Figure 8). Additionally, PE was linked to significantly elevated levels of HDL-associated apoC-II in mothers that correlated with the levels of triglycerides in HDL. Neonates born to mothers with early-onset PE exhibited elevated total plasma cholesterol levels, while neonates from late-onset PE pregnancies showed a marked reduction in HDL cholesterol efflux capacity. Overall, these findings suggest that both early- and late-onset PE have profound effects on maternal lipid metabolism, potentially contributing to the development of the disease and increasing cardiovascular risk later in life. Furthermore, PE is associated with alterations in neonatal HDL function and composition, indicating that pregnancy complications impact the lipoprotein metabolism in the neonate (5).

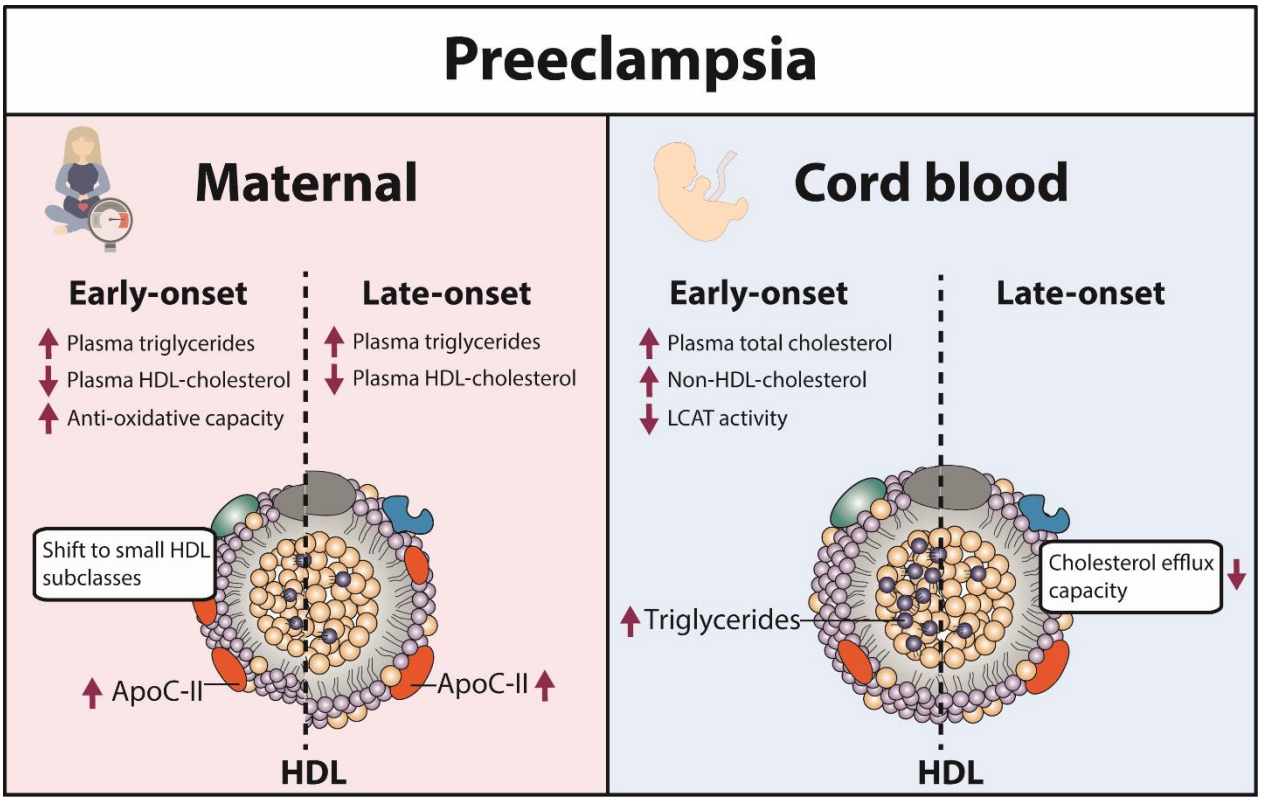

Figure 8: Preeclampsia Affects Lipid Metabolism and HDL Function in Mothers and Their Offspring. Image reproduced from Stadler et al. (5) under Creative Commons Attributions License.

Ad 6.) Finally, I did a comprehensive literature review on HDL in pregnancy, the role of HDL in the fetus, and the effects of pregnancy-related disorders on fetal HDL (6). There is growing evidence that fetal HDL is different from adult HDL in regard to size, composition and functionality (Figure 9) (214). This unique composition with enrichment of apoE, which binds to the LDL receptor, rises the possibility that fetal HDL plays a primary role in cholesterol transport to tissues, similar to the role LDL accomplishes in adults. Based on its distinctive composition, fetal HDL may also play an important role in atheroprotection. Accumulating research reports an important role of S1P on fetal HDL, which is important for preserving the feto-placental vasculature by exerting strong vasoprotective effects (293). During pregnancy disorders, such as GDM or PE, alterations in maternal HDL composition and function have been reported. Of particular interest, these pregnancy complications were also shown to affect HDL metabolism in the fetus. Disease-related changes in HDL function and composition raise the possibility that these changes may contribute to the pathophysiology of pregnancy-related disorders.

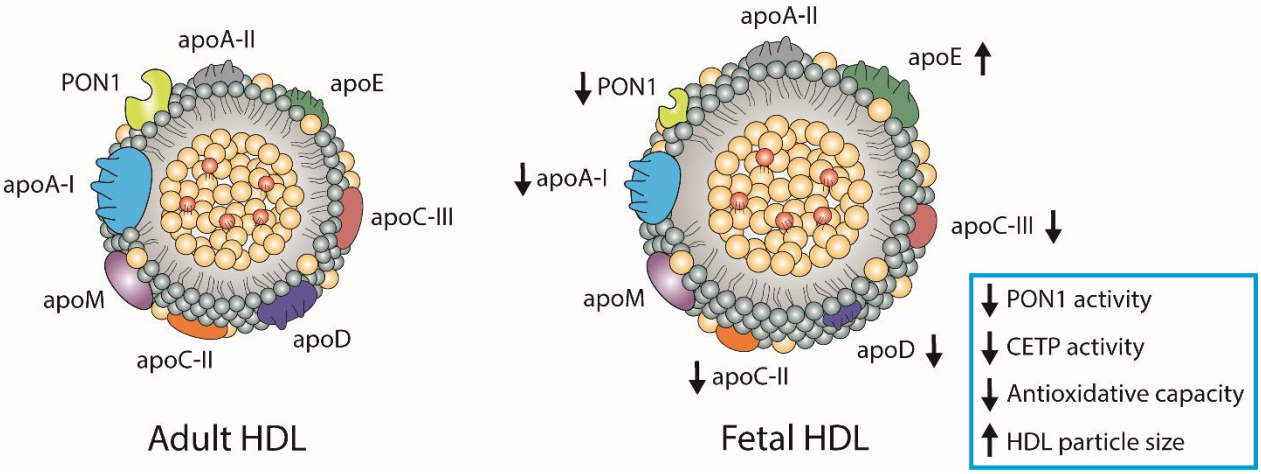

Figure 9: Differences between adult and fetal HDL metabolism, composition and function. Several changes in fetal HDL derived from cord blood have been reported and are indicated with arrows. Apo, apolipoproteins; PON1, paraoxonase-1 activity; CETP, cholesteryl-ester transferprotein. Image reproduced from Stadler et al. (6) under Creative Commons Attributions License.

3. DISCUSSION

Obesity has become a significant global health problem, and the number of affected individuals is continually increasing. Additionally, maternal obesity during pregnancy presents significant concerns and is linked with an increased risk of pregnancy-related disorders such as GDM, GHTN or PE (254,294–297). Importantly, both mothers and offspring of pregnancies affected by these disorders face an elevated long-term risk of developing cardiovascular diseases. (76,256,298–300).

During my PhD, my research focused on investigating the impact of overweight and obesity on HDL composition, function, and metabolism. Our findings revealed that obesity significantly influenced HDL metabolism, resulting in a shift in HDL subclass distribution. Additionally, we observed changes in the composition of HDL in individuals with obesity, with reduced levels of apoA-I and an increase in HDL-associated triglycerides. Notably, most of the observed alterations were only evident in individuals classified as obese, not in those classified as overweight (2). We also contributed to this important but not well established research field by writing a comprehensive literature review summarizing obesity-related changes in HDL Metabolism and function. In this review we also discussed the effect of bariatric bypass surgery or pharmacological anti-obesity interventions on HDL levels and function (1).

I further focused on investigating the impact of obesity on HDL metabolism and function in both mothers and their offspring in a study cohort of overweight and obese pregnant women. Our findings revealed that maternal obesity has a significant impact on the functional parameters of HDL in both mothers and their offspring (3). Additionally, within the same cohort, we focused on women who developed GHTN. Intriguingly, while the mothers affected by GHTN showed no significant alterations in HDL metabolism and function, their offspring exhibited substantial changes in HDL-related parameters (4). Expanding upon my previous investigations into GHTN and its effects on HDL, I extended my research to explore the alterations in maternal and neonatal HDL composition and function in the context of PE, a more severe hypertensive disorder during pregnancy. In this study, I observed substantial changes in lipid metabolism in mothers affected by both early-onset PE and late-onset PE, suggesting a potential role in the development of PE and long-term cardiovascular risk. Furthermore, PE was associated with significant modifications

in neonatal HDL function and composition, indicating that pregnancy disorders have an impact on lipoprotein metabolism in offspring as well (5).

By conducting a comprehensive review of the existing knowledge on fetal HDL particle metabolism, composition, and function, we obtained valuable insights into its crucial role in protecting the vasculature during fetal development. Additionally, our study presented a comprehensive overview of the alterations observed in the protective functions of HDL in pregnancy-related disorders, including PE and GDM. This research contributes to a better understanding of the intricate mechanisms involving HDL in pregnancy and highlights its potential implications for the pathogenesis and management of these disorders (6).

3.1. Obesity-Related Changes in High-Density Lipoprotein Metabolism and Function.

(Int J Mol Sci. 2020; 21(23): Doi: 10.3390/ijms21238985)

The prevalence of obesity has significantly increased in recent years, with excessive calorie intake and lack of physical activity being the major contributing factors. Obesity increases the risk of various diseases, including coronary artery disease, type-2 diabetes mellitus, hypertension, and dyslipidemia (301–305). In dyslipidemia associated with obesity, high levels of triglyceride-rich lipoproteins and low levels of HDL-C are commonly observed (28,306). Moreover, obesity also results in abnormal HDL metabolism, which often results in dysfunction of the HDL particles (2,307). Consequently, researchers have shifted their focus from investigating the quantity of HDL to examining its quality.

In this review article, I provide a comprehensive overview of HDL metabolism and the pathophysiological changes observed in obesity. We specifically highlighted how obesity alters HDL composition and functionality, including the relationship between HDL and the adipokine adiponectin, as well as the altered levels of the bioactive lipid S1P (308–310). The review also summarizes the effects of weight loss induced by bariatric surgery, pharmacological approaches, and Mediterranean diet, which are effective in increasing HDL-C levels and improving HDL function (311–313).

3.2. **Obesity Affects HDL Metabolism, Composition and Subclass Distribution.** (Biomedicines. 2021; 9(3): Doi: 10.3390/biomedicines903024)

Obesity is commonly associated with atherogenic dyslipidemia, characterized by elevated plasma triglycerides, reduced levels of HDL-C, and an increased risk of cardiovascular events (23,314). In addition to changes in HDL quantity, the functionality of HDL may also be compromised. However, there is a lack of comprehensive data specifically addressing the effects of obesity, independent of its associated comorbidities, on HDL function and composition. Therefore, our study aimed to examine these alterations in a young and notably healthy cohort. Women were enrolled across a range of body weights, including those with normal weight, overweight, and obesity, as classified according to the World Health Organization's recommended BMI categories for relative weight classification (7).

In the beginning, we evaluated differences in HDL constitutional parameters and examined HDL-associated proteins and lipids. The composition of HDL was found to be profoundly altered in obese women, with notable reductions in HDL-associated apoA-I, cholesteryl-ester, free cholesterol, and phospholipids, accompanied by an increase in SAA content, which suggests the presence of low-grade inflammation (315). This hypothesis was further supported by the strong correlation we observed between SAA and C-reactive protein levels. In contrast, among overweight women, we detected an increase solely in the triglyceride content of HDL. Interestingly, the activity of PON1, which has previously been reported to be affected in obese children and adolescents (138,139), did not show significant alterations in response to obesity (316,317). Despite the reduction in HDL-C levels, obesity did not have a significant impact on HDL's cholesterol efflux capacity. This paradoxical observation may be explained by the shift towards increased HDL3 and small HDL3 particles in obese women, which are known to be the most efficient mediators of cholesterol efflux (318), potentially compensating for the lower total HDL cholesterol levels.

Another important observation of our study was the significant increase in serum LCAT activity and protein concentration in obese individuals. This enzyme is thought to play a critical role in reverse cholesterol transport (319). Previous research has demonstrated that patients with type 2 diabetes have increased LCAT activity, which appears to be linked to a reduction in the anti-oxidative capacity of HDL (320). Our study observed similar results, as we found a significant correlation between HbA1c and LCAT activity, as well as a notable decrease in serum anti-oxidative capacity in obese women.

Remarkably, we also discovered a positive correlation between LCAT activity and the liver function marker cholinesterase, which is contrary to previous reports of decreased LCAT activity in patients with liver disease (321,322).

One plausible explanation for this discrepancy could be that, despite involving obese women, our study comprised young and healthy women with only slight elevations in liver markers. Our results suggest that LCAT concentration and activity may increase in response to obesity and low-grade inflammation, possibly as a compensatory mechanism. However, in severe comorbidities and pathological conditions, LCAT levels may decrease (167,320,321,323).

Our study further investigated the activity of CETP, a key enzyme in HDL metabolism that facilitates the transfer of cholesteryl-esters from HDL to triglyceride-rich lipoproteins in exchange for triglycerides (133,152). We found that CETP activity was increased in the obese group, consistent with the high expression of CETP in adipose tissue (324,325). Our findings suggest that the elevated LCAT activity observed in obese women may contribute to the accumulation of cholesteryl-esters in HDL, which are subsequently transferred to triglyceride-rich lipoproteins by CETP. This process leads to an increase in triglyceride content in HDL particles, which are then more susceptible to hydrolysis by hepatic and lipoprotein lipases, ultimately leading to the formation of smaller HDL particles (28,33,326). Notably, the rate-limiting step in this process is the slower esterification by LCAT, as compared to the transfer of cholesteryl-esters by CETP (326–328).

We next investigated the HDL subclass distribution by using native gel gradient electrophoresis and staining of neutral lipids with Sudan black. Of particular interest, a shift towards smaller HDL3 subclass and a reduction in the larger HDL2 subclass was observed in obese individuals, in line with previous research (285,286). We also observed a strong correlation between LCAT activity and the HDL3 subfraction, indicating the preferential conversion of lipid-poor pre-β particles to HDL3 by LCAT. The observed increase in CETP activity and transfer of cholesteryl-esters from HDL2 to VLDL in obese women is likely to result in decreased levels of HDL2-cholesterol.

In the state of obesity, dysregulated production of adipokines due to accumulation of fat and low-grade inflammation can lead to a reduction of adiponectin, a known regulator of HDL-C levels (329–331). In our study, we found a reduction in adiponectin levels only in the obese group, while levels in the overweight group were not different. Adiponectin plays a vital role in increasing apoA-I production and hepatic ABCA1 expression, while decreasing HL activity that catabolizes HDL2

(332,333). Consistent with this, we observed robust associations between adiponectin levels and HDL2 levels and negative correlations with HDL3 levels, which is in line with previous studies (334,335). Moreover, our findings are consistent with our previous study (336) and demonstrate a strong correlation between adiponectin and cholesterol efflux capacity.

Interestingly, a study reported an impaired macrophage cholesterol exporter ABCA1 functionality in hyperinsulinemia (337). In addition, another study demonstrated that insulin reduces HDL-mediated cholesterol efflux in macrophages by inhibiting ABCG1 and neutral cholesteryl-ester hydrolase (338). However, in our study, we did not observe a significant impact of obesity on cholesterol efflux capacity of HDL, likely because levels of HbA1c were within the normal range in overweight and obese women. Notably, serum anti-oxidative capacity was significantly lower in obese women, when compared to the normal-weight group, which aligns with previous studies showing an increase in oxidative stress with BMI (339,340).

In summary, our findings highlight the effect of obesity on HDL metabolism, resulting in notable changes in HDL composition, including a shift towards smaller HDL3 particles. We observed that LCAT activity, HDL-C, and HDL subclass distribution were strongly associated with serum adiponectin and soluble leptin receptor levels, as well as liver enzyme activities, particularly in obese women. Conversely, these alterations were not seen in overweight women, except for increased HDL triglyceride content. Our findings imply that changes in HDL composition and function associated with obesity could potentially increase the risk of cardiovascular disease.

3.3. Obesity Affects Maternal and Neonatal HDL Metabolism and Function.

(Antioxidants 2023, 12, 199. https://doi.org/10.3390/antiox12010199)

In the second part of my dissertation, I explored the effects of maternal pre-pregnancy obesity on HDL metabolism and function in mothers and their offspring. Pre-pregnancy obesity is a significant risk factor for various pregnancy complications, such as GDM or hypertensive disorders during pregnancy (254,294–297). Moreover, it is associated with an increased long-term risk of cardiovascular events in mothers and offspring (10,76,341). However, the mechanisms that contribute to these adverse outcomes are not fully understood. HDL particles play a crucial role in preventing atherosclerosis by promoting the removal of cholesterol from macrophages and reducing inflammation (168,342,343). Therefore, we hypothesized that functional impairment of

HDL may have significant implications for maternal and offspring health in obese and GDM-complicated pregnancies.

For this study, we used serum samples of the DALI (Vitamin D and Lifestyle Intervention for GDM prevention) trial in which overweight/obese pregnant women (pre-pregnancy BMI ≥ 29 kg/m^2) were enrolled and followed until birth. Among the mothers enrolled in the study, approximately 29% were diagnosed with GDM during their pregnancy. As a normal-weight control group mothers with a pre-pregnancy BMI < 25 kg/m^2 were additionally included. Maternal serum was collected at term, and umbilical cord blood samples after delivery of the child. We assessed serum lipid levels and performed measurements on HDL functional parameters as well as activity of LCAT, an important enzyme in HDL metabolism.

To begin, we evaluated serum lipid levels in maternal and cord blood samples. Our results showed that obese mothers had a reduction in total cholesterol and HDL-C levels, while serum triglycerides were significantly increased in the obese with GDM group. Although the decline in serum total cholesterol in obesity was unexpected, it aligns with results from a previous study (344). This study demonstrated that in early pregnancy, obese pregnant women exhibited a more atherogenic lipid profile (344). However, as pregnancy progresses to the late second trimester, the rise in maternal serum cholesterol is notably attenuated, resulting in higher cholesterol levels in normal-weight women compared to overweight or obese women (344). Our observed obesity-related decrease in maternal serum HDL-C is consistent with previous studies (344,345). In neonates born to overweight or obese mothers, we observed a non-significant trend towards lower levels of HDL-C in cord serum. However, triglyceride levels were significantly elevated in the obese and obese with GDM group, supporting previous studies (346). High triglyceride levels in neonates of obese mothers may be attributed to a combination of factors, including maternal hypertriglyceridemia, fetal hyperinsulinemia and placental dysfunction (296,347–349).

In the next step, we investigated the impact of maternal overweight/obesity and GDM on HDL metabolism and function. We assessed the cholesterol efflux capacity of HDL, a key anti-atherogenic metric of HDL, using a cell-based assay that involved radiolabeled cholesterol and a macrophage cell line (161,350). Contrary to our initial expectations, our findings revealed an increase in cholesterol efflux capacity in overweight/obese mothers, which was also observed in overweight/obese women with GDM, despite lower serum HDL-C levels. Previous research has shown that obesity is linked to higher levels of small pre-β HDL particles, which are particularly

efficient in facilitating cholesterol efflux (318). This increase in pre-β particle concentration may be attributed to increased activities of HL and phospholipid transferprotein, which are involved in HDL remodeling (307,351,352). Moreover, individuals with low HDL-C levels and high triglycerides have been found to have elevated pre-β HDL levels, and there is a known association between serum triglyceride levels and cholesterol efflux capacity (353–356). In line with this, we observed a correlation between maternal serum triglyceride levels and cholesterol efflux capacity in our study cohort. Although maternal obesity has been linked to changes in PON1 activity (357,358), we did not observe any differences in the activity of PON1 in pregnant mothers or their offspring with either obesity or GDM. Nonetheless, consistent with previous research, we found that cord blood PON1 activity was significantly lower than maternal PON1 activity (214,359,360).

In addition to the evaluation of HDL functional parameters, we examined LCAT activity (93,361,362). Our findings showed that there was a significant decrease in LCAT activity in the maternal overweight/obese group, compared to normal-weight control group. Furthermore, neonates born to obese mothers displayed a trend towards lower LCAT activity, although this trend did not reach statistical significance. Therefore, our results suggest that maternal obesity during pregnancy may have an impact on both maternal and fetal lipid metabolism.

We further assessed serum anti-oxidative capacity in our study by evaluating the ability of serum to inhibit the free-radicals induced oxidation of the fluorescent dye dihydrorhodamine (321). Our study findings showed that both maternal and offspring serum anti-oxidative capacity were significantly reduced in obese group, while a trend towards reduced capacity was also observed in the obese with GDM group. This observation in mothers is in agreement with a previous study, that reported a strong association of BMI with oxidative stress (339,340,363).

Another notable observation from our study is that functional properties of HDL, such as cholesterol efflux capacity and PON1 activity as well as serum anti-oxidative capacity, exhibited a strong correlation between mothers and offspring. Interestingly, serum levels of HDL-C showed only a weak correlation. This finding is unexpected since, during pregnancy, the fetus is shielded from direct exposure to external factors in the maternal circulation. Typically, it is assumed that maternal and fetal HDL metabolism is not directly connected, and lipoproteins do not efficiently cross the placenta to enter fetal circulation (6,364–366). However, mothers who are obese, the placental presence of proinflammatory macrophages may lead to an increase in proinflammatory cytokines and oxidative stress, which could impact both maternal and fetal blood parameters (60).

Moreover, shared genetic factors between the mother and fetus could also play a role in influencing the functional parameters of HDL and serum antioxidant capacity in both the mother and the offspring (367). Similar to our results, other studies have also demonstrated the correlation of PON1 activity between mothers and their offspring (358).

In summary, in this study we revealed that pre-pregnancy obesity had adverse effects on serum anti-oxidative capacity and LCAT activity in both mothers and offspring, while HDL's cholesterol efflux capacity was increased. Our findings suggest that the impact of obesity on HDL functionality is more significant than that of GDM, as we did not observe any significant alterations in HDL function and metabolism associated with GDM in obese women. Moreover, our study revealed a robust correlation between maternal and fetal HDL functionalities. However, it remains unclear when and whether this correlation persists after childbirth and further investigations are warranted.

Compared to our findings in obese non-pregnant women, we similarly observed lower levels of HDL-C in the maternal circulation among obese pregnant women. Similar to obese non-pregnant women, we did not find any differences in PON1 activity when compared to normal-weight controls. However, contrary to our results in non-pregnant obese women, pre-gravid obesity was associated with an increase in HDL cholesterol efflux capacity despite reduced levels of HDL-C. It is possible that our sample size of non-pregnant women was too small to detect significant changes in HDL cholesterol efflux capacity. Additionally, we noted an increase in LCAT activity in non-pregnant obese women, whereas pregnant obese women exhibited decreased activity of this enzyme when compared to normal-weight controls. Notably, LCAT activity has been demonstrated to be elevated in pregnant compared to non-pregnant women (368). It is important to take into account that lipid metabolism undergoes significant alterations during pregnancy to ensure adequate nutrient supply to the developing fetus (202,366), which could contribute to these divergent results.

3.4. **Gestational Hypertension and High-Density Lipoprotein Function: An Explorative Study in Overweight/Obese Women of the DALI Cohort.**

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Pregnancy initiates a range of vascular, metabolic, and physiological changes in the body aimed at ensuring a continuous supply of nutrients essential for fetal growth and development (232,369). Although these adaptations are crucial for the growth and development of the fetus, they can also create significant physiological stress on the mother and increase the risk of adverse pregnancy outcomes, such as GHTN (370,371). This hypertensive disorder is defined as new-onset of systolic blood pressure > 140 mmHg and diastolic blood pressure > 90 mmHg occurring after 20 weeks of gestation. This pregnancy-related condition can lead to several adverse pregnancy outcomes, including placental abruption, preterm birth or fetal growth restriction (372,373). Notably, GHTN is linked to an elevated risk of cardiovascular complications for both mother and offspring later in life (258,289–292). Moreover, according to the World Health Organization, GHTN is one of the leading causes of maternal and fetal morbidity and a major contributor to maternal mortality rates in Europe (374,375).Considering the substantial impact of this disease on both maternal and fetal health, enhancing our understanding of GHTN is crucial for identifying potential therapeutic targets and advancing patient care. While current management approaches such as lifestyle modification can be effective in controlling blood pressure levels and reducing the risk of complications (376,377), they do not address the underlying mechanisms that lead to GHTN. Additionally, identifying novel biomarkers for early detection of GHTN may enable earlier interventions to prevent or minimize its complications.

Several studies have suggested that inflammatory disorders can impact the composition and function of HDL (127,378,379). Furthermore, pregnancy, which is accompanied by low-grade inflammation, has also been shown to alter the structure and composition of HDL (193,380). Given the altered HDL structure and function during pregnancy, it is reasonable to assume that GHTN, which is also associated with increased inflammation (381), may further impair HDL functionality. Therefore, we used samples from the DALI trial, in which maternal serum was taken at three timepoints during pregnancy (< 20 weeks, 24-28 and 35-37 weeks of gestation) and assessed several key metrics of HDL function. Additionally, we used paired umbilical cord serum of the study cohort to evaluate whether also HDL functionality of the offspring of GHTN mothers is affected.

Our study involved 192 women, 13% of whom developed GHTN. Initially, we analyzed changes in HDL-related parameters during pregnancy and compared them with cord blood samples. Similar to the findings in the literature (190,192,382,383), our observations also revealed a significant increase in HDL-C levels from < 20 to 24-28 weeks of pregnancy. Notably, all HDL-related parameters except for HDL-C, showed significant changes from early (< 20 weeks) to late pregnancy (35-37 weeks). Specifically, we observed a significant elevation of HDL-mediated cholesterol efflux capacity, serum apoM levels and in serum anti-oxidative capacity, while PON1 activity decreased over the course of pregnancy. When we normalized for differences in HDL-C levels, we did not observe any differences in parameters of HDL function among the different timepoints of pregnancy. This suggests that the function of individual HDL particles was not changed. Compared to maternal serum, cord serum had lower levels of HDL-C, as well as reduced cholesterol efflux capacity, PON1 activity, and apoM levels. However, after normalizing for differences in HDL-C levels by calculating the ratio between each HDL functional parameter and HDL-C, we found that despite its lower concentration, HDL in cord blood appeared to have a higher apoM content and cholesterol efflux capacity, while PON1 activity remained lower. Studies have already reported the presence of higher levels of HDL-associated apoM in cord blood, which is consistent with our findings (214). Furthermore, the reduced PON1 activity in cord blood has also been reported in previous research (214,358,384).

We proceeded to evaluate HDL-related parameters in women with GHTN and their offspring. Our findings showed that GHTN was not linked to significant changes in maternal HDL-C levels, cholesterol efflux capacity, activity of PON1, or anti-oxidative capacity. Studies investigating the changes in HDL function in pregnant women complicated by GHTN are limited, since previous studies have primarily investigated changes in HDL function and structure in PE, a more severe form of GHTN. In women diagnosed with PE, HDL was found to have an increased particle diameter, reduced activity of PON1, and less effectiveness in reducing adhesion molecule expression on endothelial cells (385). This reduction in PON1 activity in PE has also been shown by others (386–388). Results on HDL-mediated cholesterol efflux capacity in preeclampsia revealed mixed results, with one study reporting increased maternal and fetal cholesterol efflux capacity (389), while another study reported a decrease in this capacity in women with a history of preeclampsia at 6 months postpartum (390). These findings indicate that in more severe GHTN cases, parameters of HDL function in maternal blood may also be impacted.

In contrast, in cord blood samples, we were able to detect some changes in the HDL parameters. Although there were no differences in HDL-C levels between controls and offspring of GHTN-complicated pregnancies, we observed a decrease in cholesterol efflux capacity and PON1 activity, which potentially could increase offspring long-term cardiovascular risk. This finding is supported by prior studies demonstrating the independent predictive value of HDL cholesterol efflux capacity and low PON1 activity with cardiovascular risk (168,391,392). However, unexpectedly, we observed an increase in cord serum anti-oxidative capacity in GHTN offspring. This might suggest a compensatory mechanism to counteract the increased oxidative stress in the neonatal circulation. Considering that the serum anti-oxidative capacity is primarily influenced by serum levels of albumin, as well as low-molecular-weight antioxidants, and to a lesser extent by HDL (393), it is reasonable to suggest that the observed increase in antioxidant capacity of cord serum in GHTN may be attributed to to a decrease in the excretion of hydrophilic antioxidants. In the more severe disorder PE, oxidative modifications have been found on both maternal and fetal lipoproteins, suggesting the transmission of oxidative stress from mother to fetus (386,394). In line with our results, GHTN-associated reduced PON1 activity in cord blood has also been demonstrated by others (394). An intriguing finding of our study was that while we observed a trend for increased serum apoM levels in mothers with GHTN at all time points during pregnancy, a non-significant trend towards a reduction in cord serum apoM levels was seen. ApoM is a lipocalin that facilitates the transport of S1P by HDL in the circulation (119). Interestingly, a previous study reported lower apoM content in HDL isolated from cord blood of preeclamptic pregnancies (395), which is consistent with our findings.

In recent years, there has been a growing interest in the research on apoM and S1P due to their significant roles in various physiological processes. ApoM and S1P have endothelium stabilizing function, promote cholesterol efflux and accelerate pre-β HDL formation (119,121,122,396). Preserving placental vascular homeostasis during pregnancy is essential for optimal perinatal and long-term offspring health. Research has shown that endothelial dysfunction, associated with impaired S1P signaling, contributes to the development of preeclampsia (397). Therefore, the observed trend towards decreased cord blood apoM levels in GHTN could potentially have certain negative impacts on neonatal endothelial function.

In summary, our study demonstrated that the variations in maternal HDL-C levels throughout pregnancy are associated with changes in HDL-functional parameters. Furthermore, we observed that GHTN did not significantly affect maternal HDL-related parameters, but it did have a notable

influence on HDL function in the offspring. Additional research is necessary to ascertain whether the observed alterations in this study revert to baseline levels postpartum or if they persist and have long-term implications for the cardiovascular health of the offspring.

3.5. Preeclampsia affects lipid metabolism and HDL function in mothers and their offspring.

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Building on the results of our previous research on GHTN-associated changes in HDL, we then aimed to investigate the effects of PE on lipid metabolism parameters and HDL composition and function (4). PE is a severe and potentially life-threatening complication of pregnancy that is characterized by high blood pressure and can also affect kidney function or leads to other organ damage (398). This disorder typically develops after 20 weeks of gestation and affects 4-5% of pregnancies worldwide (399,400). Maternal as well as fetal morbidity and mortality are significant concerns associated with this disorders, especially in low- and middle-income countries (260,269,401,402). PE typically develops after 20 weeks of gestation and is characterized by hypertension (systolic > 140 mmHg, diastolic > 90 mmHg) and proteinuria (≥ 300 mg/24 h), or in the absence of proteinuria, by other clinical diagnoses: thrombocytopenia, renal insufficiency, impaired liver function, cerebral or visual problems or pulmonary oedema (260,261). If left untreated, PE can result in severe complications and can only be cured by delivering the baby (403–405). Therefore, close monitoring is crucial in managing this hypertensive pregnancy disorder (406). Although the symptoms of PE usually return to pre-pregnancy levels, the disorder represents a significant risk factor for the later development of cardiovascular disease in both mother and child (256,257,280,407,408). PE is categorized into two subtypes depending on the gestational age at onset: early-onset PE, which is diagnosed before gestational week 34 and is generally more severe, and late-onset PE, which is diagnosed after week 34 (277,409). Both types are suggested to be caused by placental malperfusion and dysfunction of the placenta, however they may have different underlying pathophysiological mechanisms (277).

Based on our previous research on GHTN-related changes in HDL function, our next objective was to conduct in-depth analyses of lipid metabolism and parameters of HDL composition, function and subclass distribution in patients with PE. This study enrolled a total of 64 pregnant women, including 18 with early-onset PE, 14 with late-onset PE, and 32 normotensive women as

controls, of which blood was collected at term. To gain a more comprehensive understanding of the effects of PE on both the mother and child, we also collected cord blood samples.

Our study groups were matched in age and pre-pregnancy BMI in order to exclude possible confounding factors (3). As delivery in early-onset PE often occurs preterm, the gestational age in this group was lower than in the normal pregnancy group, whereas the gestational age in late-onset PE did not differ significantly from the control group. Since maternal hyperlipidemia is a risk factor for the development of PE (252,410), we initially assessed plasma lipid levels in our study cohort. Pregnancy in general is known to cause significant alterations in plasma lipid levels to meet the demands of the growing fetus (192,232). Consistent with previous studies (252,411,412), our results showed the presence of atherogenic dyslipidaemia in both maternal PE groups, characterized by increased triglyceride levels and reduced HDL-C levels. Recent research provides strong evidence linking hypertriglyceridemia with endothelial dysfunction (413–415). The rise in triglyceride levels is concomitant with an increase in free fatty acids, which are subsequently uptaken by endothelial cells and converted into triglycerides (413,416). This accumulation of triglycerides may be detrimental to endothelial cells, contributing to endothelial dysfunction in preeclampsia (413,417). Additionally, the simultaneous presence of hyperlipidemia and heightened oxidative stress in women with preeclampsia could exacerbate the formation of oxidized lipids in the arterial wall. This, in turn, could trigger inflammation and potentially elevate the susceptibility to cardiovascular disease.

Remarkably, we observed notable variances in plasma lipid levels among the offspring of pregnancies affected by early-onset preeclampsia. Notably, there was a substantial rise in total cholesterol and non-HDL cholesterol, aligning with previous research findings (394). Importantly, these changes were only evident in the offspring of early-onset PE pregnancies, which is considered a more severe form of PE (418).

Since the composition of HDL particles has a crucial influence on their functionality (419,420), we determined the major HDL-apolipoproteins and HDL-associated lipids in our study cohort. Our analysis revealed no significant differences in HDL associated apoA-I, apoA-II, apoC-III, and apoE levels in cord blood or in maternal study groups. However, we found a marked increase in apoC-II abundance on HDL in both PE groups. Moreover, we discovered a significant inverse correlation between the levels of apoC-II associated with HDL and plasma triglycerides. This finding aligns

with the role of apoC-II acting as a cofactor for lipoprotein lipase, an enzyme responsible for hydrolyzing plasma triglycerides (421,422).

Since the size of HDL particles is a key indicator of their functional capacity (155,156,318), we next assessed HDL subclass distribution in maternal and cord blood samples. We used the Quantimetrix Lipoprint© system to identify and group 10 distinct HDL subclasses, according to their size, into three categories: large, intermediate and small HDL subclasses. Although we did not find any differences in the distribution of HDL subclasses among the cord blood groups, we did observe a shift from large to small HDL subclasses in mothers with early-onset PE. This decrease in large HDL subclasses has been previously reported and is suggested to be caused by increased HL activity (198).

Based on the differences in HDL subclass distribution, we next were interested in investigating whether PE also affects LCAT activity (423). Interestingly, we did not observe any differences in plasma LCAT activity in the maternal groups, despite the changes in HDL subclass distribution. However, in cord blood of early-onset PE, LCAT activity was reduced, when compared to normal pregnancy group, suggesting alterations in HDL metabolism.

In the next step, we investigated PE-associated effects on HDL functional parameters. To investigate this ,we first assessed HDL cholesterol efflux capacity (168). In our previous study in the DALI cohort, we observed that neonates from GHTN-affected pregnancies showed decreased HDL cholesterol efflux capacity, whereas no changes were observed in their mothers (4). Building on these findings, our current study revealed a similar reduction in this HDL functionality in offspring of late-onset PE pregnancies. Remarkably, maternal samples did not exhibit significant changes in HDL cholesterol efflux capacity between study groups. Our results align with a previous study that reported a decrease in neonatal ABCA1-mediated HDL cholesterol efflux capacity associated with PE (389). However, their study observed an increase in total cholesterol efflux capacity of maternal plasma, which contrasts with our findings (389). To further investigate other HDL functionalities, we evaluated the activity of PON1 (424). Despite previous reports of reduced PON1 activity in both mothers and cord blood in PE (4,386,394,425), we did not observe any differences in our study. Importantly, these previous studies used different methods to measure PON1 activity, in contrast to our study, which measured arylesterase activity in apoB-depleted plasma.

As PE is known to be linked with elevated oxidative stress in the maternal circulation, we proceeded to assess anti-oxidative capacity of plasma. Interestingly, in mothers with early-onset PE, we observed a significant increase in plasma anti-oxidative capacity, suggesting a potential compensatory mechanism in response to the higher oxidative stress in the circulation. Notably, we also found a correlation between anti-oxidative capacity in plasma and the shift from large to small HDL subclasses in maternal early-onset PE group. This could potentially be attributed to the potent anti-oxidant activity exhibited by small and dense HDLs (155,156). Additionally, we observed a correlation between uric acid levels and anti-oxidative capacity in plasma. Uric acid is recognized for its robust antioxidant properties, acting as a scavenger of singlet oxygen and radicals (426,427). It is often found to be elevated in patients with impaired kidney function (428). Hydrophilic antioxidants, including uric acid, might explain the link between antioxidant capacity in plasma and markers of kidney dysfunction. However, further investigations are needed to substantiate this hypothesis. In cord blood of PE pregnancies, no differences in plasma anti-oxidative capacity were observed.

Women with a history of PE, have an elevated risk for developing cardiovascular complications later in life (257). As certain LDL subclasses are significantly linked to cardiovascular risk (429–431), we examined the distribution of LDL subclasses using the Quantimetrix Lipoproint© system. Since cord blood has much lower levels of triglyceride-rich lipoproteins (210), assessment of LDL subclass distribution was not possible. In maternal samples, we observed a reduction of intermediate-density lipoprotein (IDL)-C in early-onset PE, and a non-significant trend towards reduced levels in late-onset PE. However, IDL-A was significantly elevated in both maternal PE groups, suggesting alterations in metabolism of triglyceride-rich lipoproteins. Contrary to previous findings, we did not detect an elevation in small LDL particles or a reduction in large, buoyant LDL particles in our study (198). However, these findings are in line with another study that also reported no differences in small LDL particles (432).

Altogether, we demonstrated that PE is associated with profound alterations in lipid metabolism in both mothers and offspring. Of particular interest, we found that maternal plasma anti-oxidative capacity was increased in early-onset PE and correlated with the shift from large HDL to small HDL subclasses. Although early-onset and late-onset PE have distinct origins, we observed similar changes in maternal plasma lipid concentrations and HDL composition. In summary, the findings of this study suggest that both early-onset and late-onset PE have an impact on lipid metabolism in both mothers and neonates, which may play a role in the development of the

disease and increased risk of cardiovascular complications in the future. Nevertheless, further large-scale studies are required to validate these results and investigate if the observed changes persist beyond childbirth.

Compared to our findings in the GHTN-cohort, the presence of the more severe type of hypertension during pregnancy, PE, was associated with atherogenic dyslipidemia characterized by decreased levels of HDL-C and an increase in plasma triglycerides in the maternal circulation. In contrast, in the maternal GHTN group, we did not observe any differences in HDL-C levels, emphasizing that it is particularly PE that is associated with dyslipidemia.

Interestingly, in the GHTN group, we only observed changes in serum functionalities in the offspring, whereas maternal early-onset PE was linked to increased anti-oxidative capacity, which was not observed in mothers with late-onset PE. In the cord blood of the GHTN group, we observed reduced PON1 activity, a decrease in cholesterol efflux capacity, and an increase in anti-oxidative capacity. Surprisingly, we found a similar change in cholesterol efflux capacity only in the late-onset PE group, but not in the early-onset PE group. Unexpectedly, we did not detect any impairment of HDL functionality in early-onset PE, which contrasts with our initial expectations. However, it is worth noting that all the mothers in the GHTN cohort were overweight or obese, which could potentially contribute to different results and should be considered when interpreting these findings.

3.6. Fetal High-Density Lipoproteins: Current Knowledge on Particle Metabolism, Composition and Function in Health and Disease.

(Biomedicines. 2021; 9(4): Doi: 10.3390/biomedicines9040349)

Based on our obtained results and the lack of knowledge on fetal HDL, I comprehensively reviewed the current knowledge on fetal HDL particle metabolism, composition and function. Moreover, we reviewed literature on HDL in pregnancy and alterations of HDL function in pregnancy-related disorders such as GDM and PE.

In the review article I discuss the metabolism of fetal HDL, emphasizing the dynamic interplay between maternal and fetal circulation, explaining how cholesterol and other lipids are transported between the mother and fetus through specialized transport proteins and receptors. The composition of fetal HDLs is explored, highlighting the unique protein and lipid components that

contribute to their functional properties. In contrast to adults, fetal HDL represents a major cholesterol-carrying class of lipoproteins in the cord blood (214). Moreover, fetal HDL particles have a unique composition, indicating that their functions may differ from those of adult HDL particles (214–216).

In addition, the article addresses the various functions of fetal HDLs in maintaining fetal health. We explain how these lipoproteins support the maturation of organs and tissues, including the lungs, brain and immune system, by facilitating the transport of essential lipids and signaling molecules. Fetal HDLs also have antioxidant and anti-inflammatory characteristics, protecting the developing fetus from oxidative stress and inflammation.

Preserving the integrity of the fetoplacental vasculature is crucial for ensuring a sufficient supply of oxygen and nutrients to the fetus, which is important for fetal well-being (433). Neonatal HDL-associated S1P has been demonstrated to be a critical regulator of placental vascular inflammation, as well as an enhancer of endothelial barrier function and inducer of vasorelaxation, making it an important factor in maintaining vascular integrity (119,293,434–436). In addition, $LpPLA_2$, an enzyme primarily synthesized by macrophages and subsequently binding to lipoproteins upon secretion into circulation, exerts an anti-inflammatory effect and improves vascular barrier function in the placental endothelium (437). Notably, $LpPLA_2$ is predominantly associated to LDL in adults (80%), but HDL has been identified as the primary carrier of this enzyme in the fetus (437,438).

Cholesterol, both endogenous and maternally-provided, is crucial for fetal development. Despite separate lipoprotein metabolism in mother and fetus, maternal hyper- and hypocholesterolemia can impact infant health and growth. In specific, high maternal cholesterol levels have been associated with an increased risk of preterm birth and fetal growth restriction, while low levels have been linked to poor neurological development (235,249,439,440).

To conclude, the role of HDL in the fetus is a fascinating area of research that still has many unanswered questions and requires further investigation.

4. CONCLUSION

In conclusion, my research in this thesis has provided novel and valuable insights into the impact of obesity on HDL metabolism, composition, and function in women, including during pregnancy. Obesity, a significant global health concern, has been shown to disrupt various pathways involved in metabolic, vascular, and inflammatory processes. The altered activities of enzymes and changes in HDL particle composition observed in obese women highlight the dysregulation of HDL metabolism associated with obesity. Importantly, these changes were predominantly observed in women with a BMI of 30 or higher.

Furthermore, my dissertation also explored the effects of maternal obesity on HDL metabolism and function in both mothers and their offspring. The findings demonstrated that high BMI influences serum lipid profiles, HDL functionalities, and antioxidant capacity in obese mothers, and these effects can extend to their children. The strong correlation of HDL functionalities between mothers and children emphasizes the potential long-term implications of maternal obesity on offspring outcomes.

Moreover, investigating the impact of obesity-related conditions such as GHTN and PE revealed further alterations in HDL function and lipid metabolism. Impaired HDL cholesterol efflux capacity and altered PON1 activity were observed in newborns with GHTN, suggesting a potential contribution to increased risk of cardiovascular events later in life. Similarly, maternal atherogenic dyslipidemia, characterized by elevated triglyceride levels and reduced HDL-C, was associated with PE, particularly in the early-onset type. Of particular interest, changes in HDL function were also observed in the offspring of PE-affected mothers.

In this thesis, a similar obesity-related decrease of HDL-C was observed in both non-pregnant and pregnant women. However, contrary to our findings in non-pregnant obese women, pre-gravid obesity was associated with an increase in HDL cholesterol efflux capacity despite lower levels of HDL-C. In addition, we observed an increase in LCAT activity in non-pregnant obese women, while pregnant obese women exhibited decreased activity of this enzyme compared to normal-weight controls. Our findings highlight the complex interactions between obesity, pregnancy, and lipid metabolism, emphasizing the need for further research in this area.

Our studies revealed that PE was associated with atherogenic dyslipidemia with increased levels of triglycerides and lower levels of HDL-C in the maternal circulation, while these lipid abnormalities were not seen in GHTN. Furthermore, we observed differential changes in serum functionalities among the study groups. Maternal early-onset PE was linked to increased anti-oxidative capacity, while this effect was not observed in mothers with late-onset PE or GHTN. In the cord blood of the GHTN group, we observed reduced PON1 activity, a decrease in cholesterol efflux capacity, and an increase in anti-oxidative capacity. Interestingly, we identified a similar alteration in cholesterol efflux capacity only in the late-onset PE group, but not in the offspring of early-onset PE. Further investigations are warranted to elucidate the underlying mechanisms driving these variations and to explore the clinical implications of these lipid and functional changes.

Overall, this thesis contributes to our understanding of the complex relationships between obesity, HDL metabolism, and pregnancy outcomes. The findings highlight the need for effective management of obesity in women, particularly during pregnancy, as maternal health can significantly impact the intrauterine environment, affecting fetal development and the long-term health of the child. Further research in this field will provide valuable insights into potential strategies for preventing and managing obesity-related complications during pregnancy.

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Review

Obesity-Related Changes in High-Density Lipoprotein Metabolism and Function

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Abstract: In obese individuals, atherogenic dyslipidemia is a very common and important factor in the increased risk of cardiovascular disease. Adiposity-associated dyslipidemia is characterized by low high-density lipoprotein cholesterol (HDL-C) levels and an increase in triglyceride-rich lipoproteins. Several factors and mechanisms are involved in lowering HDL-C levels in the obese state and HDL quantity and quality is closely related to adiponectin levels and the bioactive lipid sphingosine-1-phosphate. Recent studies have shown that obesity profoundly alters HDL metabolism, resulting in altered HDL subclass distribution, composition, and function. Importantly, weight loss through gastric bypass surgery and Mediterranean diet, especially when enriched with virgin olive oil, is associated with increased HDL-C levels and significantly improved metrics of HDL function. A thorough understanding of the underlying mechanisms is crucial for a better understanding of the impact of obesity on lipoprotein metabolism and for the development of appropriate therapeutic approaches. The objective of this review article was to summarize the newly identified changes in the metabolism, composition, and function of HDL in obesity and to discuss possible pathophysiological consequences.

Keywords: obesity; HDL-C; HDL subclasses; cholesterol efflux; adiponectin; sphingosine 1-phosphate; bariatric surgery

1. Introduction

The increasing prevalence of obesity in the last decades has become a major health problem worldwide. In Northern America and Europe, in particular, the number of overweight and obese people is ever increasing and is becoming more common in children and adolescents [1]. The causes of obesity are multifactorial, with the most important factors being excess calorie intake and lack of physical activity. Excessive body weight increases the risk of disease development, such as coronary artery disease, hypertension, type-2 diabetes mellitus, and dyslipidemia [2–6]. High levels of triglyceride-rich lipoproteins and low levels of high-density lipoprotein cholesterol (HDL-C) commonly characterize dyslipidemia in obesity. In obesity, not only HDL levels are altered, but an altered HDL distribution pattern and abnormal HDL metabolism have also been observed, which often leads to dysfunction of the HDL particles [7–9]. Consequently, the focus has shifted from studying the quantity of HDL to studying the quality of HDL [10]. The current review will focus on HDL metabolism and the pathophysiological changes seen in obesity. Further, we will focus on obesity-induced changes in HDL composition and the concomitant changes of HDL functionality. Another aspect will be the relationship of HDL with the adipokine adiponectin as well as with the bioactive lipid sphingosine-1-phosphate (S1P), whose levels are altered in the state of obesity. We also summarize the effects of weight loss induced by bariatric surgery, Mediterranean diet and pharmacological approaches, which effectively increase HDL-C levels and improve HDL function.

2. HDL Metabolism, Structure, and Composition

2.1. HDL Metabolism

The biogenesis of HDL starts in the liver and the intestine, where apolipoprotein (apo) A-I is synthesized (Figure 1). After secretion, lipid-poor apoA-I interacts with the integral cell membrane protein ATP-binding cassette transporter A1 (ABCA1), which is abundantly expressed by hepatocytes and enterocytes [11]. Through interaction, apoA-I acquires lipids from the cellular lipid pool, generating nascent HDL particles. Additional lipids and apolipoproteins are acquired, which are derived from hydrolysis of triglyceride-rich lipoproteins. This process partly explains the strong inverse relationship of HDL-C and triglyceride levels, often observed in obese subjects [12]. The acquired cholesterol of HDL is further esterified by lecithin-cholesterol-acyl transferase (LCAT), forming mature HDL particles [13]. The reaction takes place at the surface of HDL and requires apoA-I as an activator for LCAT [14]. The generated HDL-associated cholesteryl-esters are partially transferred to apoB-containing lipoproteins by cholesteryl-ester transfer protein (CETP), usually in exchange for triglycerides. Another pathway for clearance of cholesteryl-ester in HDL is the direct uptake by the liver via scavenger receptor class B type 1 (SR-BI) [15]. After interaction of SR-BI with large cholesterol-rich HDL, cholesteryl-esters and free cholesterol are internalized and cholesterol is removed through the bile, while apoA-I dissociates [16,17].

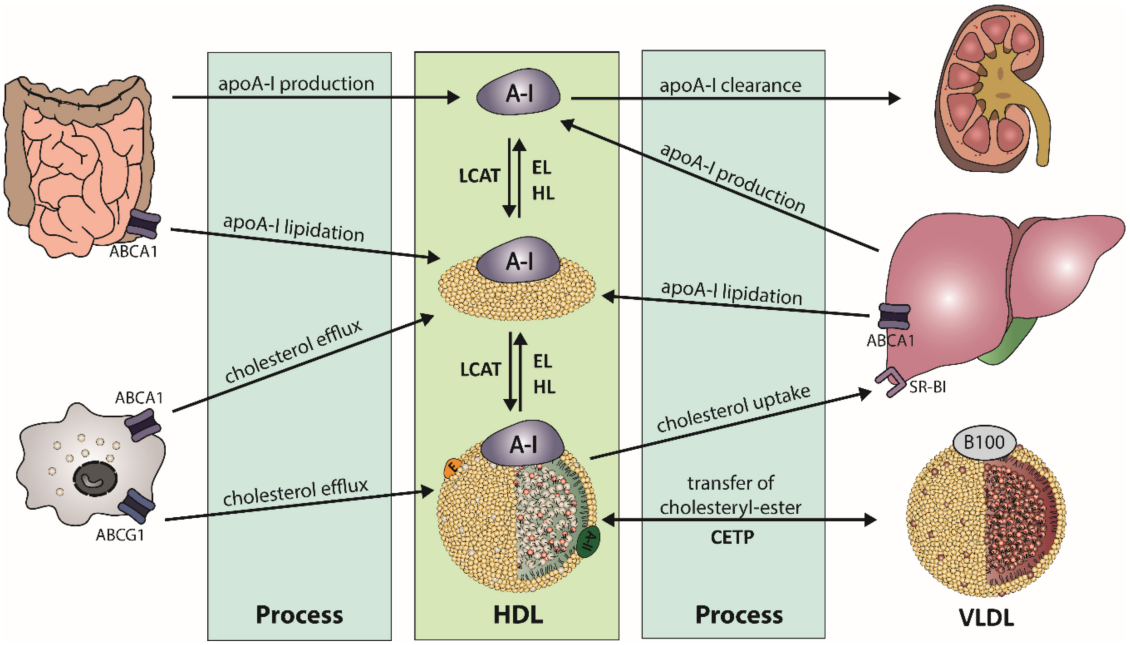

Figure 1. Schematic overview of high-density lipoprotein (HDL) metabolism. Biogenesis of apolipoprotein A-I (apoA-I) takes place in the liver and intestine. After secretion of the lipid-poor apoA-I, it interacts with ATP-binding cassette transporter A1 (ABCA1) to acquire lipids, leading to formation of nascent HDL. The enzyme lecithin-cholesterol-acyl transferase (LCAT) esterifies free cholesterol of nascent HDL to form mature HDL. Cholesteryl-esters are cleared by uptake of the liver by scavenger receptor B1 (SR-BI) or via transfer on triglyceride-rich lipoproteins by cholesteryl-ester transfer protein (CETP), in exchange of triglycerides. Triglyceride-rich HDL is susceptible to hydrolysis by endothelial lipase (EL) or hepatic lipase (HL).

HDL is enriched in triglycerides through the activity of CETP, generating HDL particles that are more susceptible to lipolysis by endothelial lipase (EL) or hepatic lipase (HL). Substrates for lipolysis are mainly phospholipids (EL) or phospholipids and triglycerides (HL), but with different specificity for phospholipids [18]. The lipolysis of triglycerides leads to the formation of smaller HDL particles, which are susceptible to faster catabolism. Another important key player of HDL metabolism is the

phospholipid transfer protein (PLTP), which transfers phospholipids between HDL particles and lipids between triglyceride-rich lipoproteins and HDL [19]. Many apolipoproteins, lipid transfer proteins, enzymes, cell surface receptors, and cellular lipid transporters are involved in the regulation of HDL metabolism and partly determine levels of plasma HDL-C. This complex metabolism produces HDL particles of varying size, density, and composition. Therefore, plasma HDL-C concentrations are not a good parameter to reflect functional properties of HDL, such as HDL-mediated reverse cholesterol transport or anti-oxidative or anti-inflammatory properties.

2.2. HDL Structure and Composition

Plasma levels of HDL-C have been associated with cardiovascular diseases for decades [20–22]. However, it is becoming widely accepted that it is not the quantity but the quality of HDL that is important, as HDL performs different functions depending on the protein and lipid composition [23–25]. ApoA-I is the most prevalent protein component of HDL, accounting for approximately 70% of the total protein [26]. ApoA-I has a variety of functions, such as activation of LCAT, interaction with cellular receptors, and anti-atherogenic activities [27–29]. ApoA-II is the second major apolipoprotein in HDL and presents about 15–20% of the total protein component [30]. The remaining 10–15% of HDL protein mass comprises minor proteins, including apoA-IV, ApoCs, which are important enzyme regulators, apoD, apoE, apoF, apoH, apoJ, ApoL-I, and apoM, and several enzymes. Paraoxonase 1 (PON1) is almost exclusively associated with HDL and has been shown to exert anti-inflammatory and anti-oxidative properties [31]. Other enzymes associated with HDL are LCAT and the platelet-activating factor acetyl hydrolase. The phospholipid transfer protein and CETP have a lipid transfer activity and are important in lipoprotein metabolism. Remarkably, it is not cholesterol that predominates the HDL lipidome, but phospholipids. Taken together, phospholipids and sphingolipids account for 40–60% of total lipids, while cholesteryl-ester (30–40%), triglycerides (5–12%), and free cholesterol (5–10%) are less abundant [23]. Similar to functions of HDL-associated proteins, HDL lipids also accomplish distinct structural functions. The lipid surface monolayer is constituted of phospholipids, while cholesteryl-ester and triglycerides form the hydrophobic core. In total, more than 200 lipids and 80 proteins are carried by different HDL subclasses, with individual HDL particles carrying only a few other proteins besides apoA-I [32–34].

2.3. HDL Subclasses

Multiple subclasses of HDL exist, depending on its stage of maturation, site of origin, and its protein and lipid composition. Thus, HDL particles are highly heterogeneous in their size, shape, structure, and density (Table 1). Pre-β HDL is structurally the simplest form of HDL. These particles consist of one or two apoA-I molecules with a phospholipid layer and a trace amount of cholesterol. These particles are discoidal shaped with a diameter of approximately 9.6 nm and a thickness of 4.7 nm [35]. Pre-β HDL particles rapidly take up cholesterol and phospholipids, which convert them into larger HDL subclasses. Therefore, pre-β HDL only accounts for about 5% of HDL in the circulation [36]. Because of their function to avidly absorb cholesterol and phospholipids, pre-β HDL particles are thought to be a major factor in preventing atherosclerotic plaque formation. Importantly, higher serum cholesterol efflux capacity is related to plasma concentrations of pre-β HDL [37]. HDL3 particles have a smaller diameter (7.5 nm) and are enriched with proteins, while HDL2 particles are larger (10 nm) and lipid rich. Most abundant apolipoproteins are apoA-I and apoA-II in both subclasses; however, apoA-II is more present in HDL3. Interestingly, the HDL-associated enzyme PON1, which has anti-oxidative and anti-inflammatory properties [31], has been shown to be more frequently associated with HDL3. This higher abundance of PON1 on HDL3 could partly explain the higher anti-oxidative capacity of the smaller HDL particles [29,38]. HDL2 and HDL3 further show differences in lipid composition. Sphingolipids are, in general, less abundant in the HDL3 subclass, affecting surface lipid fluidity, whereas the bioactive lipid sphingosine-1-phosphate (S1P) is predominantly associated with HDL3 [23]. In line, the abundance of apoM, which specifically anchors S1P to HDL particle,

shows higher abundance in HDL3 [38]. S1P maintains vascular integrity and mediates multiple effects of HDL on endothelial cells [39]. The functions of HDL to induce vasorelaxation as well as promoting barrier function have been attributed to signaling of S1P [40,41]. Taken together, it seems that smaller subclasses of HDL have a greater protective potential than larger particles [29].

Table 1. Representation of HDL heterogeneity.

HDL Subclass	Size	Shape	Abundant Components	Important Functions
Pre-β HDL	9.6 nm diameter, 4.7 nm thickness	discoidal	ApoA-I, phospholipids	ABCA1-Cholesterol efflux
HDL3	7.5 nm, 175 kDa	spherical	Protein:lipid ratio 55:45 PON1, ApoA-II, ApoM, S1P	Anti-oxidative activity Anti-inflammatory activity ABCA1-Cholesterol efflux
HDL2	10 nm, 350 kDa	spherical	Protein:lipid ratio 40:60	ABCG1- Cholesterol Efflux

Apo, apolipoprotein; ABCA1, ATP-binding cassette transporter A1; PON1, paraoxonase 1; ABCG1, ATP-binding cassette subfamily G member 1.

2.4. Important Functions of HDL

One of the main functions of HDL is its ability to promote reverse cholesterol transport, the uptake of excess cholesterol from peripheral cells, and the transport to the liver for excretion. This process is considered as the major antiatherogenic effect of HDL [42].

The reverse cholesterol transport starts with the secretion of lipid-poor apoA-I, which is released from liver or intestine into the plasma to circulate to peripheral cells from which excess cholesterol is removed, forming nascent HDL. A key role in the reverse cholesterol transport is the interaction of apoA-I with ABCA1 [43]. Studies have shown that ABCA1 preferentially lipidates small HDL, specifically apoA-I, to form nascent HDL, while ATP-binding cassette subfamily G member 1 (ABCG1) stimulates cholesterol efflux to mature HDL and not to lipid-poor apoA-I [44,45]. Cholesterol efflux includes the passive diffusion of cholesterol from cells as well as the active cellular cholesterol transfer by ABCA1, ABCG1, and SR-BI [46–48]. The absorbed cholesterol is esterified by LCAT and mature HDL is formed. HDL-associated cholesteryl-ester is partially transferred to triglyceride-rich lipoproteins by CETP and further cleared by hepatic clearance through the low-density lipoprotein (LDL) receptor or taken up together with free cholesterol by the hepatic receptor SR-BI. Therefore, the transfer of cholesterol from peripheral cells to the liver involves two routes: (1) the direct uptake via SR-BI and (2) indirect by HDL-LDL/very low-density lipoprotein (VLDL) interaction [42]. In the liver, the cholesteryl-esters are hydrolyzed, and free cholesterol is either transported by ABCG5 and ABCG8 into the bile for excretion into feces or converted into bile acids or reused for VLDL production.

This process of HDL-mediated cholesterol efflux has been of expanded research interest in recent years. A number of different cell-based assays have been developed, to measure the ability of HDL to promote cholesterol efflux, the first step of reverse cholesterol transport. In the most established assay, a mouse macrophage cell line (J774) was employed [49]. Cells are enriched with radioactively or fluorescently labeled cholesterol and cyclic adenosine monophosphate to upregulate expression of ABCA1. For these assays, isolated HDL or apoB-depleted serum from patients is added to cell medium and the proportion between labeled cholesterol in the supernatant and in the cells is calculated.

Besides the ability of HDL to promote cholesterol efflux, there is increasing evidence that HDL-mediated antiatherogenic actions toward the endothelium have physiological relevance [50–53].

The beneficial properties of HDL on the endothelium include vasodilatory activity, primarily through stimulation of nitric oxide (NO) release from endothelial cells [40,54], and also the production of prostacyclin [55,56]. The initial step for the activation of NO production involves binding of HDL to SR-BI on the endothelium. Subsequent intracellular events are mediated by endothelial protein kinase B and intracellular Ca^{2+} mobilization, increase in intracellular ceramide levels, and the phosphorylation of the endothelial NO-synthase, leading to NO release [40,57–59]. HDL reduces the activity of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the endothelium, which reduces

the cellular production of superoxide, an inactivator of NO, thereby increasing NO bioavailability [60]. Vasodilatory actions of HDL further include cholesterol efflux of cholesterol and 7-oxysterols, mediated by ABCG1, which improves formation of active endothelial NO-synthase dimers, resulting in decreased production of reactive oxygen species [61].

Another anti-atherogenic function of HDL is its ant-oxidative activity by protecting LDL from oxidative damage induced by free radicals, thus reducing its atherogenicity. ApoA-I, the major protein component of HDL may play a central role in HDL-mediated anti-oxidative activity, by reduction of lipid hydroperoxides through methionine residues [62,63]. In addition, HDL-associated PON1 was shown to decrease lipid peroxidation of LDL and HDL through a specific cysteine residue [64]. Other apolipoprotein components and HDL-associated enzymes, such as apoA-II, apoE, apoJ, lipoprotein-associated phospholipase A2, and LCAT, may further contribute to the anti-oxidative properties [29,65,66]. HDL-associated lipophilic antioxidants such as tocopherols seem to make a small contribution to the antioxidant properties of HDL [67].

Additionally, to the number of anti-oxidative effects, HDL further possesses anti-inflammatory properties. In vitro experiments have shown that HDL inhibits transmigration of monocytes [68] and inhibits cytokine-induced expression of vascular cell adhesion molecule, intercellular cell adhesion molecule, and E-selectin expression [69,70]. By modulation of the nuclear factor κB and the peroxisome proliferator-activated receptor gamma, HDL further inhibits the production of pro-inflammatory cytokines [71]. Due to these capabilities, HDL reduces the recruitment of lymphocytes, monocytes, and basophils to the vascular endothelium, thereby decelerating downstream events of inflammatory response.

3. HDL-C-Raising Therapies and Cardiovascular Outcome

The cholesterol component of HDL has been shown to be inversely associated with the risk of coronary heart disease (CHD) and is a key component of predicting cardiovascular risk in the general population [12]. The Framingham Heart Study was the first study to observe the strong association between HDL-C and CHD and, therefore, served as the basis for the hypothesis that HDL, as the "good" cholesterol, might hold protective properties against CHD [72]. However, more recent data clearly indicate that the association between HDL-C concentration and all-cause mortality is U-shaped, and both extremely high and low HDL-C concentrations are associated with an increase in mortality [73]. This leads to considerable uncertainty about the potential benefit of increasing HDL-C and may reflect or explain the disappointing results of recent clinical studies on a number of therapeutic interventions aimed at increasing HDL-C levels, such as CETP inhibitors [74–76]. Given the heterogeneity of HDL particles in terms of structure, size, lipidomic/proteomic composition, and metabolism, HDL-C values are only a snapshot of the steady-state cholesterol pool. HDL-C values provide no direct information on the rate of cholesterol efflux from vascular macrophages in liver, which is influenced by many factors beyond the mass of HDL-C alone. Furthermore, the circulating HDL-C concentrations do not provide information about the anti-inflammatory, anti-oxidant, anti-thrombotic, and endothelial function-promoting activities of HDL [77]. Therefore, considerable interest has recently focused on approaches to influence the biological functions of HDL in the search for new cardioprotective therapies [78–84]. This is based on new findings that underline the importance of HDL functionality [85–87], which has led to ongoing efforts to develop new risk markers and therapeutics that focus on HDL quality rather than quantity.

4. Obesity Alters HDL-C Levels

Obesity is commonly accompanied by low HDL-C levels and an increase in triglyceride-rich lipoproteins [88], which is often termed as atherogenic dyslipidemia. Characteristic for this dyslipidemia is a decreased clearance of triglyceride-rich lipoproteins, which is caused by a relative lack of insulin-sensitive lipoprotein lipase [89–91]. Lipoprotein lipase hydrolyzes triglycerides of chylomicrons and VLDL, leading to shrinkage of the particles and transfer of surface phospholipids and

apolipoproteins to HDL, thus increasing HDL size. During obesity, the response of lipoprotein lipase activity to glucose stimulation has been shown to be reduced [92], representing one potential factor contributing to the decrease of HDL-C in obesity.

The increase of triglyceride-rich lipoproteins is a causal factor for low HDL-C levels in obesity. The increase in the release of free fatty acids from the adipocytes caused by obesity increases their uptake by the liver, resulting in liver accumulation and enhanced production of VLDL and its release into the bloodstream (Figure 2) [93]. This increase of acceptor lipoproteins further stimulates the transfer of triglycerides on HDL in exchange for cholesteryl-esters mediated by CETP [94]. During this process, HDL is enriched in triglycerides and represents a better substrate for hepatic lipase and is hydrolyzed more rapidly [95]. In obese insulin-resistant subjects, HDL is enriched in triglycerides and the activity of hepatic lipase is increased [96–98]. Hydrolysis of triglyceride-rich HDL further leads to the formation of smaller HDL3 particles, which are susceptible to faster catabolism [99]. Interestingly, even when fasting plasma triglyceride levels are at a normal level, obese patients often display low HDL-C levels, suggesting further mechanisms leading to HDL-C lowering in obesity.

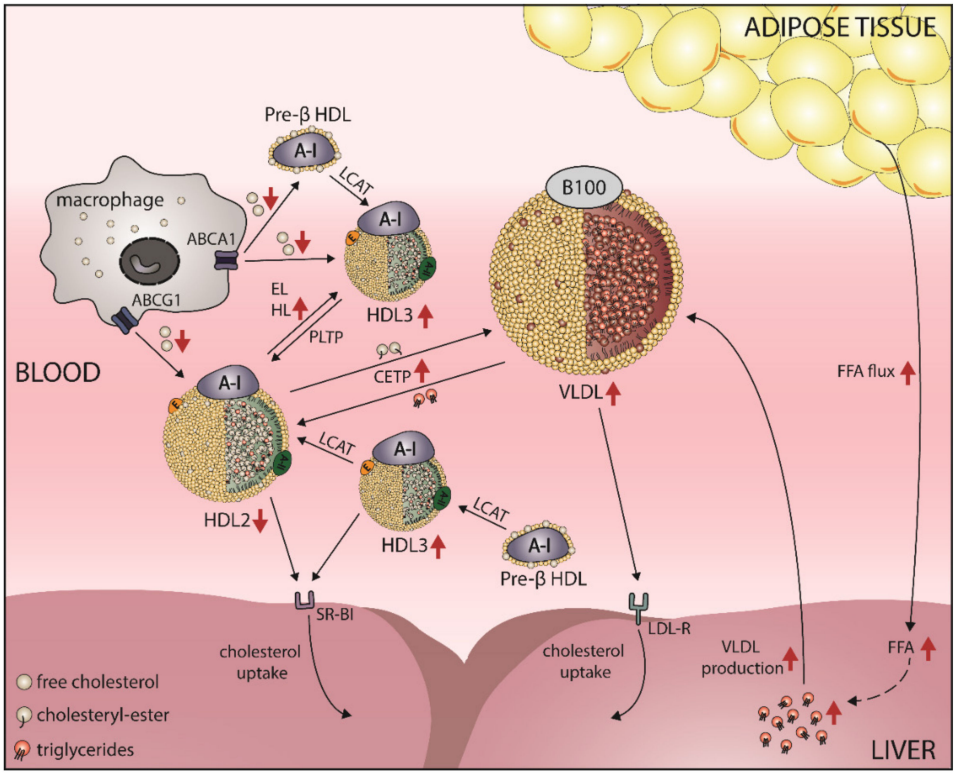

Figure 2. Proposed mechanisms involved in the obesity-induced shift in HDL subclass distribution. Pre-β HDL is rapidly lapidated, leading to the formation of HDL3. The ingested free cholesterol is esterified by lecithin-cholesterol-acyl transferase (LCAT), which leads to maturation of HDL particles. Phospholipid transfer protein (PLTP) transfers phospholipids onto HDL, whereas HDL-associated cholesteryl-esters are transferred to very low-density lipoproteins (VLDL) in exchange of triglycerides by cholesteryl-ester transfer protein (CETP). Pre-β HDL and small HDL3 remove excess cholesterol via ABCA1, while HDL2 removes cholesterol via ABCG1. Endothelial lipase (EL) and hepatic lipase (HL) hydrolyze HDL-associated triglycerides and phospholipids. Cholesteryl-esters of HDL are transported back to the liver by HDL via scavenger receptor B1 (SR-BI) or by VLDL via the LDL receptor. In obesity (indicated with red arrows), there is an increased flux of free fatty acids (FFA) from adipocytes to the liver, which leads to accumulation of triglycerides and an increased secretion of VLDL, resulting in elevated transfer of triglycerides to HDL via CETP. Triglyceride-rich HDL is rapidly hydrolyzed via HL, which shows increased activity, resulting in formation of smaller HDL particles. The ability of HDL to promote cholesterol efflux from lipid-loaded cells is reduced in obesity.

Another characteristic of obesity is an imbalance of adipokines, including leptin. Leptin is mainly produced by adipocytes and is elevated in overweight and obese individuals [100,101]. Interestingly, a study in children showed that plasma leptin levels correlated with HDL-C [102]. Further, a correlation between leptin and HDL-associated triglycerides and with HDL particle size has been reported in adults [103]. In vivo experiments in leptin-deficient (ob/ob) mice suggest that leptin upregulates hepatic SR-BI and thereby influences levels of HDL-C [104].

In the state of obesity, increased CETP levels are correlated with leptin levels [105], in line with the fact that adipose tissue is one of the major sources of CETP expression [106]. Therefore, the obesity-associated increase in CETP production is thought to affect HDL-C levels [107].

Another molecule, secreted from adipose tissue, which may have a direct impact on HDL metabolism, is the adipokine adiponectin. Studies have shown that levels of adiponectin, which are reduced in the state of obesity, are directly correlated with plasma HDL-C levels [108–112]. Furthermore, an intervention study showed that levels of adiponectin as well as of HDL-C are increasing after weight loss and that this improvement was independent of changes in insulin sensitivity and fat mass [113]. The relationship of HDL with adiponectin will be discussed in Section 5.3 in more detail.

As mentioned above, the activity of hepatic lipase is increased in obesity and insulin resistance [96–98,114,115], leading to faster clearance of triglyceride-rich HDL [116], which is produced by CETP-mediated transfer. The triglyceride-enriched HDL is a more susceptible substrate for hepatic lipase and, therefore, undergoes rapid hydrolysis [99,117]. The mechanisms underlying the increase in hepatic lipase activity in obese states are not yet understood, but it appears that hepatic insulin resistance plays an important role [118]. However, further studies are needed to clarify the link between HDL metabolism and hepatic lipase expression in obesity and insulin resistance.

Another lipase, which may affect HDL-C levels in obesity or insulin resistance is endothelial lipase. Experiments with rodents already revealed the impact of endothelial lipase on HDL metabolism: Inhibition or genetic deletion of endothelial lipase resulted in elevated levels of HDL-C by reduction of catabolism rate [119–121], while overexpression of endothelial lipase caused a reduction of HDL-C by increased catabolism rate [119,122,123]. Human studies further have shown that some rare genetic variants in the endothelial lipase gene are linked with high HDL-C levels and that they are correlated to levels of plasma endothelial lipase mass [124,125]. In obesity, levels of endothelial lipase have been shown to be significantly elevated, proposing an upregulation of endothelial lipase during obese states, which may contribute to the reduced HDL-C levels [124]. Obesity is characterized by low-grade inflammation, leading to infiltration of immune cells into adipose tissue [126,127]. The obesity-induced inflammation may decrease HDL-C levels by upregulation of endothelial lipase. However, the significance of endothelial lipase on low levels of HDL-C in the obese state further needs to be investigated.

Another factor affecting HDL-C is cholesterol released by adipocytes. In humans, adipose tissue is a major site for cholesterol storage and contains up to 25% of total body cholesterol in normal-weight subjects and approximately half of it in obese states [128,129]. In adipose tissue, nearly all of the cholesterol is stored in the unesterified form, as free cholesterol, which makes adipocytes unique among cells [129–132]. It is well reported that adipocytes express the major cholesterol transporters ABCA1 and SR-BI as well as ABCG1, but in a much lesser extent [133]. Adipocytes are promoting cholesterol transfer to HDL via ABCA1 and SR-BI, representing a direct factor for modulation of HDL-C levels. Importantly, Zhang et al. demonstrated that lack of adipose ABCA1 resulted in reduced levels of HDL-C and caused a backlog of cholesterol within adipose tissue [134]. Further, they showed that adipocyte inflammation, which is a hallmark of central obesity, downregulates ABCA1 and SR-BI expression and impairs cholesterol efflux from adipocytes to HDL. Therefore, their results suggest a direct impact of adipose tissue on modulation of HDL-C and that obesity-induced inflammation of adipocytes may result in impaired cholesterol efflux to HDL, contributing to reduced HDL-C levels.

Concluding, several factors and mechanisms are involved in the reduction of HDL-C levels in the obese state, but further research on these mechanisms is of importance to find novel treatment strategies improving HDL quality and quantity.

5. Obesity, HDL, and Cardiovascular Risk

Obesity is one of the major risk factors for cardiovascular disease, which is associated with atherogenic dyslipidemia. These alterations in plasma lipid and lipoprotein levels contribute to the manifestation of such a severe morbidity.

5.1. Obesity Leads to a Shift in HDL Subclass Distribution

As described above, plasma HDL-C levels do not adequately reflect protective functions of HDL and greater protective potential is attributed to the smaller, more dense HDL particles. Recent studies of Woudberg et al. assessed HDL subclass distribution in normal-weight and obese white and black South African women. In obese study participants, a shift from large HDL toward increased levels of intermediate and small HDL subclasses was seen, whereby the effect was more pronounced in white women [135]. In a 5.5-year follow-up study they showed that the shifts in HDL subclass distribution were related to increasing central adiposity, suggesting a link between body fat distribution and lipid metabolism [8]. Based on the observed changes in HDL subclass distribution in obese individuals, Woudberg et al. explored the effect of exercise training on HDL subfractions. Interestingly, 12 weeks of exercise intervention altered the distribution of small HDL in obese women [136].

In adolescents suffering from type 2 diabetes mellitus, Davidson et al. determined the risk factors associated with the depletion of large HDL particles and simultaneous accumulation of small particles [137]. The authors investigated the distribution of HDL subclasses of individuals who differed in body mass index and insulin sensitivity and found that obesity is the major risk factor linked to the altered HDL subclasses. An increased CETP-mediated transfer of triglycerides on HDL and the subsequent hydrolysis of triglyceride-enriched HDL by hepatic lipase appeared to be the mechanism underlying the shift of large HDL to small and dense HDL particles [137].

5.2. Obesity Affects HDL Function

It is known that HDL functionality is severely impaired in certain diseases and HDL may even have inflammatory or pro-atherogenic properties. This was clearly demonstrated in HDL from patients suffering from chronic kidney disease [138,139], diabetes [140], cardiovascular disease [86], liver disease [141], psoriasis [142], or even atopic dermatitis [143] and allergic rhinitis [144]. Obesity-associated complications, such as inflammation or diabetes, have been shown to render HDL dysfunctional. HDL isolated from type 2 diabetes patients did not reduce endothelial oxidant stress and did not improve endothelium-dependent vasodilatation when compared to HDL isolated from healthy subjects [145]. Vasodilatory activity of HDL has been shown to be inversely correlated with triglyceride content of HDL, which is elevated in obesity [146]. A reduction of the overall capacity of HDL to promote cholesterol efflux from fibroblasts in obese, compared to lean, normal-weight, subjects was reported [147]. Of particular interest, cholesterol efflux capacity appears to be significantly inversely correlated with the body mass index [148,149]. Since cholesterol efflux capacity is the main metric of HDL function and has strong inverse association with coronary artery disease [85,150,151], the reduction of efflux capacity in obesity may have a crucial impact on the development of cardiovascular disease.

5.3. Adiponectin and HDL

It has been well reported that plasma HDL-C concentrations show a strong correlation with levels of adiponectin, independent of body mass index, distribution of body fat, and insulin sensitivity [108–112]. Adiponectin is mainly secreted by adipocytes, shows anti-atherogenic properties, and modulates glucose metabolism [152,153]. Studies with mice overexpressing or lacking adiponectin as well as in vitro studies suggest a causal relationship with HDL-C levels.

Adiponectin increases the production of apoA-I as well as hepatic ABCA1, which increases HDL-C levels (Figure 3) [154,155]. The enhanced expression of ABCA1 has been suggested by activation of liver X receptor alpha and peroxisome proliferator-activated receptor gamma [156–158]. Plasma levels of adiponectin show a negative correlation with fractional catabolic rate of apoA-I in individuals with metabolic syndrome and control subjects [159]. Besides ABCA1, adiponectin upregulates ABCG1 expression, increases cholesterol efflux capacity, and efficiently promotes lipidation of apoA-I, leading to formation of nascent HDL [160].

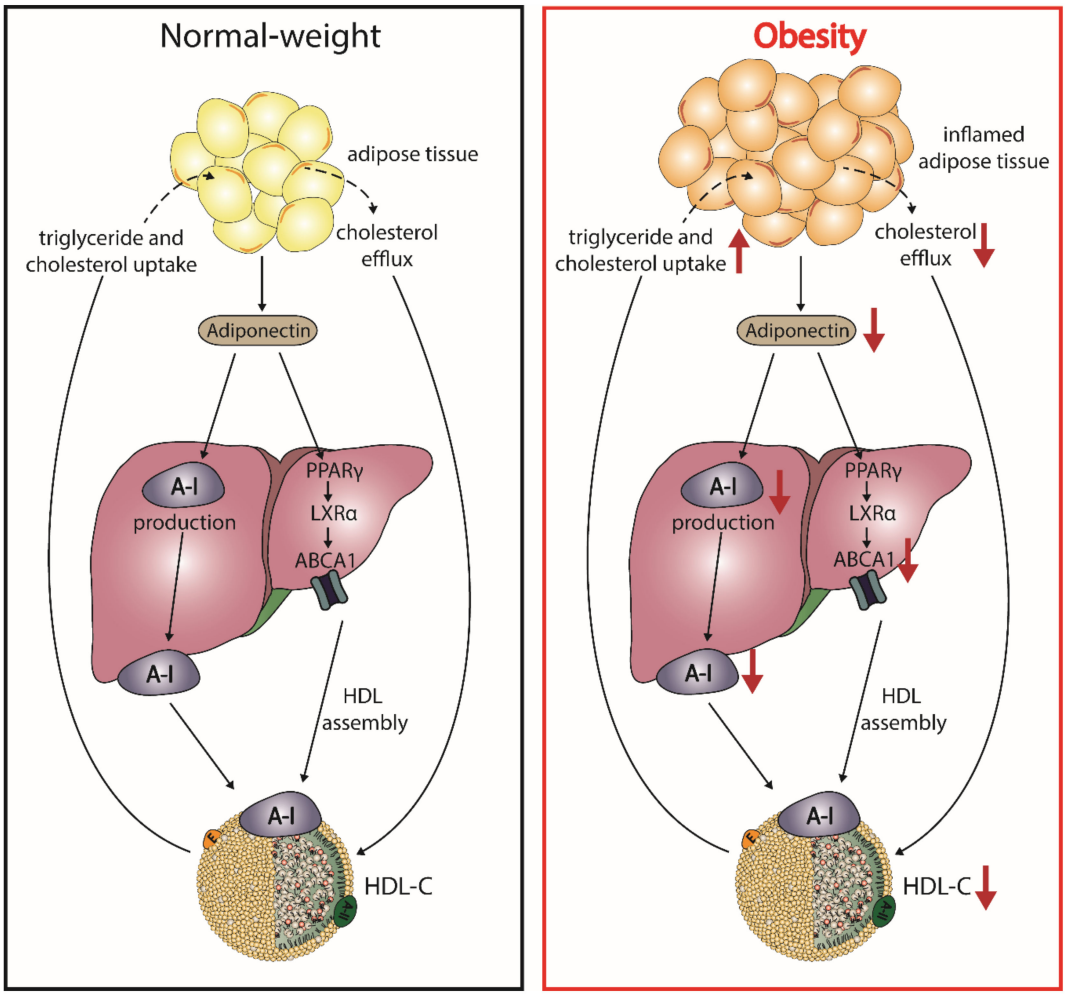

Figure 3. Postulated effects of obesity on adiponectin and HDL metabolism. In normal-weight subjects, adipocytes produce adiponectin, which enhances expression of the ATP-binding cassette transporter A1 (ABCA1) through activation of peroxisome proliferator-activated receptor gamma (PPARγ) and liver X receptor alpha (LXRα), leading to HDL assembly. Further, adiponectin increases the hepatic production of apoA-I. During the state of obesity, adipocytes manifest several altered properties, which play a role in the reduction of HDL-C. Increased inflammation and fat accumulation in the adipocytes reduces the production of adiponectin and impairs cholesterol flux to HDL. The reduction of adiponectin downregulates apoA-I production and ABCA1 expression in hepatocytes, thus reducing HDL assembly.

Adiponectin has been consistently reported to be associated with cholesterol efflux capacity of HDL [148,161,162]. Other studies have shown an inverse association of hepatic lipase with serum adiponectin levels [163,164]. Adiponectin might inhibit the hepatic lipase-mediated hydrolysis of triglycerides and phospholipids of HDL2 particles. This is in line with studies showing an association of adiponectin with HDL particle size and HDL2 [165–168]. However, further studies are needed to prove causality. Another further mechanism suggested that adiponectin increases lipoprotein

lipase activity, thereby accelerating clearance of triglyceride-rich particles. This, in turn, would lead to less exchange of triglycerides and cholesteryl-ester by CETP and, thus, to cholesteryl-ester-enriched HDL2 particles. A positive correlation between circulating adiponectin and post-heparin lipoprotein lipase activity has been reported [169,170], but causality has to be proven to draw firm conclusions. Low-grade inflammation and fat accumulation cause a dysregulated adipokine production [171,172] and markedly reduce adiponectin levels [173,174]. Therefore, low adiponectin observed in obesity levels may explain, at least in part, the shift of large HDL to small HDL particles.

5.4. Obesity and HDL-Associated Sphingosine-1-Phosphate (S1P)

Of particular importance, the complete sphingolipid metabolism is altered in obesity [175]. In obese individuals the levels of ceramides, sphingosine, sphinganine, and S1P are increased in adipocytes when compared to lean controls [175]. The bioactive lipid S1P is mainly carried via apoM anchored to HDL (about 65%) and to a lesser extent via albumin (about 25%) or LDL/VLDL (about 10%) [39]. The half-life of S1P is prolonged when it is associated with HDL, when compared with albumin-associated S1P [176]. S1P is a member of the sphingolipid family, a large group of molecules with a wide range of physiological functions. S1P in the circulation is mainly derived from erythrocytes, vascular endothelial cells, and platelets [177,178]. S1P activates five different G protein-coupled receptors, termed S1P receptors 1–5 (S1PR1–5), in an autocrine or paracrine manner [179].

Kowalski et al. observed that levels of S1P are elevated in plasma of obese humans and rodents and that the levels correlate with metabolic abnormalities such as adiposity and markers of insulin resistance [180]. However, this increase of S1P could not be confirmed in a study comparing levels between overweight and lean adolescents [181]. More recently, the group of Green et al. analyzed the liver metabolome of mice after caloric restriction and revealed that caloric restriction had an impact on S1P signaling [182]. The authors observed that as a response to caloric restriction, liver expression of S1P was significantly increased. S1P levels were negatively associated with decreasing body mass, leptin, and insulin-like growth factor-1. Another study investigated the role of S1P/S1PR1 signaling in the regulation of energy homeostasis in rodents [183]. The authors showed that S1PR is highly expressed in the hypothalamus and that a fasting period of 12 h could reduce S1PR level, whereas refeeding restored the protein levels of the receptor in the hypothalamus. Altogether, their results indicated that the S1P/S1PR1 axis plays a critical role in energy balance and represents a potential target for treatment of obesity. The potential role of S1P signaling in energy metabolism was strengthened by Christoffersen et al., showing that lack of apoM in mice increases the amount of brown adipose tissue and that the turnover of fat is increased, resulting in low white adipose tissue mass and low body weight [184]. These effects of apoM knockout suggest that pharmacological modulation of S1PRs may be a promising approach for the treatment of obesity and associated diseases in the future [185].

Noteworthily, only a small number of studies investigated plasma levels of S1P in obese or overweight human subjects. While obesity is associated with a shift from large HDL to small and dense HDL, S1P has to be increasingly transported with alternative chaperones, reducing the effectiveness of S1P [8]. In line with this hypothesis, Frej et al. showed that a shift in apoM/S1P between HDL particles in women was associated with impaired anti-inflammatory effects of the apoM/S1P complex [186].

6. Bariatric Surgery Improves HDL Levels and Function

Bariatric surgery has been demonstrated as the most effective intervention for patients with severe obesity, which induces sustained long-term weight reduction associated with decreased obesity-associated comorbidities and cardiovascular mortality [187–190]. The standard bariatric surgeries are Roux-en-Y gastric bypass (RYGB), where most of the stomach is bypassed, creating a small gastric pouch; whereas sleeve gastrectomy resects the gastric fundus and most of the gastric body [191]. RYGB surgeries resulted in significant improvements of plasma lipid levels, decreased risk of cardiovascular disease, and overall mortality [192–195]. Further, after RYGB, levels of circulating adiponectin increased, insulin sensitivity improved, and blood pressure levels were reduced [196–198].

Of particular interest is that the plasma levels of HDL-C after bariatric surgery were remarkably improved compared to the preoperative values and compared to people who only received medical therapy for weight loss [195,199–202]. In the Surgical Treatment and Medications Potentially Eradicate Diabetes Efficiently (STAMPEDE) clinical trial, obese patients with type 2 diabetes mellitus were randomly assigned to receive intensive medical therapy alone or in combination with RYGB or sleeve gastrectomy. Five years after surgical procedures, the levels of HDL-C were increased by 32%, 30%, and 7% in the RYGB, sleeve gastrectomy, and medical therapy alone groups, respectively [201]. In a substudy, Lorkowski et al. investigated serum HDL function, by determining the apoA-I exchange rate and cholesterol efflux capacity in the STAMPEDE study. The apoA-I exchange rate is determined by adding labeled apoA-I to serum samples and recording labeled apoA-I incorporation into serum HDL [203]. This apoA-I exchange rate has been linked with risk of major adverse cardiovascular events [203]. HDL in both RYGB and sleeve gastrectomy groups showed improved functionality, by increased apoA-I exchange rate after one and five years compared to baseline. Moreover, also cholesterol efflux capacity after five years was improved when compared to pre-operative samples (Figure 4). Improvement of cholesterol efflux capacity appears to depend on the procedure, with an improvement only with sleeve gastrectomy, but not with RYGB at six months after surgery [204]. However, after 12 months both operations resulted in improved cholesterol efflux capacity [204].

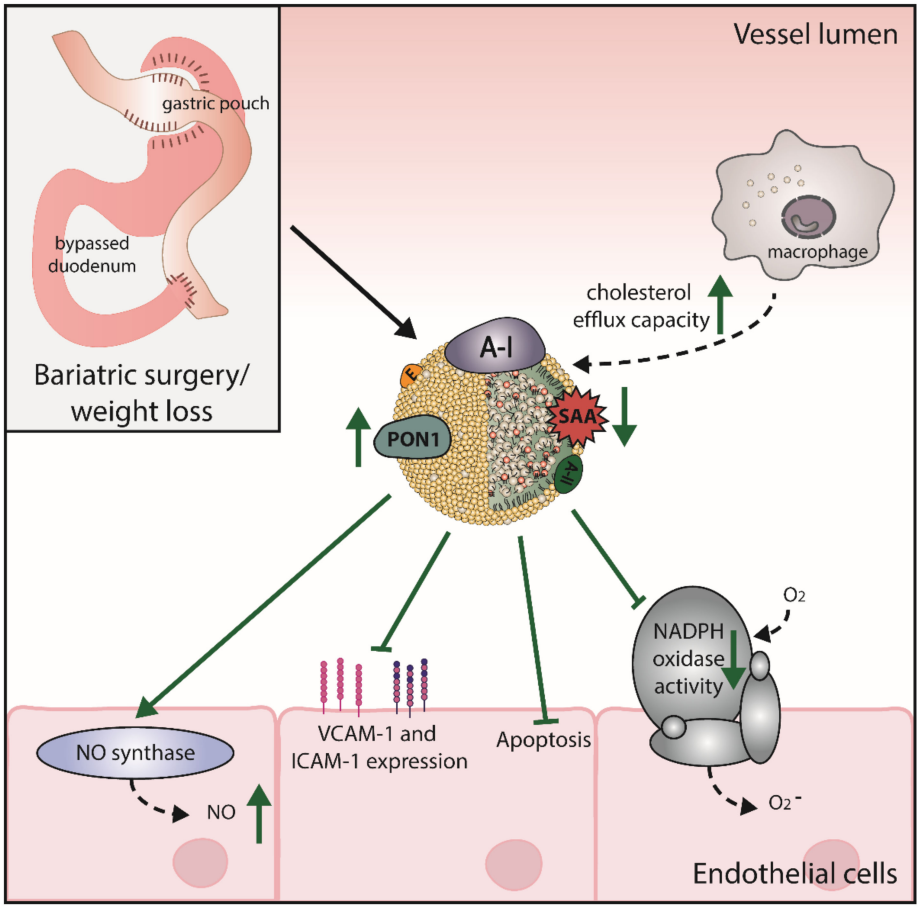

Figure 4. Proposed effects of bariatric surgery on metrics of HDL function. After surgical procedure, HDL shows increased cholesterol efflux capacity, improved paraoxonase 1 (PON1), and reduced levels of serum amyloid A (SAA). Further, HDL of patients after bariatric surgery inhibits expression of the vascular adhesion molecule (VCAM-1) and intracellular adhesion molecule (ICAM-1), improves endothelial anti-apoptotic properties, and reduces nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. Bariatric surgery also improved HDL-mediated production of nitric oxide (NO) and, thus, improved endothelial function.

In addition, other metrics of HDL function were assessed in morbidly obese patients after bariatric procedure. Six months after surgery, the antioxidant potential of HDL was increased, accompanied by an increase in PON1 protein levels. Further, alterations in the distribution of HDL subpopulations with a shift toward more mature HDL as well as an increase in apoA-I/apoE ratio was found [205].

Laparoscopic adjustable gastric banding is another type of weight-loss surgery, which is minimally invasive and associated with low rates of associated complications and mortality rates [206]. Recently, the impact of laparoscopic adjustable gastric banding on HDL subclass distribution was studied [207]. The authors observed an increase in large HDL and intermediate HDL subclasses and a decrease of the small HDL subfraction [207]. Similar to this, another study observed an increase in the large HDL subfractions after laparoscopic adjustable gastric banding and a reduction of HDL-associated pro-inflammatory serum amyloid A [208].

Another study evaluated whether RYGB restores protective properties of HDL and reverses the obesity-induced endothelial dysfunction [209]. In a rat model of RYGB as well as in human samples, endothelium protective activities of HDL were improved and associated with increased plasma levels of the gut hormone glucagon-like peptide-1 and bile acids. HDL isolated from patients after RYGB led to restored endothelial nitric oxide synthase, increased nitric oxide release and, in parallel, a reduction of endothelial nicotinamide adenine dinucleotide phosphate oxidase, and decrease in endothelial apoptosis and vascular adhesion molecule expression. Moreover, the ability of HDL to induce cholesterol efflux from macrophages as well as PON1 activity was enhanced. Interestingly, 12 weeks after RYGB, the properties of HDL were improved to levels of healthy subjects, although the patients were still obese [209]. A recently published study confirmed the improvement of cholesterol efflux capacity and PON1 activity 12 months after RYGB and observed an association of miR-222 and miR-223, both reported to play an important role in the pathophysiology of obesity [210,211], with markers of HDL function [212].

Altogether, the current state of research suggests that the marked increase in HDL quality and quantity observed after bariatric surgery is likely linked to reduction of obesity-related comorbidities and cardiovascular mortality.

7. Effects of Pharmacological Anti-Obesity Interventions on HDL Levels and Function

Changes in dietary and physical lifestyle have been shown to result in a limited reduction in bodyweight (3–10%) and that most people regained weight again [213]. Therefore, besides bariatric surgery, complementary treatments with anti-obesity drugs are a strategy to achieve permanent weight loss in pathologically obese individuals. In 1959, the first anti-obesity drug, termed phentermine was approved by the United States Food and Drug Administration Nowadays, a number of pharmacotherapies have become available to treat obesity.

Phentermine belongs to the group of sympathomimetics and is the most commonly prescribed anti-obesity drug in the USA [214]. Twelve weeks of administration of phentermine reduced body weight and decreased levels of total cholesterol in Korean obese subjects [215].

A combination therapy of phentermine with topiramate has been shown to induce greater weight loss than either drug alone and showed fewer occurrence of side effects [216]. Administration of phentermine and topiramate in overweight and obese patients with dyslipidemia showed improvements in HDL-C levels and non-HDL-C levels vs. the placebo group at week 56 [217]. Another study designed to evaluate the long-term efficacy of phentermine/topiramate treatment found that the HDL-C levels of study participants increased more than in the placebo group [218].

Orlistat is an intestinal lipase inhibitor that prevents breakdown of triglycerides and has an excellent long-term safety record [216]. Interestingly, orlistat causes a 25% reduction in cholesterol absorption [219]. Regarding orlistat-induced changes in HDL-C levels, studies are inconsistent. Some studies reported a significant increase of HDL-C in patients receiving orlistat [200–222], while others observed no significant changes [223–225].

Noteworthily, food intake only minimally affects HDL-C [226,227], which might explain the inconsistent effects of orlistat on HDL-C levels.

Lorcaserin is a serotonin 2c receptor agonist available in the USA that increases central serotonin release and has been shown to be effective for long-term weight management [228,229]. A recent study showed that lorcaserin treatment for six months resulted in decrease of LDL-C, while plasma levels of HDL-C were increased [230]. Lipid subfraction analysis further revealed an increase in HDL particle size.

Liraglutide is a glucagon-like peptide-1 receptor agonist widely used to treat type 2 diabetes. This drug further increases satiety, slows gastric emptying, and also decreases body weight, besides reducing glucose concentration [231]. Long-term treatments with liraglutide have been shown to reduce body weight and waist circumference, but also to improve plasma lipid levels, including an increase in HDL-C levels [232,233].

Overall, most pharmacological approaches for obesity treatment increase HDL-C. Further studies examining potential effects of anti-obesity treatment on metrics of HDL function are warranted.

8. Effects of Dietary Approaches on HDL Levels and Function

Other strategies to treat obesity, besides pharmacological treatments and surgical procedures, are hypocaloric diets, such as intermittent fasting and caloric restriction. Furthermore, dietary patterns including Mediterranean diet are commonly used to induce weight loss and improve cardiovascular health in obese individuals [234,235].

Caloric restriction is the most common form of dietary restriction, in which subjects strive to decrease their daily energy intake by 15–40% of baseline needs each day [236]. In a 16-week intervention trial in which obese diabetic participants were given a very low calorie diet (450 kcal/day), caloric restriction was shown to reduce CETP activity and increase ApoA-I levels, but did not affect HDL-C levels or HDL cholesterol efflux capacity [237]. Another recently published study compared the effect of an 8-week intermittent caloric restriction regimen to continuous caloric restriction in overweight and obese subjects. They observed that these interventions similarly reduced body weight and fat mass and improved plasma triglycerides but had no effect on levels of HDL-C [238]. Interestingly, Liang et al. observed that a 3-month intervention of caloric restriction, together with moderate physical activity, resulted in weight reduction in obese subjects with metabolic syndrome but decreased PON1 levels [239]. In line with this, another study with obese participants observed that a low-calorie diet reduced PON1 enzyme activity [240]. Furthermore, weight loss through caloric restriction has been shown to decrease LCAT activity in obese [241] as well as in normal-weight subjects [242].

Alternate-day fasting (ADF) regimens consist of a “feeding day”, with ad libitum feeding and a “fasting day”, with complete abstinence of food and drink intake, except for water for 24 h. These regimens are less common than caloric restriction but were created to facilitate compliance with dietary restriction protocol, as these regimens require energy restriction only every-other day. In a modified ADF study, in which obese participants were allowed to consume 25% of their regular energy needs on the fasting day, body weight and body fat decreased and also levels of triglycerides, total cholesterol, and LDL-C decreased, whereas levels of HDL-C remained unchanged [243]. Varady et al. demonstrated that the same ADF regimen was effective in both weight reduction and cardioprotection in normal-weight and overweight subjects [244]. After 12 weeks of ADF, the study participants showed decreased body weight and fat mass, but no changes in the levels of HDL-C were observed. Similar results were observed in another ADF intervention study in normal-weight participants [245].

Mediterranean diet is a dietary approach to induce weight loss and to prevent cardiovascular events [234]. This diet pattern is generally characterized by high consumption of vegetables, fruits, nuts, legumes, wheat-based cereals, olive oil, and fish; moderate consumption of dairy products and poultry; and low consumption of red and processed meats [246]. In the Prevention with Mediterranean Diet study (PREDIMED), individuals with high cardiovascular risk were assigned to a Mediterranean

diet supplemented with extra-virgin olive oil or nuts and had lower incidence of cardiovascular events than the control group, assigned to a reduced-fat diet [247]. A substudy, including volunteers of the PREDIMED trial, concentrated on examining the effect of this anti-oxidant-rich dietary pattern on HDL function. Of particular interest, they observed that a 1-year Mediterranean diet, enriched with olive oil or nuts, increased the HDL cholesterol efflux capacity, PON1 activity, and HDL vasodilatory activity [248]. Similarly, another study showed that 12 weeks of Mediterranean diet and exercise improved HDL cholesterol efflux capacity and improved HDL function by inhibiting myeloperoxidase-mediated oxidative stress in subjects with metabolic syndrome [249].

9. Conclusions

Obesity leads to a depletion of HDL-C, due to a marked shift from large cholesteryl-ester-rich HDL to small and dense triglyceride-rich particles. The mechanisms underlying this shift are multifactorial, including elevated CETP activity linked to increased levels of triglyceride-rich lipoproteins, lower adiponectin levels, and increased clearance of large HDL particles. These changes in HDL subspecies are accompanied by changes in composition and functionality. S1P will potentially be attached to alternative chaperones, resulting in attenuated multiple beneficial effects of S1P. Bariatric surgery is currently the most effective treatment for raising HDL-C levels and, more importantly, it also significantly improves HDL functionality and may be related, at least in part, to the reduction in mortality observed in observational studies. In addition, there is accumulating evidence that Mediterranean diet, especially when enriched with virgin olive oil, significantly enhances parameters of HDL atheroprotective functions. Further studies are warranted to identify specific components in olive oil or other nutrients that improve HDL function. Most pharmacological approaches for obesity treatment increase HDL-C but further studies examining potential effects of anti-obesity treatment on metrics of HDL function are needed. The data of caloric restriction strategies are inconsistent and even show negative effects on some metrics of HDL functionality.

Considerable interest has recently focused on approaches to influence the biological functions of HDL in the search for new cardioprotective therapies and might establish novel treatment strategies in obese individuals.

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Obesity Affects HDL Metabolism, Composition and Subclass Distribution

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Abstract: Background: Obesity increases the risk of coronary heart disease, partly due to its strong association with atherogenic dyslipidemia, characterized by high triglycerides and low high-density lipoprotein (HDL) cholesterol levels. Functional impairment of HDL may contribute to the increased cardiovascular mortality, but the effect of obesity on composition, structure, and function of HDL is not well understood. Design and Methods: We determined HDL composition, HDL subclass distribution, parameters of HDL function, and activities of most important enzymes involved in lipoprotein remodeling, including lecithin–cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) in relatively young normal weight (n = 26), overweight (n = 22), and obese (n = 20) women. Results: Obesity (body mass index (BMI) $\geq$ 30) was associated with noticeable changes in LCAT and CETP activities and altered HDL composition, such as decreased apolipoprotein A-I, cholesterol, and phospholipid content, while pro-inflammatory HDL serum amyloid a content was increased. We observed a marked shift towards smaller HDL subclasses in obesity linked to lower anti-oxidative capacity of serum. LCAT activity, HDL subclass distribution, and HDL-cholesterol were associated with soluble leptin receptor, adiponectin, and liver enzyme activities. Of note, most of these alterations were only seen in obese women but not in overweight women. Conclusions: Obesity markedly affects HDL metabolism, composition, and subclass distribution linked to changes in liver and adipose tissue. HDL dysfunction may contribute to increased cardiovascular risk in obesity.

Keywords: obesity; HDL-C; HDL subclasses; LCAT; CETP; adiponectin; soluble leptin receptor

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1. Introduction

One of the strongest predictors of cardiovascular disease in obesity and obesity-associated metabolic syndrome is a low level of high-density lipoprotein cholesterol (HDL-C) and increased triglyceride-rich lipoproteins (TRGLs) [1], which are components of a

constellation, often referred to as "atherogenic dyslipidemia". As a consequence of dietary changes and reduced physical activity, the increasing prevalence of obesity is becoming a severe burden for general public health [2]. Several diseases are known to be closely linked to obesity, such as cardiovascular disease, non-alcoholic fatty liver disease, cancer, and type 2 diabetes [3–6]. Reduced plasma levels of HDL-C, but increased triglyceride levels are often observed in obese individuals [7], together with an imbalance of adipokines, such as leptin and adiponectin [8]. Both peptide hormones are produced by adipose tissue and play an important role in the regulation of energy metabolism [9]. In obese individuals, adiponectin levels are decreased and associate with decreased HDL-C and HDL cholesterol efflux capacity, an integrated metric of HDL quantity and quality [10,11]. Interestingly, the most efficient treatment for low HDL-C levels in morbidly obese patients is bariatric surgery [12,13]. This surgical procedure leads to a remarkable increase in plasma HDL-C levels, but also to improved HDL function, independent of body weight [11,14,15].

Reduced HDL function may play a role in a variety of diseases, as HDL particles have anti-inflammatory [16], anti-oxidant [17], and anti-thrombotic [18] activities and promote reverse cholesterol transport [19]. HDL particles contain a considerable number of proteins and lipids and are structurally and functionally heterogeneous. Apolipoprotein (apo)A-I and apoA-II, which stabilize HDL, as well as apoC-II and apoC-III, act as cofactors for enzymes involved in lipid metabolism [20,21]. Furthermore, several enzymes are associated with HDL, such as paraoxonase, which is a hydrolytic enzyme with a wide range of substrates and is partly responsible for the anti-oxidant and anti-inflammatory properties of HDL [22]. Lecithin–cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) are key enzymes involved in the remodeling of HDL. By esterifying the free cholesterol of HDL, LCAT increases the HDL particle size and provides substrates for CETP [23]. Disturbances in the activity of these enzymes have been described in insulin resistance, type 2 diabetes, and liver disease [24,25]. Effects of obesity on HDL metabolism and function remain unclear.

In this cross-sectional study in relatively young and healthy women, we assessed whether obesity affects HDL metabolism, composition, and subclass distribution as well as metrics of HDL function.

2. Materials and Methods

2.1. Recruitment and Group Characteristics

A total of 68 participants were enrolled: 26 normal weight, 22 overweight, and 20 obese women aged 18–39 years. All participants gave their written informed consent. The study population was a subgroup of a larger cross-sectional study (5 groups of different energy status, n = 107) [13,26,27] and was conducted according to the Declaration of Helsinki and was approved by the ethics committee of the Medical University of Graz on 2 June 2014 (MUG-26-383ex13/14). The study population was enrolled according to the following inclusion criteria: female, aged between 18 and 40 years. The participants were assigned to the groups according to the WHO-recommended body mass index (BMI) categories for relative weight classification for normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obesity ($\geq$30 kg/m^2) [28]. We included only metabolically healthy women with the following exclusion criteria: acute or chronic illnesses or infections, alcohol or drug abuse, statin medication, severe cognitive deficits, history of digestive tract diseases, history of gastrointestinal surgery, treatment with antibiotics and taking prebiotics or probiotics within the last 2 months, pregnancy or breastfeeding.

2.2. Body Mass Index (BMI) and Body Fat Measurement

The BMI (body weight [kg]/body height [m]^2) was calculated and used for group allocation in accordance with the WHO categories. To obtain information on the fat distribution of participants, we measured the thickness of subcutaneous adipose tissue (SAT) at 8 clearly defined body sites by the standardized ultrasound method [29]. The sum of these 8 digits was calculated (D_{INCL}), which is a reliable and representative measure of body fat percentage, even in obese people [30].

2.3. Plasma Lipids

Plasma lipids such as total cholesterol, triglycerides, and HDL-C were measured by enzymatic photometric transmission measurement (Roche Diagnostics, Mannheim, Germany). The concentrations of LDL-cholesterol were calculated by the Friedewald's formula [31].

2.4. VLDL

Plasma levels of very low density lipoprotein (VLDL) were measured using the Lipoprint System (Quantimetrix Corp., Redondo Beach, CA, USA), according to the manufacturer's instructions. Serum was loaded on gel tubes and mixed with 200 µL of Lipoprint loading gel, containing a lipophilic dye, which binds proportionally to the lipids in the sample. Photopolymerisation was carried out for 30 min. Electrophoresis was performed for 60 min at 3 mA per gel tube and at a maximum delivery of 500 V. After a rest period of 30 min, gel tubes were scanned and analyzed using the Lipoware Software (Lipoware HDL Research LW03-v.16-134).

2.5. Markers of Inflammation

Markers of inflammation, namely, C-reactive protein and interleukin-6, were analyzed by a particle-enhanced turbidimetric assay and an electrochemiluminescent immunoassay (ECLIA), respectively, on a Cobas 6000 chemical routine analyzer (Roche Diagnostics, Mannheim, Germany).

2.6. ApoB-Depleted Serum

ApoB-depleted serum was prepared by the addition of 40 µL polyethylenglycol (Sigma-Aldrich, Darmstadt, Germany) (20% in 200 mmol/L glycine buffer) to 100 µL serum followed by gentle mixing [32]. Serum samples were incubated at room temperature for 20 min, and after centrifugation at 10,000 rpm for 30 min at 4 °C, the supernatant was collected, and samples were stored at −70 °C until usage.

2.7. HDL-Associated Proteins and Lipids

HDL-associated apoA-I, apoA-II, apoC-II, apoC-III, and apoE were determined by immunoturbidimetry [33]. Lipids including cholesterol, phospholipids, and triglycerides were determined using enzymatic methods, as previously described [34]. Cholesteryl ester was calculated as the difference between total cholesterol and free cholesterol, measured in apoB-depleted serum. All lipoprotein analyses were performed on an Olympus AU680 analyzer (Beckman Coulter, Brea, CA, USA), as previously described [33]. Serum amyloid A (SAA) was quantified using a commercially available kit (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions.

2.8. HDL Particle Size

ApoB-depleted serum (2 µL) was separated by native gradient gel electrophoresis (4–16% NativePage; Life Technologies, Carlsbad, CA, USA). The gels were run for 120 min at constant voltage of 150 in NativePage running buffer (Life Technologies, Carlsbad, CA, USA). Subsequently, standard (NativeMark, Life Technologies, Carlsbad, CA, USA) of the gels was fixed with 25% isopropanol/10% acetic acid for 10 min and stained with protein staining solution (PageBlue, Thermo Scientific, Waltham, MA, USA). Separated neutral

lipids of the samples were stained with Sudan black (Sigma-Aldrich, Darmstadt, Germany). Size distribution of HDL was analyzed using Image Lab software (version 5.2), as described previously [35].

2.9. Arylesterase Activity of Paraoxonase

Arylesterase activity of HDL-associated paraoxonase was determined with a photometric assay using phenylacetate as substrate, as described elsewhere [36].

2.10. Anti-Oxidative Capacity of HDL

The anti-oxidative activity of serum was determined with a fluorometric assay, as previously described [37]. The ability of serum samples to inhibit oxidation of dihydrorhodamine was monitored.

2.11. Total Oxidative Capacity (TOC)

TOC captures the total peroxide concentration in serum. TOC was determined by a rapid enzymatic in vitro diagnostic assay (TOC, Labor Diagnostic Nord, Nordhorn, Germany). The assay uses a peroxide/peroxidase reaction with 3,5,3′,5′-tetramethylbenzidine as substrate. The results were calculated from the linear hydrogen peroxide standard curve and peroxide levels were specified as micromole H_2O_2 equivalents [38].

2.12. LCAT Activity

LCAT activity of serum was assessed by a commercially available kit (Merck, Darmstadt, Germany) according to the manufacturer's instructions. Specifically, serum samples were incubated with the LCAT substrate for 4 h at 37 °C. The fluorescent substrate emits fluorescence at 470 nm. When the substrate is hydrolyzed by LCAT, a monomer is released that emits fluorescence at 390 nm. The LCAT activity is assessed over time and expressed in change of 470/390 nm emission intensity.

2.13. LCAT Protein Concentration

LCAT protein concentration in serum samples was measured using a commercially available ELISA kit (BioVendor, Brno, Czech Republic) according to the manufacturer's instructions.

2.14. CETP Activity

CETP activity of serum was measured using a commercially available kit (Abcam, Cambridge Science Park, Cambridge, UK), according to the manufacturer's instructions. Specifically, the assay uses a donor molecule containing a fluorescent self-quenched neutral lipid that is transferred to an acceptor molecule in the presence of CETP. The CETP-mediated transfer of the fluorescent lipid to the acceptor molecule results in an increase in fluorescence intensity (excitation: 465 nm; emission: 535 nm).

2.15. Cholesterol Efflux Capacity of ApoB-Depleted Serum

Cholesterol efflux capacity was performed as described elsewhere [19,39]. J774.2 cells (Sigma-Aldrich, Darmstadt, Germany) were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Carlsbad, California, USA) in the presence of 10% fetal bovine serum and 1% penicillin/streptomycin. A total of 300,000 cells per well were plated on 48-well plates (Greiner Bio-One, Kremsmünster, Austria), cultured for 24 h, and labelled with 0.5 µCi/mL radiolabeled [^{3}H]-cholesterol (Hartmann Analytic, Braunschweig, Germany) in Dulbecco's modified Eagle's medium supplemented with 2% fetal bovine serum and 1% penicillin/streptomycin in the presence of 0.3 mM 8-(4-chlorophenylthio)-cyclic adenosine monophosphate (Sigma-Aldrich, Darmstadt, Germany) overnight. Cyclic adenosine monophosphate was used to upregulate ATP-binding cassette transporter A1. The day after labelling, cells were rinsed with serum-free Dulbecco's modified Eagle's medium containing 1% penicillin/streptomycin and equilibrated with serum-free Dulbecco's modi-

fied Eagle's medium containing 1% penicillin/streptomycin and 2 mg/mL bovine serum albumin (Sigma-Aldrich, Darmstadt, Germany) for 2 h. Subsequently, [^{3}H]-cholesterol efflux was determined by incubating cells for 3 h with 2.8% apoB-depleted serum. Cholesterol efflux capacity was expressed as the radioactivity in the medium relative to total radioactivity in medium and cells. All steps were performed in the presence of 2 µg/mL of the acyl- coenzyme A cholesterol acyltransferase inhibitor Sandoz 58-035 (Sigma-Aldrich, Darmstadt, Germany).

2.16. Adipokines

Leptin, leptin receptors, and adiponectin were determined in serum samples by specific enzyme-linked immunosorbent assays in accordance with the user manual (all BioVendor, Brno, Czech Republic). In brief, samples were incubated in microplate wells pre-coated with polyclonal anti-human leptin antibody, monoclonal anti-human leptin receptor antibody, or polyclonal anti-human adiponectin antibody. After incubation and washing of recombinant human leptin, leptin receptor or adiponectin together with polyclonal anti-human leptin antibody, monoclonal anti-human leptin receptor antibody, or polyclonal anti-human adiponectin antibody conjugated with horseradish peroxidase was added to the wells and incubated with the captured analytes. After another washing step, the horseradish peroxidase conjugate bound to leptin, leptin receptor, or adiponectin immobilized on the wells reacted with the added substrate solution. By adding an acidic solution, we found that the reaction stopped, resulting in a yellowish product. The absorbance was measured photometrically, whereas the concentrations of the analytes leptin and leptin receptor were proportional and the concentration of adiponectin was inversely proportional to the absorbance. Concentrations were determined using standard curves.

2.17. Statistical Analysis

Statistical analyses were performed using SPSS Statistics (version 26) and R (version 3.6.1). Differences between the overweight or obese group with the normal weight control group were analyzed using Wilcoxon rank sum test. Data are presented as medians with interquartile range. Correlations were determined using Spearman's correlation coefficient *rho* and were Bonferroni corrected.

3. Results

3.1. Obesity Was Associated with Alterations in HDL Composition and Subclass Distribution

In total, 26 normal weight, 22 overweight, and 20 obese women were included in this study. Subject characteristics are presented in Table 1.

Despite the reported key role of lipids and apolipoproteins, specifically apoA-I, in HDL metabolism, the composition of HDL particles in obesity is currently unknown. We first assessed whether overweight or obesity affected the lipid composition of HDL. Remarkably, we observed substantially lower levels of HDL-associated free cholesterol, cholesteryl-esters, and phospholipids in the obese group compared to the normal weight group (Figure 1A–C), while there was a trend towards increased HDL triglyceride content ($p = 0.055$) (Figure 1D). Interestingly, we observed no changes in the lipid composition of HDL in overweight women (BMI 25.0–29.9 kg/m^2), except for HDL triglycerides, which were significantly elevated ($p < 0.001$) (Figure 1D).

Table 1. Clinical characteristics of the study cohort.

Population Characteristics	Normal Weight (n = 26)	Overweight (n = 22)	p-Value	Obese (n = 20)	p-Value
Age (years)	24 (23–27)	24 (23–29)	0.976	26 (22–32)	0.373
BMI (kg/m^2)	21.8 (20.5–23.3)	27.0 (26.3–27.4)	<0.001	33.0 (31.4–35.3)	<0.001
D_{INCL} (mm)	83.6 (66.1–99.1)	140.8 (120.2–162.0)	<0.001	196.9 (189.7–223.7)	<0.001
HDL-cholesterol (mg/dL)	80.0 (67.0–86.5)	75.0 (61.2–81.8)	0.239	58.0 (48.8–68.0)	<0.001
LDL-cholesterol (mg/dL)	80.0 (64.5–101.5)	82.0 (65.5–105.0)	0.740	106.5 (79.2–120.8)	0.031
VLDL (mg/dL)	21.0 (16.5–24.0)	22.5 (19.0–25.5)	0.309	27.5 (24.0–33.5)	<0.001
Triglycerides (mg/dL)	66.0 (50.0–88.0)	78.5 (65.5–122.8)	0.057	105.0 (79.2–143.5)	0.005
HbA1c (mmol/mol)	31.0 (30.0–32.5)	33.0 (31.0–33.0)	0.069	34.0 (32.0–36.5)	<0.001
CRP (mg/L)	1.3 (0.6–2.4)	1.4 (0.8–3.5)	0.345	5.3 (3.0–8.2)	<0.001
IL-6 (pg/mL)	1.5 (1.5–2.1)	2.0 (1.5–2.5)	0.119	3.6 (2.8–4.5)	<0.001
Leptin (ng/mL)	10.9 (7.8–14.9)	23.9 (19.1–39.2)	<0.001	49.1 (37.1–50.0)	<0.001
sOB-R (ng/mL)	19.5 (16.4–21.5)	15.1 (12.4–18.0)	0.003	10.2 (9.3–12.2)	<0.001
Adiponectin (µg/mL)	11.7 (9.4–15.5)	10.8 (9.4–12.1)	0.287	8.4 (7.0–10.5)	0.018
ALP (U/L)	50.0 (43.0–54.5)	51.0 (47.5–64.0)	0.136	60.5 (55.0–80.0)	<0.001
GGT (U/L)	12.0 (10.5–16.0)	13.0 (11.0–16.8)	0.408	17.0 (15.8–23.0)	0.002
CHE (U/L)	7225.0 (6604.0–7872.5)	7378.5 (6219.5–8535.0)	0.695	8253.5 (7682.8–9609.8)	<0.001
ALT (U/L)	14.0 (12.0–19.5)	18.0 (13.0–21.8)	0.197	23.0 (19.0–26.0)	<0.001

Clinical characteristics of the study cohort. Data are presented as median (Q1–Q3). Differences between normal weight women and either overweight or obese women were assessed by Wilcoxon rank sum test. n, number of subjects; BMI, body mass index; D_{INCL}, thickness of subcutaneous adipose tissue at eight measured body sites; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, glycated hemoglobin A1c; CRP, C-reactive protein; IL-6, interleukin-6; sOB-R, soluble leptin receptor; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; CHE, cholinesterase; ALT, alanine transaminase.

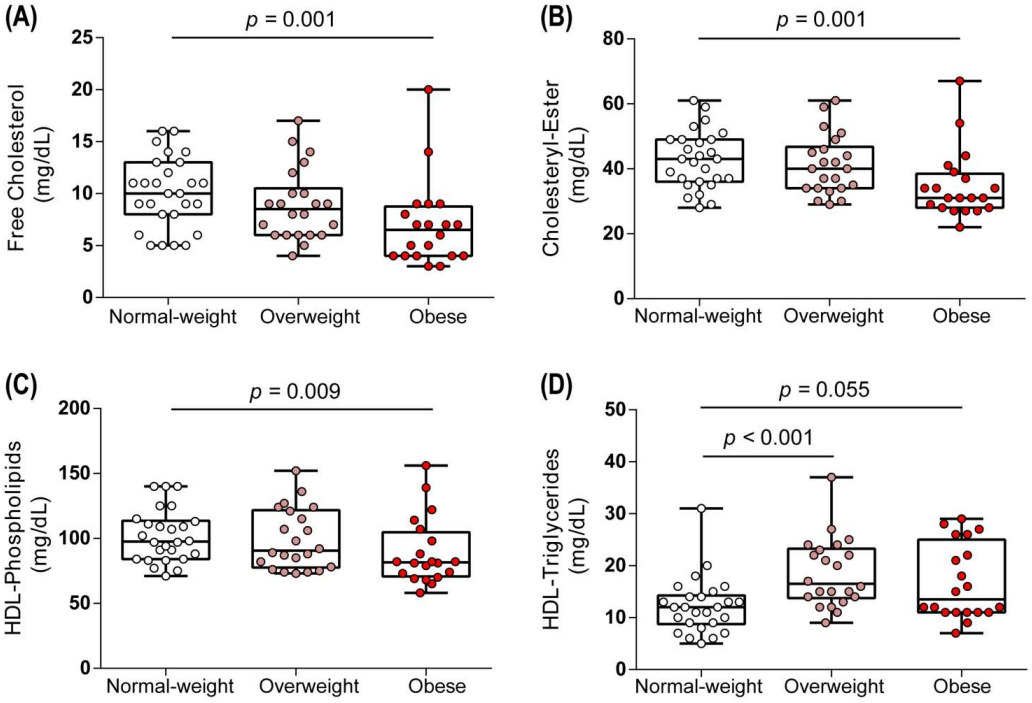

Figure 1. Lipid composition of HDL. Free cholesterol (**A**), cholesteryl ester (**B**), phospholipids (**C**), and triglycerides (**D**) were determined in study subjects. Differences between the two groups were analyzed by Wilcoxon rank sum test. Individual data are presented on top of boxplots displaying median and interquartile range as well as minimum and maximum values.

In addition to the alterations in the lipid composition, HDL of obese women showed decreased levels of apoA-I when compared to normal weight women (Figure 2A), while the contents of apoA-II, apoC-II, apoC-III, and apoE were not altered (Figure 2B–E). We detected increased levels of the acute phase protein serum amyloid a (SAA) in HDL of obese women (Figure 2F), suggesting low-grade inflammation, in line with increased C-reactive protein (CRP) and interleukin-6 (IL-6) levels in obese women (Table 1).

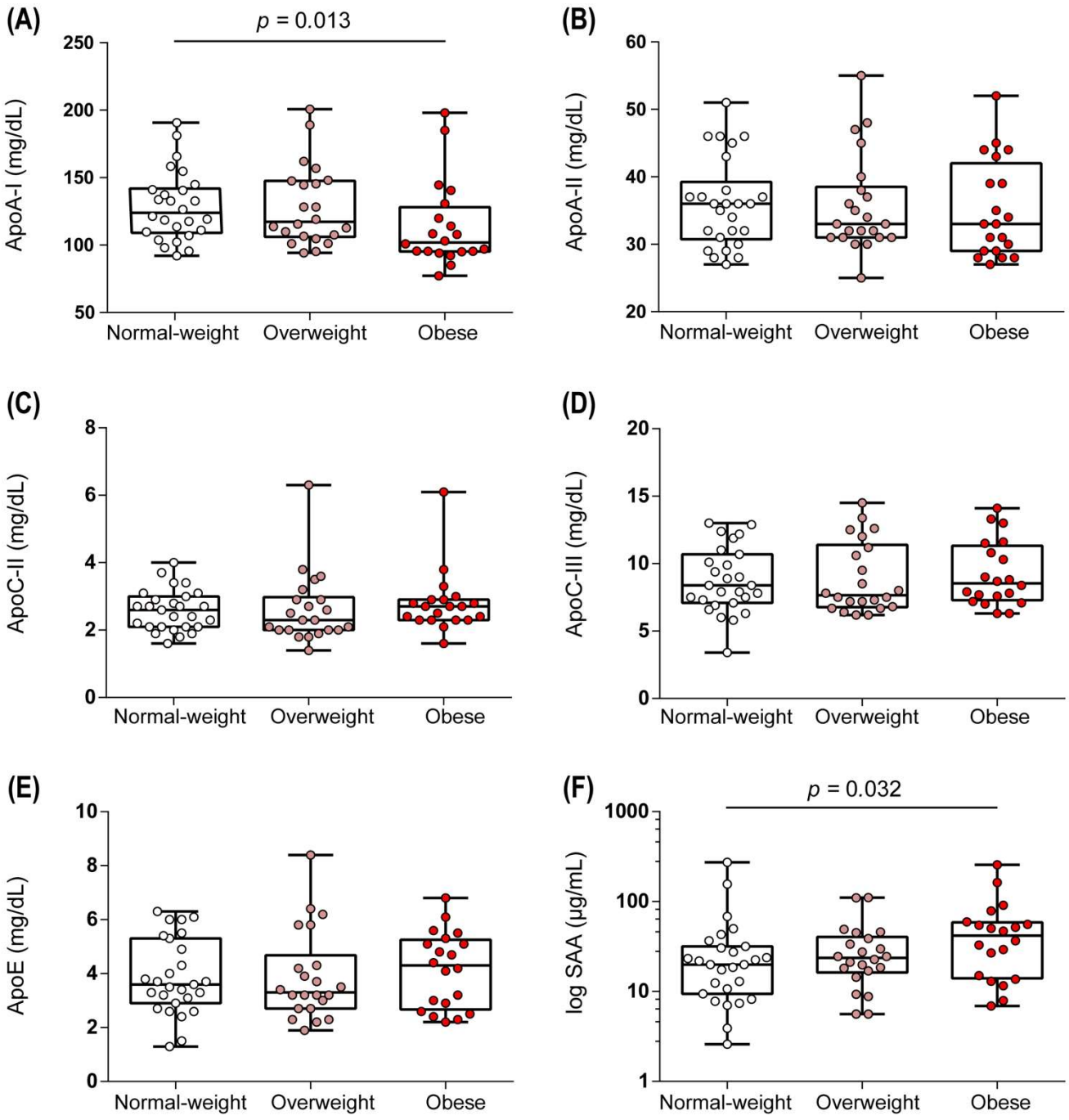

Figure 2. Protein composition of HDL. HDL-associated proteins, including apoA-I (**A**), apoA-II (**B**), apoC-II (**C**), apoC-III (**D**), apoE (**E**), and SAA (**F**) were determined for the study subjects. Differences between the two groups were analyzed by Wilcoxon rank sum test. Individual data are presented on top of boxplots displaying median and interquartile range as well as minimum and maximum values. ApoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; apoC-II, apolipoprotein C-II; apoC-III, apolipoprotein C-III; apoE, apolipoprotein E; SAA, serum amyloid A.

The HDL size distribution reflects the complex intravascular metabolism of these lipoproteins. On the basis of the differences in density and size, we were able to subdivide HDL particles into large and lipid-rich HDL2 particles and protein-rich smaller HDL3 particles. When assessing HDL particle distribution, we observed increased levels of the HDL3 (Figure 3A) and the small HDL3 (Figure 3B) subclasses and decreased levels of the HDL2 subclass (Figure 3C) in the obese compared to normal-weight group. A representative gradient gel electrophoresis of HDL subfractions is shown in Figure 3D.

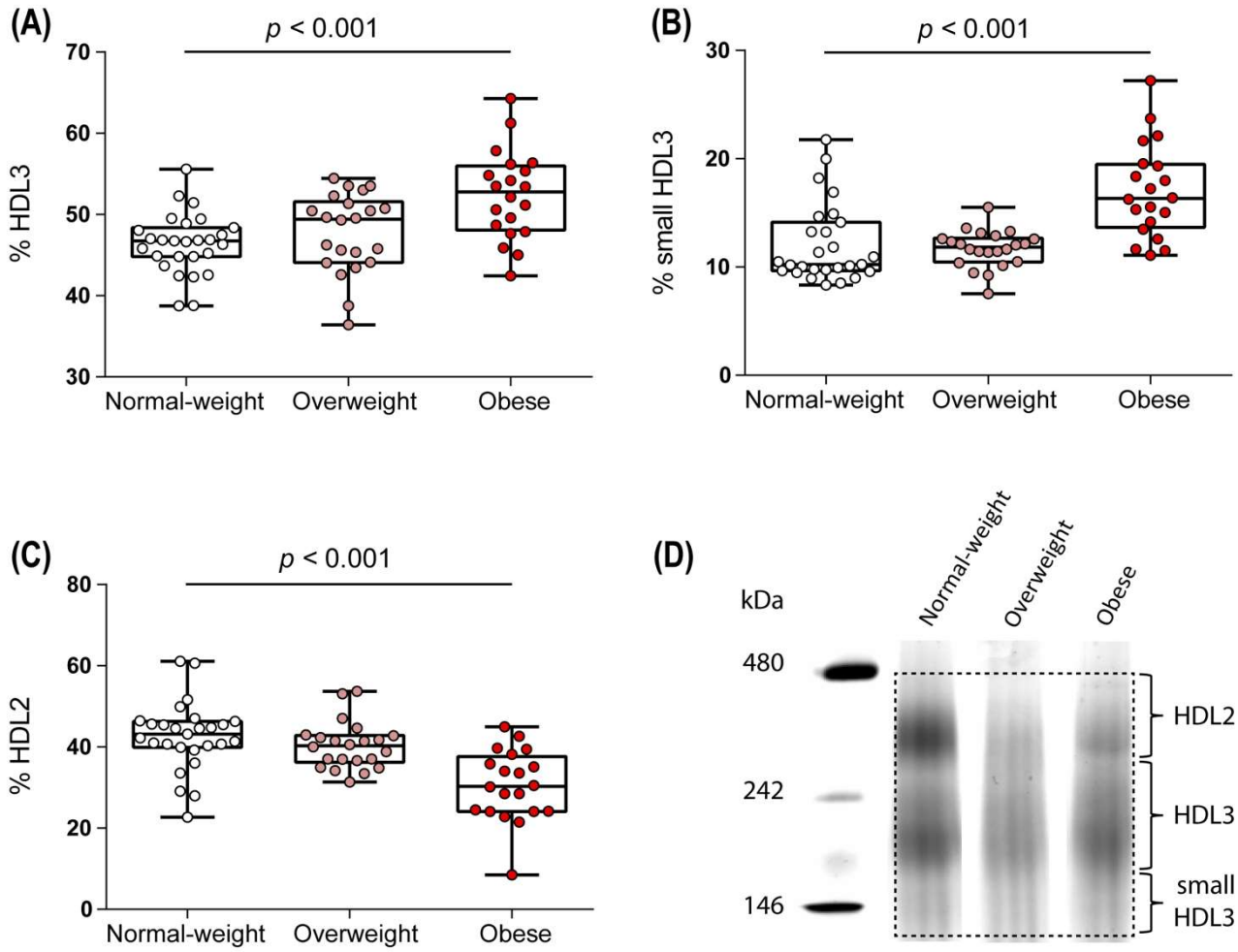

Figure 3. HDL subclass distribution. The distribution of HDL3 (**A**), small HDL3 (**B**), and HDL2 (**C**) subfractions was assessed by native gradient gel electrophoresis. A representative gradient gel electrophoresis of apoB-depleted serum of one normal weight, one overweight, and one obese woman is shown (**D**). Differences between the two groups were analyzed by Wilcoxon rank sum test. Individual data are presented on top of boxplots displaying median and interquartile range as well as minimum and maximum values.

3.2. Obesity Altered Activities of Enzymes Involved in HDL Metabolism

Having observed marked changes in HDL structure and composition in obese women, we next investigated whether obesity affected activities of key enzymes involved in HDL metabolism. HDL-associated cholesteryl ester can subsequently be transferred to apoB-containing lipoproteins via CETP in exchange for triglycerides. Notably, we observed increased activity of CETP in obese women when compared to the normal weight control group ($p = 0.044$), while the activity remained unchanged in the overweight group (Figure 4A). Through LCAT-induced esterification of HDL-associated free cholesterol to cholesteryl-ester, nascent HDL is converted into mature HDL, an important step in reverse cholesterol transport [40]. Interestingly, LCAT activity and protein levels were markedly

increased in obese subjects when compared to the normal weight group (Figure 4B,C) but were not significantly altered in the overweight group (Figure 4B,C). LCAT activity correlated inversely with the ratio between free cholesterol and total cholesterol (Figure 4D).

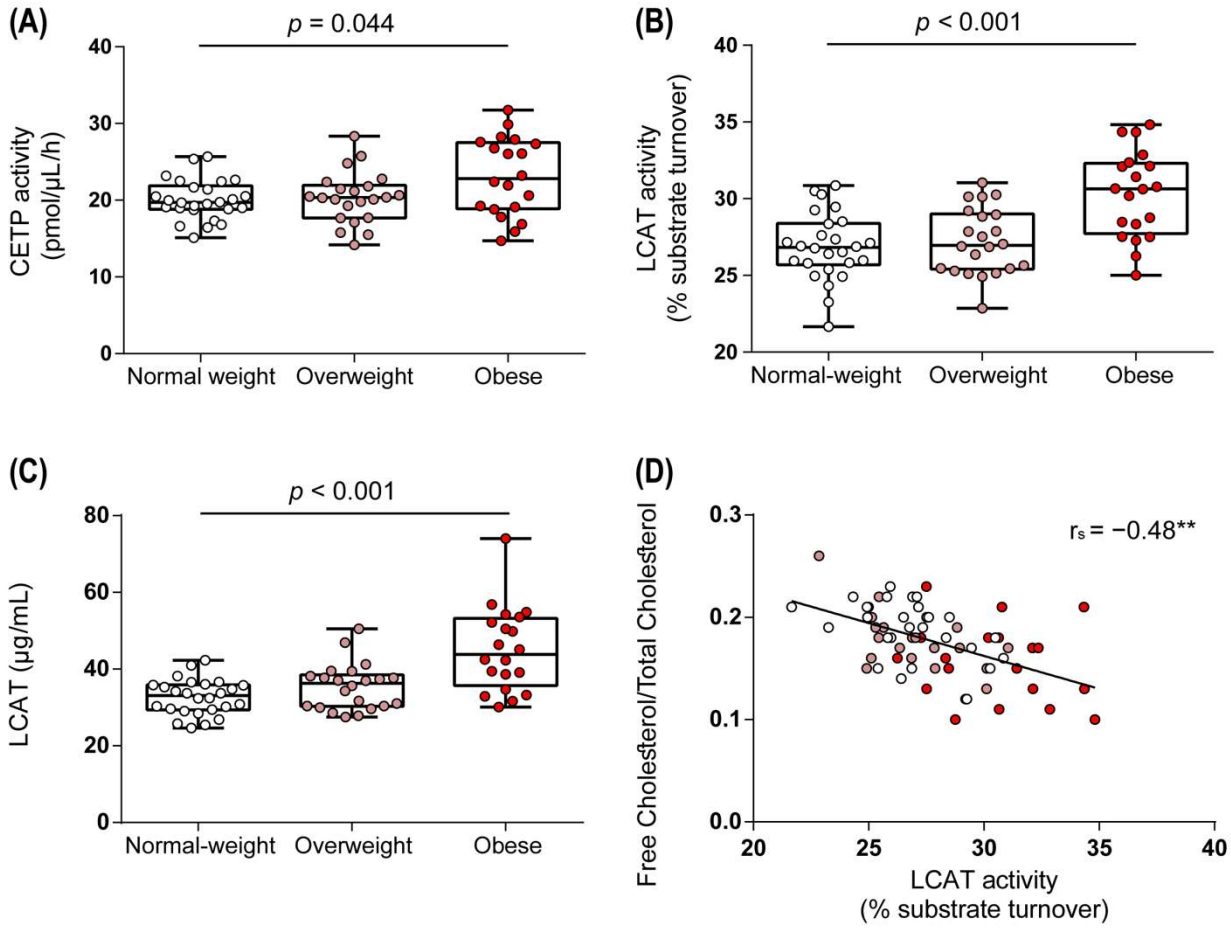

Figure 4. Obese women show increased serum activities of cholesteryl ester transfer protein (CETP) and lecithin–cholesterol acyltransferase (LCAT). Activities of CETP (**A**), LCAT (**B**), and LCAT protein levels (**C**) in serum samples. (**D**) shows the correlation of the ratio between free cholesterol and total cholesterol with LCAT activity. Differences between the two groups were analyzed by Wilcoxon rank sum test. Individual data are presented on top of boxplots, displaying median and interquartile range, as well as minimum and maximum values. Correlation was determined using Spearman's correlation coefficient rho. LCAT, lecithin–cholesterol acyltransferase; CETP, cholesteryl ester transfer protein. (** $p < 0.01$).

3.3. Functional Metrics of HDL in Overweight and Obese Women

HDL exhibits potent anti-oxidative properties [41] contributing to anti-oxidative capacity of serum. We observed a notable reduction in the ability of serum of obese women to inhibit free radical-induced oxidation of the fluorescent dye dihydrorhodamine ($p = 0.005$) (Figure 5A). This was paralleled by a marked increase in the total amount of peroxides (TOC) in serum of obese women (Figure 5B). The anti-oxidative capacity of serum correlated negatively with TOC ($r_S = -0.58$, $p < 0.001$). We observed no significant change in other metrics of HDL function, such as arylesterase activity of HDL-associated paraoxonase and HDL cholesterol efflux capacity in overweight or obese women (Figure 5C,D).

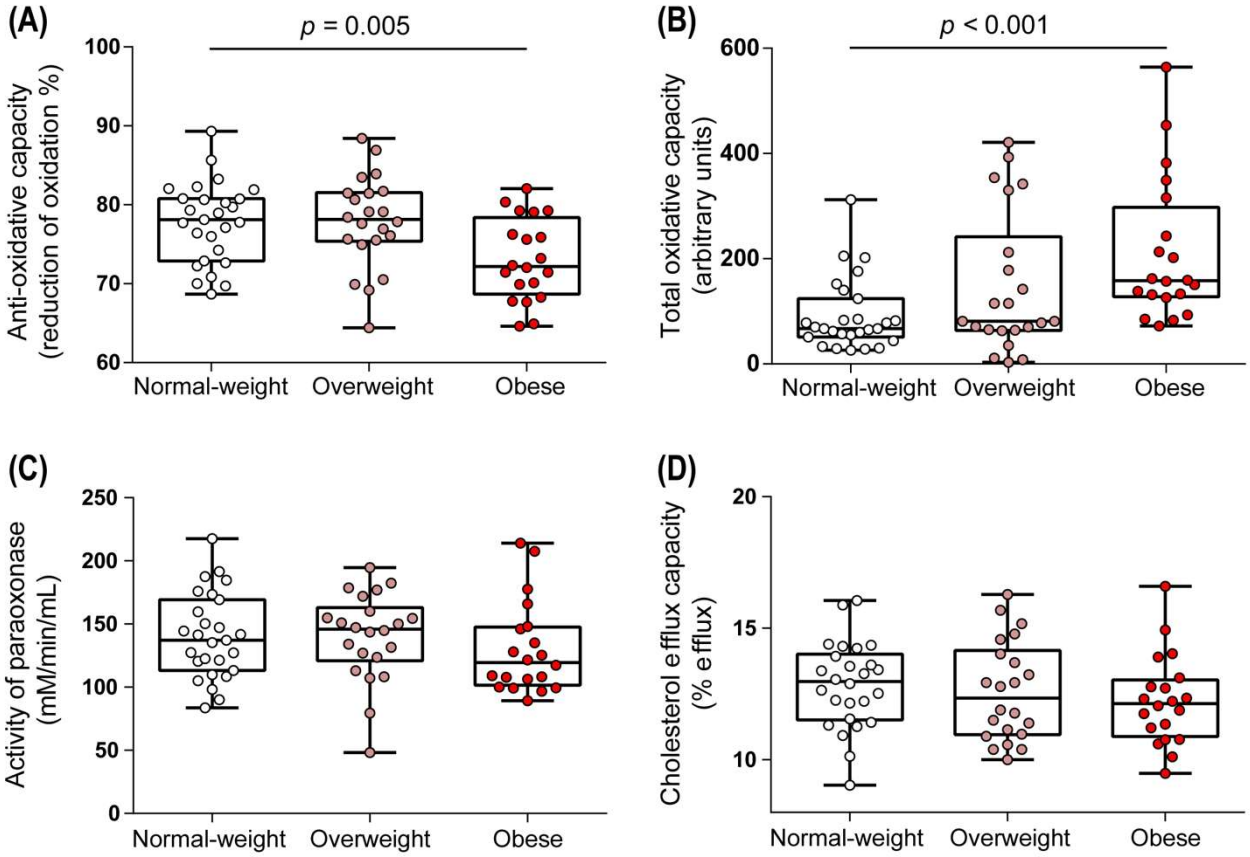

Figure 5. Metrics of HDL function of normal weight, overweight, and obese women. Anti-oxidative capacity of serum (**A**) and the number of total peroxides (total oxidative capacity) (**B**) were determined. ApoB-depleted sera of women were used to assess arylesterase activity of HDL-associated paraoxonase (**C**) and the ability to promote cholesterol efflux (**D**). Differences between the two groups were analyzed by Wilcoxon rank sum test. Individual data are presented on top of boxplots displaying median and interquartile range as well as minimum and maximum values.

3.4. Correlation of Lipoprotein Parameters with Obesity-Related Factors

We observed multiple robust and complex associations of obesity-related factors with metrics of HDL structure and metabolism, depicted in a heat-map (Figure 6). Of particular interest, we observed that the HDL2 subclass inversely correlated with several liver enzymes, including alkaline phosphatase, gamma-glutamyl transpeptidase, cholinesterase, and serum alanine transaminase. Interestingly, LCAT activity showed a positive correlation with cholinesterase, as well as a positive correlation with the soluble leptin receptor. Further correlation analysis revealed that LCAT activity correlated positively with glycated hemoglobin A1c (HbA1c; $r_S = 0.35$, $p = 0.004$), HDL3 ($r_S = 0.57$, $p < 0.001$), and small HDL3 ($r_S = 0.44$, $p < 0.001$), and negatively with HDL2 subclass ($r_S = -0.55$, $p < 0.001$). Somewhat unexpected, LCAT protein levels correlated with C-reactive protein levels ($r_S = 0.37$, $p = 0.01$) and total oxidative capacity ($r_S = 0.41$, $p < 0.001$), suggesting a compensatory mechanism. CETP showed a significant correlation with hip circumference ($r_S = 0.25$, $p = 0.44$) and with D_{INCL} ($r_S = 0.28$, $p = 0.25$). Further, CETP correlated significantly with VLDL ($r_S = 0.35$, $p = 0.004$) and with plasma triglycerides ($r_S = 0.38$, $p = 0.002$).

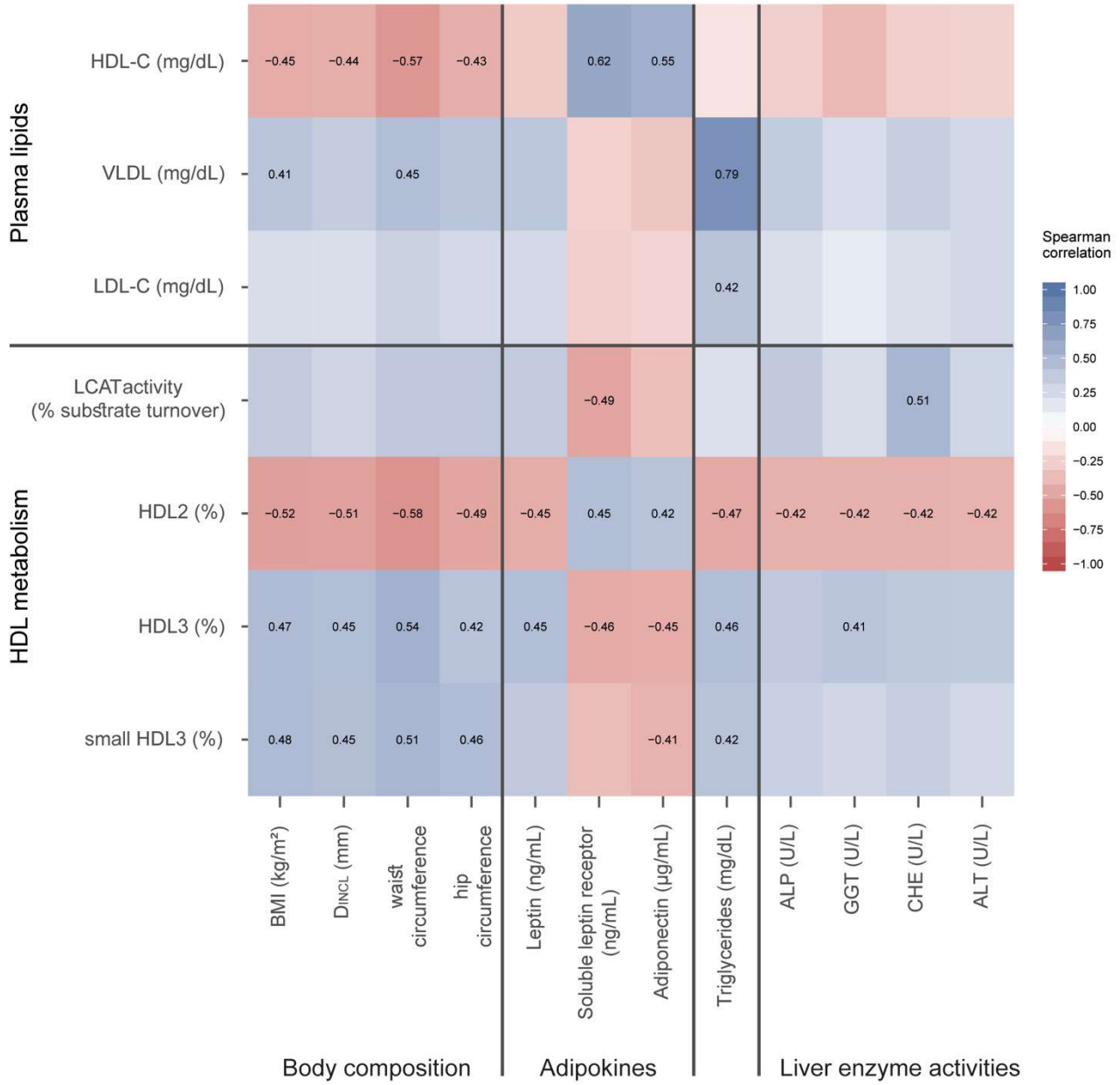

Figure 6. Correlation between plasma lipid levels and parameters of HDL metabolism with body composition, adipokines, serum triglycerides, and liver enzyme activities. Each cell of the heatmap represents pairwise Spearman correlation between the two parameters indicated in the respective row and column. Correlations that reached significance after Bonferroni correction are indicated with the corresponding Spearman rank correlation coefficient. Non-significant correlations can still be inferred from the color but are not explicitly indicated. HDL-C, high-density lipoprotein cholesterol; VLDL, very low density lipoprotein; LDL-C, low-density lipoprotein cholesterol; LCAT, lecithin cholesteryl acyltransferase; CETP, cholesteryl-ester transfer protein; BMI, body mass index; D_{INCL} thickness of subcutaneous adipose tissue at eight measured body sites; SOB-R, soluble leptin receptor; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; CHE, cholinesterase; ALT, alanine transaminase.

Adiponectin levels correlated positively with HDL-C and the large HDL2 subclass, but negatively with HDL3 and small HDL3 subclasses. Levels of the soluble leptin receptor (which plays an important role in maintaining leptin sensitivity [42]) showed a correlation pattern like adiponectin. In contrast, leptin levels showed opposite correlations.

ApoC-III is a potent inhibitor of lipoprotein lipase [43] and showed a strong correlation with serum triglycerides ($r_S = 0.59$, $p < 0.001$) and HDL-associated triglycerides ($r_S = 0.51$,

$p < 0.001$). As expected, we observed a positive correlation of SAA with C-reactive protein levels ($r_S = 0.66$, $p < 0.001$).

BMI and measurements of body fat, such as D_{INCL}, waist circumference, and hip circumference, correlated positively with HDL3 and small HDL3, whereas large HDL2 correlated negatively and the strongest correlations were observed with the measurement of waist circumference.

4. Discussion

The identification of factors that modulate HDL particle distribution and subsequent HDL function is crucial for a better understanding of the complex relationship between HDL and cardiovascular risk. In the present study, we provide evidence that obesity significantly affects HDL metabolism, subclass composition, and distribution in association with changes in liver and adipose tissue. Alterations in HDL metabolism, structure, and function in obesity could contribute to increased cardiovascular risk.

In the present study, we found that obesity was associated with marked changes in CETP and LCAT activities and altered HDL composition, such as decreased apoA-I, cholesterol, and phospholipid levels, while serum amyloid a levels were increased. We observed a marked shift towards smaller HDL subclasses in obesity linked to lower anti-oxidative capacity of serum. Of note, most of these alterations were only seen in obese women but not in overweight women. We noted a strong association of the small HDL subclasses with serum triglyceride levels and an inverse association with the larger HDL2 subclass. Our results are in good agreement with a previous study showing that levels of small HDL3 particles are significantly increased in hypertriglyceridemic individuals and directly correlated with the extent of hypertriglyceridemia [44].

Of particular importance is our observation that LCAT activity and protein concentration were significantly increased in obese individuals. LCAT is reported to be closely involved in reverse cholesterol transport, an anti-atherogenic process by which excess cholesterol is removed from cells by HDL and delivered to the liver for excretion [45]. It was shown previously that LCAT activity is increased in patients suffering from type 2 diabetes and that it is supposedly connected to reduced anti-oxidative capacity of HDL [46]. Similar to this study, we observed a significant correlation of HbA1c with LCAT activity and a significant reduction in the anti-oxidative capacity of serum in obese women. Somewhat unexpected, LCAT protein levels correlated with C-reactive protein levels and total oxidative capacity. Moreover, we observed a robust positive correlation of LCAT activity with the liver function marker cholinesterase. This is somewhat surprising considering that LCAT activity was reported to be substantially decreased in patients with liver disease [35,47]. An explanation for the positive correlation of LCAT activity with cholinesterase in our study could be the fact that we determined LCAT activity in obese but otherwise young and healthy women with only slightly increased liver markers. Thus, it appears that the concentration and activity of LCAT increases with obesity and obesity-associated low-grade inflammation, suggesting a compensatory mechanism, but appears to decrease when it comes to severe comorbidities and pathological conditions [35,47–49].

CETP, another important enzyme involved in HDL metabolism, mediates the exchange of cholesteryl esters from HDL to triglyceride-rich lipoproteins in exchange for triglycerides [50]. In our study, we observed an increase in CETP activity in the obese group, which is consistent with the fact that adipose tissue is one of the major sources of CETP expression [51,52]. Our data suggest that higher LCAT activity in obese women may lead to increased formation of cholesteryl esters in HDL and subsequent CETP-mediated transfer to triglyceride-rich lipoproteins in exchange for triglycerides. This increases the triglyceride content of HDL, which accelerates the hydrolysis of HDL particles by hepatic and lipoprotein lipases, promoting the formation of smaller HDL particles [53–55]. In good agreement with this notion, we observed a robust association of CETP activity with small HDL3 subclass and subcutaneous fat mass. Moreover, levels of the triglyceride-rich VLDL particles correlated significantly with CETP activity. It has to be noted that the esterification

by LCAT proceeds more slowly than the transfer of cholesteryl esters by CETP and thus represents a rate-limiting step [53,56,57].

In agreement with previous studies, we observed a shift towards the smaller HDL3 subclass and a reduction in the larger HDL2 subclass in obese individuals [58,59]. The HDL3 subfraction showed a strong correlation with LCAT activity, which is in agreement with the preferential conversion of lipid-poor pre-β particles to HDL3 catalyzed by LCAT. Increased CETP activity and increased cholesteryl ester transfer from HDL2 to VLDL in obese women is expected to decrease HDL2-cholesterol levels.

We found that VLDL levels were elevated in obese individuals compared to the normal weight group, whereas VLDL levels were not altered in overweight women. This is of importance, given that VLDL-cholesterol in particular accounts for the increased risk of myocardial infarction [60].

It is well known that in the state of obesity, fat accumulation and low-grade inflammation causes a dysregulated production of adipokines, leading to a reduction of adiponectin. We observed that adiponectin levels were reduced only in the obese group, but not in the overweight group. Noteworthy, adiponectin directly affects HDL cholesterol levels [61] by increasing apoA-I production and hepatic ATP-binding cassette A1 (ABCA1) expression [62] and by decreasing hepatic lipase activity, which reduces catabolism of HDL2 [63]. In good agreement, we observed robust associations of adiponectin with HDL2 levels and negative correlations with HDL3. Similar results have been reported in other studies [64,65].

We observed that leptin and soluble leptin receptor levels differed significantly in overweight and obese women when compared to normal weight women. Serum levels of the soluble leptin receptor were inversely associated with serum leptin levels but positively with HDL-C and serum adiponectin levels. In good agreement, similar results were observed in healthy Japanese subjects [66].

Interestingly, HDL composition was profoundly altered in obese women. We observed reduced levels of HDL-associated apoA-I, free cholesterol, cholesteryl ester, and phospholipids, whereas SAA content was increased in obese women, suggesting low-grade inflammation. In line with this assumption, we observed a robust correlation of SAA with C-reactive protein levels. Of note, in overweight women, only the triglyceride content of HDL was increased. We observed no significant effect of obesity on paraoxonase activity, which was previously reported in children and adolescents [67,68]. Furthermore, cholesterol efflux capacity was not affected by obesity despite reduced HDL cholesterol levels. A possible explanation for this paradoxical observation is the shift towards increased HDL3 and small HDL3 particles in obese women. HDL3 and small HDL3 particles are the most efficient mediators of cholesterol efflux [69] and might compensate for lower total HDL cholesterol levels.

In good agreement with our previous study [39], we observed a robust association of adiponectin with cholesterol efflux capacity.

Interestingly, an impaired functionality of the macrophage cholesterol exporter ABCA1 was reported in hyperinsulinaemia [70]. Furthermore, a previous study showed that insulin reduces HDL-mediated cholesterol efflux by inhibiting ATP-binding cassette transporter G1 and neutral cholesteryl ester hydrolase in macrophages [71]. In the present study, we did not observe a significant effect of obesity on HDL cholesterol efflux capacity. It has to be noted that HbA1c levels were in the normal range in overweight and obese women of our study.

The anti-oxidative capacity of serum was significantly decreased in the obese group compared to the normal weight group, consistent with a previous study, showing that oxidative stress increases with BMI [72].

We acknowledge limitations to this study. Due to the complex experiments and analyses, we had to keep the number of participants rather small. Further studies in larger cohorts are needed to confirm our findings.

Strengths of our study are that we measured multiple metrics of HDL metabolism, composition, structure, and function in relatively young and healthy individuals, excluding a major contribution of gender and age-associated diseases to structural and compositional alterations of HDL.

In conclusion, we demonstrated that obesity profoundly affects HDL metabolism and leads to changes in HDL composition and a shift towards small HDL3 particles. Importantly, we observed robust associations of LCAT activity, HDL subclass distribution, and HDL-cholesterol with soluble leptin receptor and adiponectin and liver enzyme activities. Of note, these alterations, with the exception of increased HDL triglyceride content, were not seen in overweight women. Obesity associated alterations in HDL subclasses, composition, and function may increase cardiovascular risk in obesity.

Author Contributions: Conceptualization, G.M. and J.T.S.; investigation, J.T.S., S.L., and N.M.-A.; writing—original draft preparation, J.T.S., S.L., S.J.H. and G.M.; writing—review and editing, J.T.S., S.L., S.M., A.T., H.S., A.B., W.W., H.M., S.Z., N.M.-A., S.J.H. and G.M.; visualization, J.T.S. and A.B.; supervision, G.M. and S.J.H.; funding acquisition, G.M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the Declaration of Helsinki and was approved by the ethics committee of the Medical University of Graz on 2 June 2014 (MUG-26-383ex13/14).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ABCA1	ATP-binding cassette A1
ALP	alkaline phosphatase
ALT	alanine transaminase
Apo	apolipoprotein
BMI	body mass index
CHE	cholinesterase
CETP	cholesteryl ester transfer protein
GGT	gamma-glutamyl transpeptidase
HbA1c	glycated haemoglobin
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
LCAT	lecithin–cholesterol acyltransferase
LDL	low-density lipoprotein
SAA	serum amyloid A
SAT	subcutaneous adipose tissue
SOB-R	soluble leptin receptor
TGRL	triglyceride-rich lipoproteins
VLDL	very low density lipoprotein

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antioxidants

MDPI

Article

Obesity Affects Maternal and Neonatal HDL Metabolism and Function

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Abstract: Pregravid obesity is one of the major risk factors for pregnancy complications such as gestational diabetes mellitus (GDM) and an increased risk of cardiovascular events in children of affected mothers. However, the biological mechanisms that underpin these adverse outcomes are not well understood. High-density lipoproteins (HDLs) are antiatherogenic by promoting the efflux of cholesterol from macrophages and by suppression of inflammation. Functional impairment of HDLs in obese and GDM-complicated pregnancies may have long-term effects on maternal and offspring health. In the present study, we assessed metrics of HDL function in sera of pregnant women with overweight/obesity of the DALI lifestyle trial (prepregnancy BMI $\geq$ 29 kg/m^2) and women with normal weight (prepregnancy BMI < 25 kg/m^2), as well as HDL functionalities in cord blood at delivery. We observed that pregravid obesity was associated with impaired serum antioxidative capacity and lecithin–cholesterol acyltransferase activity in both mothers and offspring, whereas maternal HDL cholesterol efflux capacity was increased. Interestingly, functionalities of maternal and fetal HDL correlated robustly. GDM did not significantly further alter the parameters of HDL function and metabolism in women with obesity, so obesity itself appears to have a major impact on HDL functionality in mothers and their offspring.

Keywords: obesity; pregnancy; gestational diabetes mellitus; cholesterol efflux capacity; paraoxonase-1; LCAT; antioxidative capacity

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1. Introduction

Obesity poses a serious health concern during pregnancy, and maternal obesity is associated with difficulties in conceiving, adverse perinatal outcomes, and represents a long-term risk to the health of the child [1,2]. Maternal obesity increases the risk of pregnancy complications such as gestational diabetes mellitus (GDM) [3] and gestational hypertension [4]. GDM is the most common pregnancy disorder affecting up to 22% of all pregnancies [5,6] and is characterized by the onset of glucose intolerance first diagnosed in the second and third trimesters of pregnancy [7]. Women with a history of GDM have an increased risk of developing type 2 diabetes mellitus, hypertension, metabolic syndrome, hyperlipidemia, and cardiovascular disease later in life [8–12]. Maternal obesity is further associated with an increased risk in their children of congenital anomalies, including congenital heart disease, together with premature death from cardiovascular events, compared with children born to women without obesity during pregnancy [13,14].

The exact pathophysiology of this increased CVD risk in the offspring of obese women remains to be determined [13].

The inverse association of high-density lipoprotein (HDL) cholesterol with cardiovascular risk is well established [15,16]; however, recent research has shown that steady-state HDL cholesterol concentrations may provide limited information regarding the potential antiatherogenic and anti-inflammatory functions of HDL. There is clear evidence that HDL composition determines its functional properties rather than the levels of circulating HDL cholesterol. The HDL-associated enzyme paraoxonase-1 (PON1), for example, protects HDL from lipid oxidation and shows anti-inflammatory activities independent of HDL cholesterol [17]. The first step of HDL to promote reverse cholesterol transport, which can be measured in a cell-based assay as cholesterol efflux capacity, has been shown to be inversely associated with an increased risk of cardiovascular events independent of HDL cholesterol levels [18]. In general, HDL particles exert anti-inflammatory, antioxidative, and vasoprotective effects through associated enzymes and proteins [17,19,20].

Obesity has significant effects on the metabolism, composition, and function of HDL [21–23]. In the present study, we investigated the effects of overweight/obesity and obesity + GDM on HDL functionality in pregnant women and their offspring, which has not been previously studied. We assessed metrics of HDL function in sera of pregnant women with overweight/obesity of the DALI lifestyle trial (prepregnancy BMI $\geq$ 29 kg/m^2) and women with normal weight (prepregnancy BMI < 25 kg/m^2). In addition, we analyzed HDL functionalities in umbilical cord blood at delivery, representing neonatal blood [24,25].

2. Materials and Methods

2.1. Study Cohorts

Pregnant women < 20 weeks gestation with a singleton pregnancy, aged $\geq$ 18 years with a prepregnancy BMI of $\geq$29 kg/m^2 were invited to participate. These women were recruited within the multicenter randomized controlled trial study "vitamin D and lifestyle intervention for GDM prevention (DALI)," which was conducted between 2012 and 2015 at 11 study sites in nine European countries (Austria, Belgium, Denmark (Odense, Copenhagen), Ireland, Italy (Pisa, Padua), Netherlands, Poland, Spain, and United Kingdom). The study was registered under trial registration number ISRCTN70595832 and was approved by all local ethics committees (Netherlands, Amsterdam, 2012/400; Belgium, Leuven, S52171; Italy, Padua, n. 200 del 27/03/2013; Italy, Pisa, nr. 3266; Denmark, Odense and Copenhagen, H-4-2013-005; Ireland, Galway, Ref 7/12; UK, Cambridge, 11/EE/0221; Spain, Barcelona, 13/006 (OBS); Poland, Poznan, Nr 1165/12; Austria, Vienna, 2022/2012).

Women were excluded if they had GDM at baseline according to the International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria (fasting venous plasma glucose $\geq$ 5.1 mmol/L and/or 1 h glucose $\geq$ 10 mmol/L and/or 2 h glucose $\geq$ 8.5 mmol/L) [26] or if they had a history of diabetes, chronic diseases, or psychiatric disorders. Other exclusion criteria included inability to walk 100 m safely, requirement for a complex diet, and inability to communicate with the lifestyle coach due to a lack of language skills.

Following written informed consent, women were randomly assigned to three (pilot study) or four (lifestyle trial) groups receiving counseling for healthy eating (HE), physical activity (PA), healthy eating + physical activity (HE + PA), and—in the lifestyle trial—a control group receiving usual care (UC). For this analysis, data from 186 participants were combined into one cohort and analyzed. At baseline, women were screened, and fasting blood samples were collected before and during an oral glucose tolerance test. This was repeated at 24–28 weeks and at 35–37 weeks. From women with GDM at 24–28 weeks, only fasting blood samples were taken at 35–37 weeks, as previously described [27,28]. Whole blood samples were separated into serum and stored at −20 °C or −80 °C to be further handled in the ISO-certified central trial laboratory in Graz, Austria. GDM was defined according to IADPSG/WHO2013 criteria (oral glucose tolerance test, venous

plasma glucose: fasting $\geq$ 5.1 mmol/L, 1 h $\geq$ 10 mmol/L and/or 2 h $\geq$ 8.5 mmol/L) at <20 weeks, at 24–28 weeks, and 35–37 weeks gestation.

Normal-weight women (prepregnancy BMI < 25 kg/m^2) were recruited at the Medical University Graz and gave informed written consent at the time of delivery (26–333 ex 13/14). Included women had normal blood pressure levels and absence of medical complications during pregnancy. Included women were matched to the DALI cohort in maternal age and offspring sex. Venous blood from pregnant women was collected before delivery, while corresponding umbilical cord blood was taken longest 10 min after delivery. Serum samples were stored at −80 °C.

2.2. Biochemical Analyses

Plasma glucose was measured using the hexokinase method (DiaSys Diagnostic Systems, Holzheim, Germany) with a lower limit of sensitivity of 0.1 mmol/L.

Insulin was quantified using a sandwich immunoassay (ADVIA Centaur, Siemens Healthcare Diagnostics Inc., Vienna, Austria) with an analytical sensitivity of 0.5 mU/L, intra-assay CVs of 3.3–4.6%, and interassay CVs of 2.6–5.9%. All assays were carried out following the manufacturer's instructions. HOMA-IR was calculated as [glucose∗insulin]/22.5 mmol/L∗µU/mL.

Total cholesterol and triglycerides were measured using colorimetric enzymatic assays using reagents from DiaSys Diagnostic Systems (Holzheim, Germany) and were calibrated using secondary standards from Roche Diagnostics (Mannheim, Germany). HDL-C was measured with a homogenous assay from DiaSys Diagnostics, and LDL cholesterol (LDL-C) was calculated according to the Friedewald formula (LDL-C = TC − HDL-C − TG/5). Nonesterified fatty acids (FFAs) were quantified using an enzymatic reagent and standards from Wako Chemicals (Neuss, Germany). All lipid analyses were performed on an Olympus AU640 automatic analyzer (Beckman Coulter, Brea, CA, USA).

2.3. ApoB-Depletion of Serum

To analyze HDL composition and function, we used serum HDL (apoB-depleted serum). A 20% stock solution of polyethylene glycol (P1458, Sigma-Aldrich, Darmstadt, Germany) was prepared in 200 mmol/L glycine and 40 µL added to 100 µL serum. The mixture was mixed gently and incubated for 20 min at room temperature. The samples were centrifuged at 10,100× *g* for 30 min at 4 °C, and the supernatant was collected. Samples were stored at −70 °C until usage.

2.4. Cholesterol Efflux Capacity

Cholesterol efflux capacity of apoB-depleted serum was measured as described elsewhere [22]. Briefly, J774.2 macrophages (Sigma-Aldrich, Darmstadt, Germany) were cultured in DMEM media (containing 10% FBS, 1% PS). The cells were seeded on 48-well plates (300,000 cells/well), maintained for 24 h, and loaded with 0.5 µCi/mL radiolabelled [^{3}H]-cholesterol (ART0255, Hartmann Analytics, Braunschweig, Germany) in medium (containing 2% FBS, 1% PS, and 0.3 mM 8-(4-chlorophenylthio)-cyclic AMP (c3912, Sigma-Aldrich, Darmstadt, Germany)) overnight. Cells were then rinsed and equilibrated in serum-free media supplemented with 0.2% BSA (A7030, Sigma-Aldrich, Darmstadt, Germany) for 2 h. To determine [^{3}H]-cholesterol efflux, cells were incubated with 2.8% apoB-depleted serum for 3 h at 37 °C. Cholesterol efflux capacity was calculated as radioactivity in cell culture supernatant relative to total radioactivity in supernatant and cells.

2.5. Lecithin–Cholesteryl Acyltransferase (LCAT) Activity

LCAT activity of serum was assessed using a commercially available kit (MAK107, Merck, Darmstadt, Germany) according to the manufacturer's instructions. In brief, serum samples were incubated with the LCAT substrate at 37 °C for 4 h. The substrate emits fluorescence at 470 nm. Hydrolysis of the substrate by LCAT releases a monomer that emits fluorescence at 390 nm. Activity of LCAT is measured over time and expressed as the change in emission intensity at 470/390 nm.

2.6. Arylesterase (AE)—Activity of Paraoxonase1 (PON1)

The Ca^{2+}-dependent AE activity of PON1 was assessed using a photometric assay and the substrate phenylacetate (108723, Sigma-Aldrich, Darmstadt, Germany), as described elsewhere [29]. In brief, apoB-depleted serum was diluted (1:10), and 1.5 µL was added to 200 µL reaction buffer (100 mM Tris, 2 mM $CaCl_2$, 1 mM phenylacetate) in absorbance at 270 nm. Activities were calculated from the slopes of the kinetic diagrams of three independent experiments measured in duplicate.

2.7. Antioxidative Capacity of Apob-Depleted Serum

As described [29], the antioxidative capacity of apoB-depleted serum was determined with a fluorometric assay by using the fluorescent dye dihydrorhodamine (D1054, Sigma-Aldrich, Darmstadt, Germany). The dye was dissolved in DMSO (50 mM stock), diluted in HEPES (20 mM HEPES, 150 mM $NaCl_2$, pH 7.4) containing 1 mM 2,2′-azobis-2-methyl-propanimidamide-dihydrochloride (440914, Sigma-Aldrich, Darmstadt, Germany) to yield a 10 µM working reagent. Into a 384-well plate, 10 µL apoB-depleted serum dilution (1:10) was added, and 90 µL of working reagent was added. The increase in fluorescence as a result of dihydrorhodamine oxidation was monitored at 538 nm for 90 min. The increase in dihydrorhodamine fluorescence per minute in absence of apoB-depleted serum was set at 100%, and the individual apoB-depleted serum samples were expressed as a percentage of inhibition of dihydrorhodamine oxidation.

2.8. Statistical Analyses

The characteristics of study participants are presented as mean and standard deviation (SD), median and interquartile range (IQR), or as count and proportion.

Maternal and neonatal characteristics, as well as HDL-related parameters, were compared between normal-weight controls, obesity group, and women with GDM using ANOVA or Kruskal–Wallis test, depending on the distribution of the variable.

Spearman correlation coefficients between maternal and cord blood levels of HDL parameters were calculated.

All analyses were performed in IBM SPSS (Version 27.0. IBM Corp, Armonk, NY, USA). A p-value of <0.05 was used to determine statistical significance.

3. Results

3.1. Characteristics of the Study Population

A detailed description of the study participants is provided in Table 1. In this study, 186 women of the DALI cohort were included, of whom 54 (29%) developed GDM. Normal-weight controls (n = 34) provided maternal and cord serum. Maternal age and blood pressure did not differ between the groups.

Neonates of overweight/obese women had higher birthweights when compared with the offspring of normal-weight mothers, while birthweight was further elevated in the GDM group. Besides that, no significant differences in maternal or neonatal characteristics between women of the DALI study with or without GDM were observed.

While the analyzed groups were matched in maternal age and offspring sex, most of the included normal-weight women underwent delivery by cesarean section, while this was the case for 32% of women in the overweight/obese group. However, no significant

relationship was observed when comparing the mode of delivery and antioxidant capacity or HDL-related parameters in mothers and neonates (Supplementary Figure S1).

Table 1. Clinical characteristics of study cohort. Data are presented as mean and standard deviation, median and interquartile range, or as count and proportion. Differences between normal-weight and overweight/obese subjects, as well as comparisons between obese and GDM groups, were calculated: BMI, body mass index; GDM, gestational diabetes mellitus.

Maternal Characteristics	Normal Weight N = 34	Overweight/Obese N = 132	P Normal Weight vs. Overweight/Obese	GDM N = 54	P Overweight/Obese vs. GDM
Maternal age, year	29.9 ± 5.1	31.9 ± 5.2	0.13	31.1 ± 5.8	0.66
BMI, kg/m^2	21.5 ± 1.7	34.2 ± 4.6	**<0.001**	34.1 ± 4.4	0.99
Systolic blood pressure, mm Hg	117 ± 11	120 ± 10	0.491	118 ± 10	0.472
Diastolic blood pressure, mm Hg	74 ± 9	78 ± 9	0.45	75 ± 7	0.104
HOMA-IR	-	3.3 (2.4–4.4)	-	5.1 (3.0–6.6)	**<0.001**
Neonatal characteristics					
Birth weight, g	3177 ± 498	3495 ± 518	**0.03**	3768 ± 483	**0.003**
Gestational age at birth, week	38.6 ± 1.7	39.6 ± 1.4	**<0.001**	39.8 ± 1.2	0.71
Placenta weight, g	591 ± 95	633 ± 138	0.24	686 ± 133	0.06
Female sex,	19 (56%)	61 (46%)	0.31	28 (51%)	0.64

3.2. Serum Plasma Lipids of Normal-Weight, Overweight/Obese, and GDM Mothers and Their Neonates

We first examined lipid levels in our study cohort. We observed an obesity-related reduction in total cholesterol in maternal serum (Figure 1A), while no difference was observed in the cord blood (Figure 1B). Compared with normal-weight pregnant women, serum HDL-C levels were reduced in overweight/obese women of the DALI cohort, whereas HDL-C was even lower in the obese and GDM group. A trend for lower HDL-C levels in the neonates of overweight/obese mothers was observed ($p = 0.072$). Triglycerides were increased in the obese + GDM maternal group compared with the normal-weight pregnant women. Interestingly, we observed that obesity also was significantly associated with fetal triglyceride concentrations, which were elevated in both the obese and obese and GDM groups.

3.3. Obesity- and GDM-Associated Changes in Parameters of HDL Metabolism and Function

We next assessed whether overweight/obesity in the absence or presence of GDM in pregnant women of the DALI cohort was associated with maternal as well as neonatal functional metrics of HDL. The ability of HDL to remove cholesterol from macrophages (cholesterol efflux capacity) was increased in both maternal obese and obese GDM groups when compared with normal-weight women (Figure 2A). Interestingly, HDL cholesterol efflux capacity was not significantly altered in the neonates of obese mothers with or without GDM; in fact, we found a trend toward lower capacity compared with the normal-weight group ($p = 0.053$) (Figure 2B). Of particular interest, maternal serum triglycerides correlated with maternal cholesterol efflux capacity ($r_S = 0.240$, $p < 0.01$).

Lecithin–cholesterol acyltransferase (LCAT) is an enzyme important for HDL particle maturation [30,31]. We observed an obesity-related reduction in LCAT activity in maternal serum as well as a nonsignificant trend in cord blood ($p = 0.077$) (Figure 2C,D).

To gain insight into the changes in serum antioxidant and anti-inflammatory activities associated with obesity/GDM, we examined the activity of the HDL-associated enzyme PON1 and the total antioxidative capacity of serum. Serum antioxidant capacity was significantly reduced in overweight/obese mothers and tended to be lower in obese and GDM mothers, which was also observed in their offspring (Figure 2E,F). Interestingly, neither obesity nor GDM was associated with altered PON1 activity (Figure 2G,H).

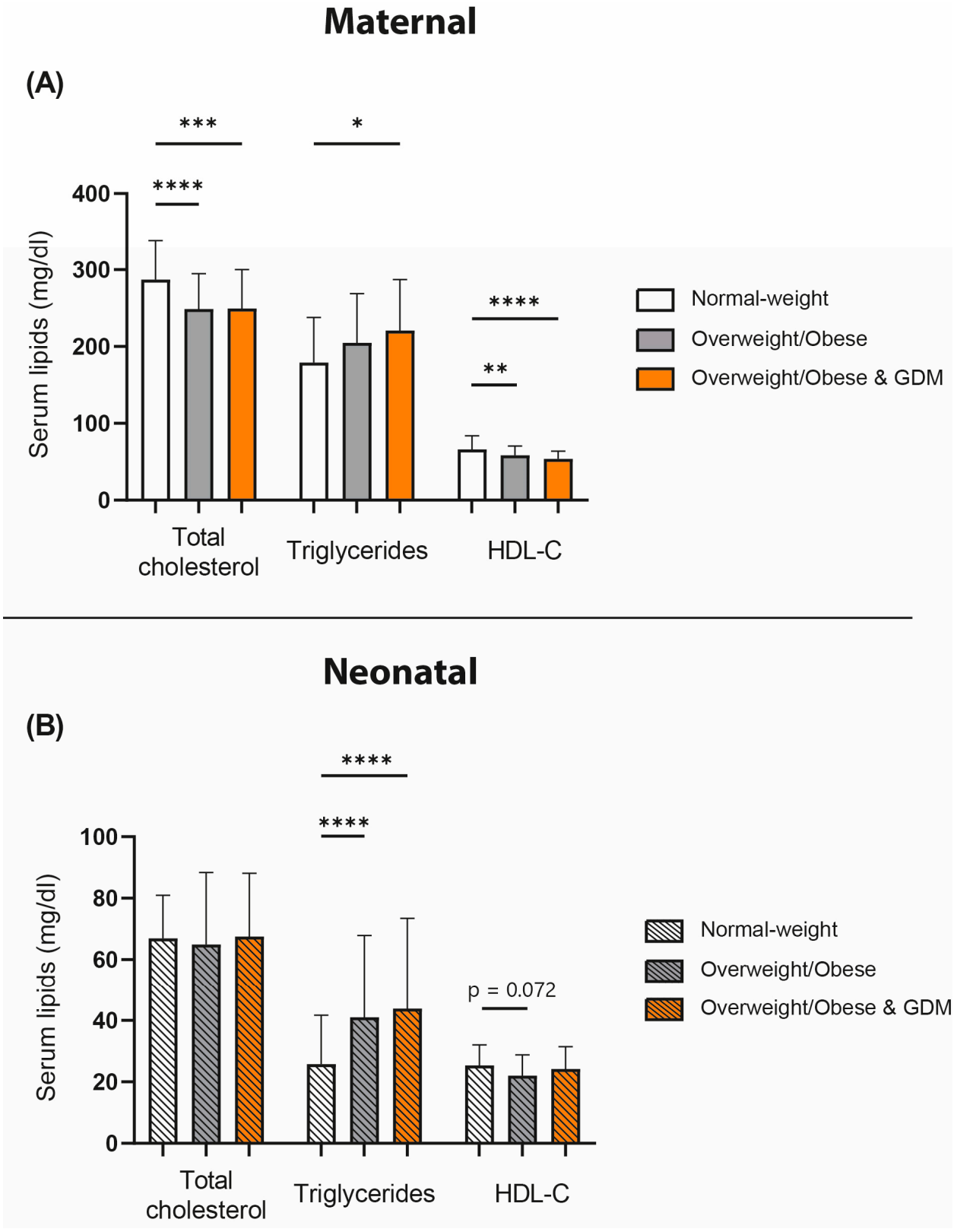

Figure 1. Differences between serum lipid levels in normal-weight and overweight/obese pregnant women and in women diagnosed with GDM and its impact on their offspring: (**A**) shows serum total cholesterol levels, triglycerides, and HDL-C of mothers and (**B**) of neonates. Data are presented as mean and standard deviation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

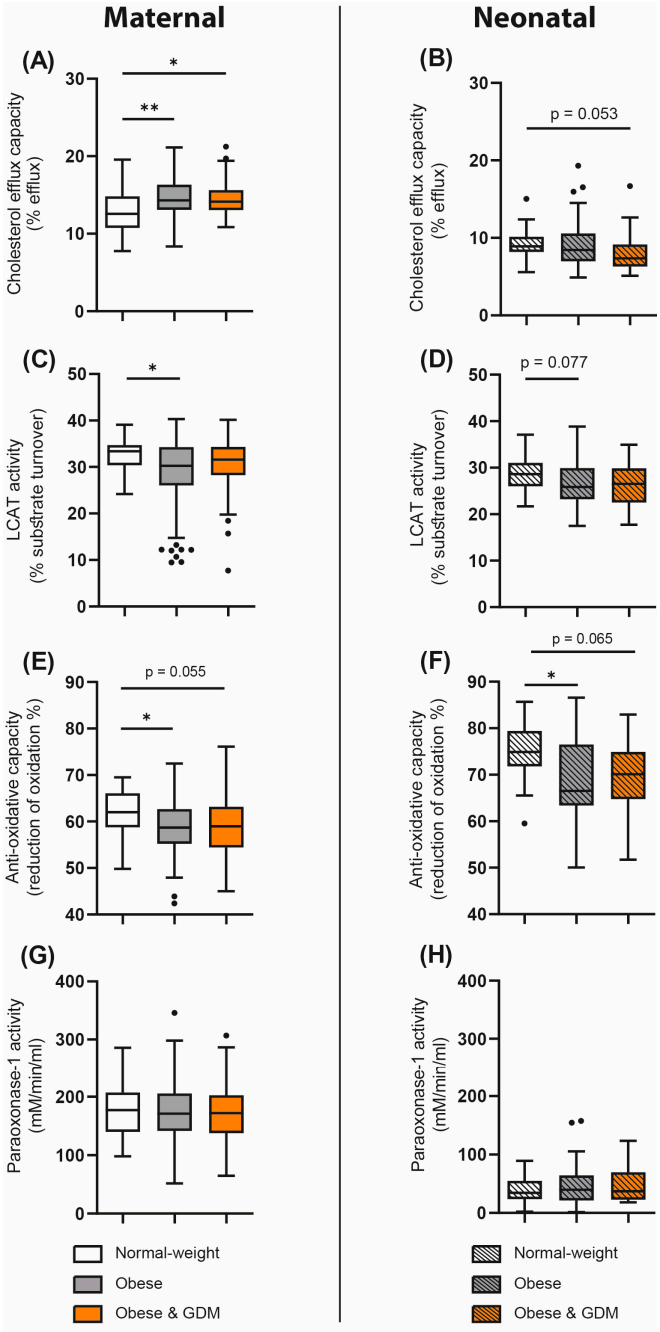

Figure 2. Differences in serum functionalities in normal-weight and overweight/obese pregnant women and in women with GDM and their offspring. Cholesterol efflux capacity was assessed with a cell-based assay (**A**,**B**). The activity of LCAT in mothers (**C**) and offspring (**D**) was evaluated. Serum antioxidative capacity was assessed in mothers (**E**) as well as in paired umbilical cord blood (**F**). Activity of HDL-associated anti-inflammatory enzyme paraoxonase-1 in mothers (**G**) and neonates (**H**) was evaluated. Data are presented as Tukey boxplots showing the median and interquartile ranges as well as minimum and maximum values and outliers. Differences were analyzed by ANOVA or Kruskal–Wallis test based on the distribution of the variable. * $p < 0.05$, ** $p < 0.01$.

3.4. Correlations of Maternal and Neonatal HDL-Related Parameters

We were next interested to see whether serum functional parameters between mothers and offspring of the DALI cohort are linked. For that purpose, we calculated Spearman correlation coefficients for correlations between maternal samples and the corresponding umbilical cord samples. The maternal and neonatal HDL functional parameters correlated

significantly. Maternal and cord blood cholesterol efflux capacity correlated robustly ($r_S = 0.44$, $p < 0.01$) (Figure 3B), even though we observed divergent results when comparing maternal and fetal cholesterol efflux capacities. PON1 activity and antioxidative capacity of serum also correlated significantly between mothers and their offspring (Figure 3C,D), whereas maternal HDL-C levels showed a weaker correlation with cord blood levels ($r_S = 0.18$, $p < 0.05$) (Figure 3A).

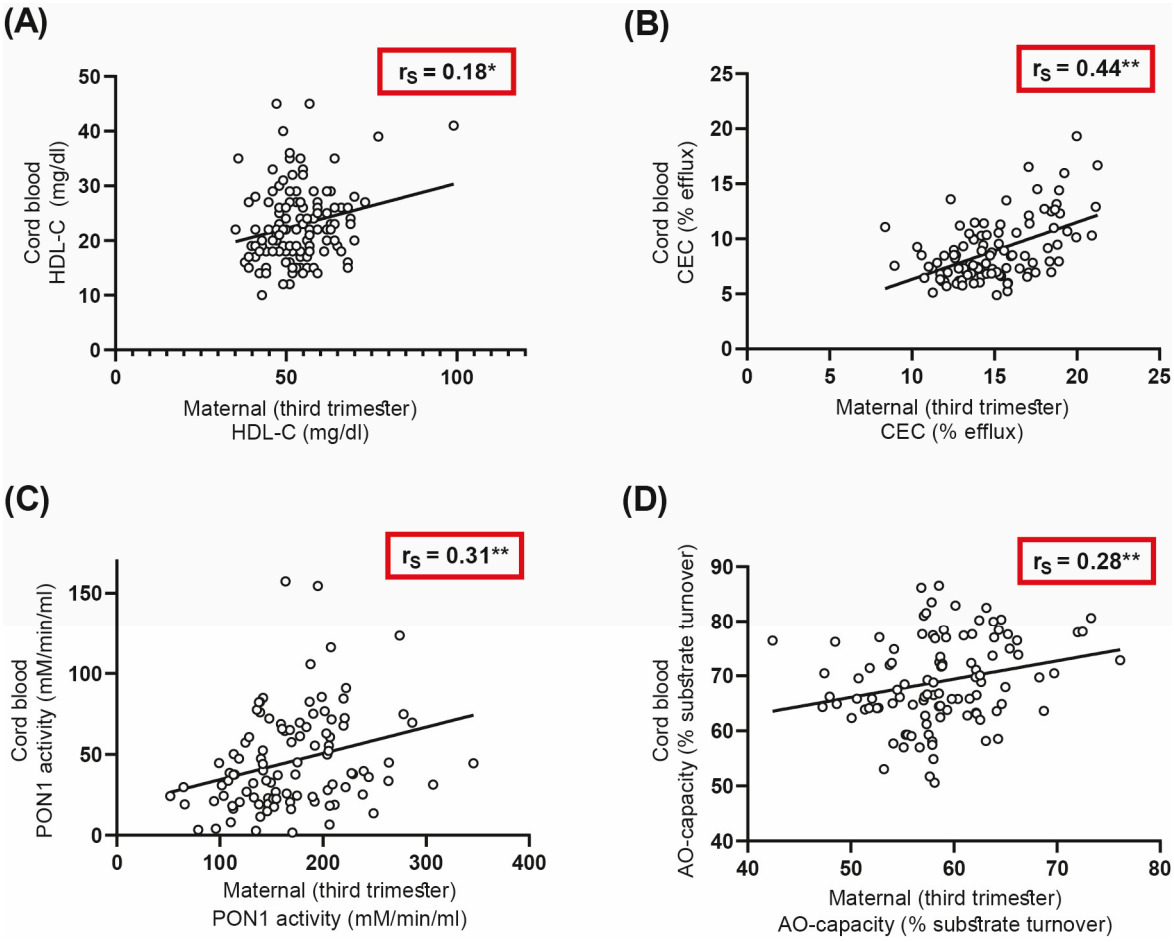

Figure 3. Spearman correlation analyses between maternal and cord blood HDL-related parameters and serum functionalities (**A**) HDL-C, (**B**) CEC (cholesterol efflux capacity), (**C**) paraoxonase-1 (PON1) activity, and (**D**) serum antioxidative capacity. * $p < 0.05$, ** $p < 0.01$.

4. Discussion

In this study, we investigated whether overweight/obesity with or without GDM was associated with changes in functional parameters of HDL in pregnant women as well as in their offspring. We observed that serum antioxidant capacity was decreased in obese mothers and neonates when compared with normal-weight controls. GDM did not further affect the antioxidative capacity of serum in mothers and their offspring. In addition, LCAT activity was decreased in sera of overweight/obese and overweight/obese and GDM mothers and offspring, suggesting significant alterations in HDL maturation. Remarkably, we observed that the ability of HDL cholesterol efflux capacity was increased in overweight/obese mothers with or without GDM. Differences in the mode of delivery or weight gain during pregnancy were not associated with changes in HDL-related parameters of the antioxidative capacity of serum.

An important finding of the present study was that overweight/obese women with or without GDM showed comparable changes in functional metrics of HDL. This suggests that overweight/obesity itself, largely independent of GDM, affects parameters of HDL

function and metabolism in both mothers and offspring. Another interesting observation of this study was that maternal HDL functions such as cholesterol efflux capacity, PON1 activity, and antioxidant capacity in serum correlate robustly with parameters in cord blood, whereas HDL-C showed a weaker association.

Obesity is generally associated with a decrease in HDL-C levels, and obese individuals depict lower HDL2 and higher HDL3 particle concentrations [22,23,32–34]. This was also observed in previous studies in obese pregnant women, who tended to have higher total cholesterol and triglyceride levels but lower serum HDL-C levels [35,36]. Similarly, we found that HDL-C levels in overweight/obese pregnant women with or without GDM were decreased. HDL-C levels in neonates of overweight/obese mothers showed only a nonsignificant trend to be lower.

We observed no significant differences in maternal serum triglyceride levels between the normal-weight and overweight/obese groups, while cholesterol levels were lower. This observation seems surprising but is in good agreement with a previous study [35]. In general, obese pregnant women have a more atherogenic lipid profile in early pregnancy compared with normal-weight women [35]. Maternal serum total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides increase in all women from the first to the late second trimester. However, in overweight/obese women, the increase in maternal serum cholesterol is significantly attenuated between the first and late second trimester [35]. This may lead to late-second-trimester cholesterol levels often being higher in normal-weight women than in overweight or obese women [35]. Regarding total cholesterol in the cord blood, we detected no significant differences between the samples from the offspring of normal-weight and overweight/obese groups. Consistent data were shown in a previous study [37]. Also in good agreement with a previous study [38], we observed that triglyceride levels were increased in the offspring of overweight/obese mothers.

The association of overweight/obesity with changes in the HDL-mediated cholesterol efflux capacity of pregnant women has not been studied previously. One study reported that the cholesterol efflux capacity of HDL is increased in pregnant women when compared with nonpregnant controls [39].

Unexpectedly, in the present study, we observed an overweight/obesity-related increase in HDL cholesterol efflux capacity. This was also observed in overweight/obese women with GDM, although overweight/obesity was associated with lower serum HDL-C levels. A few studies have investigated the association of BMI with the cholesterol efflux capacity of HDL [40,41]. Levels of small preβ-1 HDL particles, which depict the highest cholesterol efflux capacity [42], are increased in obese subjects when compared with lean subjects. This has been explained by increased hepatic lipase and phospholipid transfer protein activities [23,43]. An increase in preβ-1 HDL particles was also observed in individuals with elevated serum triglyceride levels but low HDL-C levels [44,45], and HDL cholesterol efflux capacity was positively associated with serum triglycerides [46,47]. Similarly, we observed that maternal serum triglyceride concentrations correlated with cholesterol efflux capacity.

LCAT is a key enzyme involved in HDL remodeling. By esterifying free cholesterol on the HDL surface, LCAT increases HDL particle size and leads to HDL maturation [48]. Previous studies reported that LCAT activity is higher in pregnant women [49].

We observed a decrease in LCAT activity in obese pregnant women and a trend in their offspring when compared with normal-weight controls. Thus, maternal obesity in pregnancy appears to affect both maternal and fetal lipid metabolism [50].

In addition to parameters of HDL metabolism, we also examined serum antioxidant capacity. Of particular interest, we observed a marked obesity-related reduction in serum antioxidant capacity in mothers and in the cord blood. This observation in the mothers is consistent with a previous study, showing that BMI is markedly associated with oxidative stress [51]. Obesity has been shown to affect PON1 activity [52], but we neither detected obesity nor GDM-related changes in PON1 activity in pregnant mothers or their offspring.

However, similar to previous studies, we observed that cord blood PON1 activity was markedly lower compared with maternal PON1 enzyme activity [53–55].

An intriguing observation of this study was that maternal serum and HDL functionalities such as cholesterol efflux capacity, serum antioxidant capacity but also PON1 activity correlated strongly with cord blood functional parameters, whereas HDL-C levels correlated only weakly. This observation is surprising, given that during pregnancy, the fetus is protected from direct contact with external factors in maternal circulation. Maternal and fetal HDL metabolism are not directly linked [56], and it is assumed that lipoproteins do not cross the placenta efficiently to enter fetal circulation [57,58]. Maternal lipoprotein cholesterol is thought to be initially taken up by trophoblasts, transported by a number of sterol transport proteins, and secreted from the basal site. This cholesterol is then taken up by the endothelium and effluxed to acceptors within the fetal circulation [59]. However, in obese mothers, an infiltration of proinflammatory macrophages in the placenta is observed, increasing proinflammatory cytokines and oxidative stress [60]. Therefore, we suggest that this proinflammatory environment affects maternal as well as fetal blood parameters. In addition, genetic factors [61] shared between mother and fetus may also impact parameters of HDL function and serum antioxidative capacity on both sides.

Some limitations must be acknowledged. The samples of the normal-weight control group were collected at the Medical University of Graz, whereas the samples of the DALI cohort were collected all over Europe. Therefore, we cannot exclude that the different lifestyles in other countries may have an influence on our results. In addition, the normal-weight control subjects differed from the DALI cohort subjects by mode and gestational age at delivery, as most of the infants born to the women included in this study were delivered by cesarean section. Since participants in the DALI study were well controlled during pregnancy, we cannot confidently state whether this cohort is representative of the general population. Because this study was originally designed as an intervention study to prevent GDM in overweight/obese pregnant women, normal-weight GDM controls were not included.

The strengths of this study are that we examined several HDL functional parameters in maternal serum and in paired umbilical cord blood serum of the offspring. In addition, we included a normal-weight (BMI < 25 kg/m^2) control cohort to determine the relationship of obesity with changes in HDL functional parameters. Further, it should be noted that although the samples were collected at different centers, the serum lipid levels were measured at the same core laboratory. To our knowledge, this is the first large study to examine the effects of obesity and GDM on HDL-related parameters in mothers and offspring.

5. Conclusions

We observed that pregravid obesity was associated with impaired serum antioxidative capacity and lecithin–cholesterol acyltransferase activity in both mothers and offspring, whereas maternal HDL cholesterol efflux capacity was increased. GDM did not significantly further alter the parameters of HDL function and metabolism in women with obesity, so obesity itself appears to have a major impact on HDL functionality in mothers and their offspring. Interestingly, functionalities of maternal and fetal HDL correlated robustly. Follow-up studies are needed to clarify whether and when this correlation disappears after childbirth. Understanding the link between maternal and cord blood parameters could provide novel mechanistic links for therapeutic options.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox12010199/s1, Figure S1: Effect of delivery mode on parameters of HDL function and serum anti-oxidative capacity in **(A)** mothers and **(B)** neonates.

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Informed Consent Statement: Informed written consent was obtained from all subjects involved in the study at baseline.

Data Availability Statement: The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, on request to the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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Gestational Hypertension and High-Density Lipoprotein Function: An Explorative Study in Overweight/Obese Women of the DALI Cohort

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Abstract: Gestational hypertension (GHTN) is associated with an increased cardiovascular risk for mothers and their offspring later in life. High-density lipoproteins (HDL) are anti-atherogenic by promoting efflux of cholesterol from macrophages and suppression of endothelial cell activation. Functional impairment of HDL in GHTN-complicated pregnancies may affect long-term health of both mothers and offspring. We studied functional parameters of maternal and neonatal HDL in 192 obese women (pre-pregnancy BMI $\geq$ 29), who were at high risk for GHTN. Maternal blood samples were collected longitudinally at <20 weeks, at 24–28 and 35–37 weeks of gestation. Venous cord blood was collected immediately after birth. Maternal and cord blood were used to determine functional parameters of HDL, such as HDL cholesterol efflux capacity, activity of the vaso-protective HDL-associated enzyme paraoxonase-1, and levels of the HDL-associated anti-inflammatory apolipoprotein (apo)M. In addition, we determined serum anti-oxidative capacity. Thirteen percent of the women were diagnosed with GHTN. While we found no changes in measures of HDL function in mothers with GHTN, we observed impaired HDL cholesterol efflux capacity and paraoxonase-1 activity in cord blood, while serum antioxidant capacity was increased. Of particular interest, increased maternal paraoxonase-1 activity and apoM levels in early pregnancy were associated with the risk of developing GHTN. GHTN significantly impairs HDL cholesterol efflux capacity as well as HDL PON1 activity in cord blood and could affect vascular health in offspring. Maternal paraoxonase-1 activity and apoM levels in early pregnancy associate with the risk of developing GHTN.

Keywords: gestational hypertension; HDL; cholesterol efflux capacity; paraoxonase-1; apolipoprotein M; pregnancy; obesity

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1. Introduction

During pregnancy, various metabolic, vascular and physiological changes occur to ensure a continuous supply of essential nutrients for fetal growth and development [1]. The physiological stress of pregnancy can also lead to the development of adverse maternal pregnancy outcomes, such as gestational hypertension (GHTN). GHTN is defined as systolic blood pressure > 140 mmHg and diastolic blood pressure > 90 mmHg. According to the WHO, this pregnancy disorder is one of the main causes of maternal and fetal

morbidity, and one of the most common causes of maternal death in Europe [2,3]. Obesity is one of the main risk factors for developing GHTN and the more severe condition of preeclampsia [4]. This is of particular importance given the increasing prevalence of obesity, which is a serious public health problem associated with the lifestyle prevalent in the developed world [5]. Hypertensive pregnancy disorders have public health implications that extend far beyond affected pregnancies, as GHTN is associated with an increased risk of cardiovascular complications later in life for mothers and their offspring [6–8].

Serum levels of high-density lipoprotein (HDL)-cholesterol (HDL-C) are inversely associated with the risk of developing cardiovascular disease. HDL particles promote reverse cholesterol transport, i.e., the uptake of excessive cholesterol from peripheral cells and its delivery to the liver for excretion. This cholesterol efflux capacity of HDL has been shown to be inversely associated with the incidence of cardiovascular events, independent of HDL-C levels [9]. Lipoproteins not only serve as lipid transporters but are also known to exert important anti-inflammatory and immunomodulatory functions [10]. HDL exhibits vascular-protective activities and anti-oxidative and anti-inflammatory functions through HDL-associated enzymes and apolipoproteins [11–13]. The bioactive lipid sphingosine-1-phosphate (S1P) is mainly bound to apolipoprotein M (apoM) of HDL and mediates many beneficial effects in hypertension and cardiac hypertrophy on the vasculature via G protein–coupled S1P receptors [14,15]. Moreover, HDL associated paraoxonase-1 (PON1) shows anti-inflammatory properties and is an important determinant for the capacity of HDL to stimulate endothelial nitric oxide production [16].

An increasing number of studies have demonstrated a significant effect of inflammatory disorders on HDL composition and function [17–20]. Since pregnancy is a low-grade systemic inflammatory condition [21], changes in HDL structure occur and an increase of HDL particle size and changes in HDL protein composition were reported in pregnant women (18–24 weeks of gestation) [22].

We hypothesized that HDL functionality would be impaired in women diagnosed with GHTN and in their offspring. In this longitudinal study, obese women considered at high risk for pregnancy complications were followed from their recruitment early in pregnancy until delivery. We assessed several key functions of HDL, including the ability to remove cholesterol from macrophages (cholesterol efflux capacity), the activity of the HDL-associated enzyme paraoxonase-1 (PON1), an vaso-protective anti-inflammatory enzyme [23], and serum levels of HDL associated anti-inflammatory apolipoprotein M (apoM) [24]. In addition, we assessed neonatal and maternal serum total anti-oxidative capacity. We further investigated whether HDL function in early pregnancy is related to GHTN risk.

2. Methods

This is a secondary analysis of the vitamin D And Lifestyle Intervention for gestational diabetes mellitus prevention (DALI) study, a randomized controlled trial (ISRCTN70595832) [25,26]. Further details including recruitment and inclusion criteria are provided in the Supplementary Material. All local ethics committees provided ethical approval and written informed consent was signed by all participants prior to data collection. Maternal blood samples were longitudinally collected <20 weeks, 24–28 and 35–37 weeks of gestation, and venous cord blood was collected immediately after birth.

2.1. Definition of GHTN

Information on GHTN was collected from medical records. GHTN was defined as systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg without proteinuria after 20 weeks of gestation.

2.2. Biochemical Analyses

Glucose was measured using the hexokinase method (DiaSys Diagnostic Systems, Holzheim, Germany) with a lower limit of sensitivity of 0.1 mmol/L.

Insulin was quantified by a sandwich-immunoassay (ADVIA Centaur, Siemens Healthcare Diagnostics Inc., Vienna, Austria) with an analytical sensitivity of 0.5 mU/L, intra-assay CVs of 3.3–4.6% and inter-assay CVs of 2.6–5.9%. All assays were carried out following the instructions of the manufacturer. HOMA-IR was calculated as [glucose*insulin]/22.5 mmol/L*UI/mL.

Total cholesterol (cord blood only) and triglycerides, were measured using colorimetric enzymatic assays using reagents from DiaSys Diagnostic Systems (Holzheim, Germany) and were calibrated using secondary standards from Roche Diagnostics (Mannheim, Germany). HDL-C was measured with a homogenous assay from DiaSys Diagnostics, and LDL cholesterol (LDL-C) was calculated according to the Friedewald formula (LDL-C = TC − HDL-C − TG/5). Non-esterified fatty acids (FFAs) were quantified using an enzymatic reagent and standards from Wako Chemicals (Neuss, Germany). All lipid analyses were performed on an Olympus AU640 automatic analyzer (Beckman Coulter, Brea, CA, USA).

2.3. ApoB-Depletion of Serum

For the analyses of HDL composition and function, serum HDL (apoB-depleted serum) was used. Polyethylene glycol (Sigma Aldrich, Darmstadt, Germany) (40 µL; 20% in 200 mmol/L glycine buffer) was added to 100 µL serum, mixed gently, and then incubated for 20 min at room temperature. After a centrifugation step at 10,100× *g* for 30 min at 4 °C, the supernatant was collected. Samples were stored at −70 °C until use.

2.4. Cholesterol Efflux Capacity

The cholesterol efflux capacity of apoB-depleted serum was assessed as described previously [27]. In brief, J774.2 macrophages (Sigma-Aldrich, Darmstadt, Germany) were maintained in DMEM media (containing 10% FBS, 1% PS). Cells were seeded on 48-well plates (300,000 cells/well), cultured for 24 h and loaded with 0.5 µCi/mL radiolabelled [^{3}H]-cholesterol in medium containing 2% FBS, 1% PS and 0.3 mM 8-(4-chlorophenylthio)-cyclic AMP overnight. After that, cells were washed and equilibrated in serum-free media supplemented with 0.2% BSA for 2 h. To determine [^{3}H]-cholesterol efflux, cells were incubated with 2.8% apoB-depleted serum for 3 h at 37 °C. Cholesterol efflux capacity was expressed as radioactivity in cell culture supernatant relative to total radioactivity in cells and supernatant.

2.5. Arylesterase (AE)—Activity of Paraoxonase1

The Ca^{2+}-dependent AE-activity of PON1 was determined with a photometric assay using the substrate phenylacetate as described elsewhere [28]. Briefly, apoB-depleted serum was diluted 10-fold and 1.5 µL were added to 200 µL reaction buffer (100 mM Tris, 2 mM $CaCl_2$, 1 mM phenylacetate). The rate of hydrolysis of the substrate was monitored by the increase of absorbance at 270 nm.

2.6. Anti-Oxidative (AO)—Capacity of ApoB-Depleted Serum

As previously described [28], the AO-capacity of apoB-depleted serum was assessed with a fluorometric assay using the fluorescent dye dihydrorhodamine. The dye was suspended in DMSO (50 mM stock), which was diluted in HEPES (20 mM HEPES, 150 mM $NaCl_2$, pH 7.4) containing 1 mM 2,2′-azobis-2-methyl-propanimidamide-dihydrochloride (AAPH) to a 10 µM working reagent. In a 384-well plate, 10 µL apoB-depleted serum dilution (1:10) were placed and 90 µL working reagent was added. The increase in fluorescence due to oxidation of dihydro-rhodamine was monitored for 90 min at 538 nm. The increase in dihydro-rhodamine fluorescence per minute in the absence of apoB-depleted serum was set at 100%, and individual apoB-depleted serum samples were calculated as the percentage of inhibition of dihydro-rhodamine oxidation.

2.7. Serum Levels of Apolipoprotein M (apoM)

Quantification of serum apoM was performed using a sandwich ELISA based assay as previously described [29].

2.8. Statistical Analyses

Participant characteristics are presented by mean and standard deviation (SD), median and interquartile range (IQR) or count and proportion. Maternal and neonatal characteristics were compared between the groups of women with and without GHTN using unpaired *t*-test and chi-square tests. Characteristics of included and excluded participants were compared using unpaired *t*-test and chi-square test. Differences in HDL parameters between time points were tested by paired sample *t*-tests.

The differences in HDL-related parameters between women with GHTN and women without this complication were tested using Student's *t* test (maternal samples) or Mann–Whitney U test (cord blood samples). We performed logistic regression analysis to identify possible associations between HDL-related parameters measured at baseline (<20 weeks) and the pregnancy complication GHTN. For better comparison, z-scores of the HDL-related parameters were used in the models. This means that the ORs are the chance of developing GHTN with one SD increase in the HDL-related parameter. Models were adjusted for maternal age, BMI and HOMA-R. In sensitivity analyses, possible confounding by maternal smoking, intervention allocation, or (for cord blood HDL parameters) mode of delivery was assessed.

To assess the association of GHTN with functional parameters of HDL in cord blood, we performed linear regression analyses, adjusted for maternal age, parity and gestational age at birth.

All analyses were performed in IBM SPSS (Version 27.0. Armonk, NY, USA: IBM Corp). A p value of <0.05 was used for determining statistical significance.

3. Results

3.1. Study Cohort Characteristics

A description of study cohort characteristics is provided in Table 1. Participants were selected based on the availability of serum samples from all time points of pregnancy (<20 weeks, 24–28, and 35–37 weeks of gestation; n = 192). As information on GHTN prevalence was not available from all women, 185 participants were selected for the analyses between GHTN and no GHTN. Women included in these analyses were higher educated, smoked less frequently, and their neonates had a higher birth weight compared to the total DALI study population in the pilot and lifestyle trials (Table S1). During pregnancy, 24 (13%) women were diagnosed with GHTN. There were no significant differences in maternal or neonatal characteristics between women with or without GHTN.

Table 1. Characteristics of study cohort.

Maternal Characteristics	Total n = 192	No GHTN n = 161	GHTN n = 24	p
Age, years	31.7 ± 5.4	31.9 ± 5.3	30.4 ± 5.3	0.19
Prepregnancy BMI, kg/m^2	34.1 ± 4.5	33.9 ± 4.3	36.2 ± 5.8	0.07
Gestational weight gain, kg	8.4 ± 4.8	8.3 ± 5.0	8.9 ± 4.0	0.59
Primiparous	102 (53%)	82 (51%)	16 (67%)	0.15
Married/living with partner	181 (94%)	151 (94%)	23 (96%)	0.69
High education	119 (62%)	103 (64%)	14 (58%)	0.59
European descent	170 (89%)	142 (88%)	22 (92%)	0.62
Smoking	18 (9%)	17 (11%)	0 (0%)	0.09

Table 1. *Cont.*

Maternal Characteristics	Total $n = 192$	No GHTN $n = 161$	GHTN $n = 24$	p
GDM	55 (29%)	44 (28%)	7 (29%)	0.87
GHTN	24 (13%)	–	–	–
Preeclampsia	6 (3%)	5 (3%)	1 (4%)	0.78
Neonatal characteristics				
Gestational age at birth	39.7 ± 1.4	39.7 ± 1.4	39.5 ± 1.3	0.50
Birthweight	3575 ± 520	3587 ± 527	3521 ± 462	0.56
Female sex	90 (47%)	74 (46%)	15 (63%)	0.13

3.2. Changes of HDL-Related Parameters during Pregnancy

Maternal serum levels of HDL-C increased modestly in our study between <20 weeks and 24–28 weeks. Interestingly, all HDL-related parameters, besides HDL-C, changed from early to late pregnancy, with a significant increase in the HDL cholesterol efflux capacity and serum apoM levels, whereas PON1 activity decreased from <20 weeks to 35–37 weeks of pregnancy. In addition, the anti-oxidative capacity of serum was increased (Figure 1). After correction for differences in HDL-C levels (by normalization to HDL-C), no differences in parameters of HDL function were observed between the time points of pregnancy (Figure S1).

3.3. HDL-Related Parameters in Cord Blood

Compared to maternal serum, anti-oxidative capacity of serum was higher in circuit of the offspring, while PON1 activity, cholesterol efflux capacity and serum apoM levels were markedly lower compared to maternal levels (Figure 1). To determine the functionality of individual HDL particles, we normalized the measured functional parameters to HDL-C levels (Figure S1). Compared with mothers, the HDL cholesterol efflux capacity of individual HDL particles in cord blood was significantly higher, indicating differences in the structure and composition of fetal HDL. ApoM content of HDL particles in cord blood was significantly increased (Figure S1). ApoM improves cholesterol efflux capacity of HDL [30,31], consistent with the increased cholesterol efflux capacity of individual HDL particles in cord blood. Interestingly, PON1 activity of individual HDL particles was lower in cord blood (Figure S1). We observed no sex differences in HDL-related parameters in the offspring cohort.

3.4. GHTN-Associated Changes in HDL-Related Parameters in Mothers and Cord Blood

Next, we assessed parameters of HDL function in women diagnosed with GHTN and their offspring.

We observed that GHTN was not associated with altered maternal HDL-C, HDL cholesterol efflux capacity, PON1 activity or anti-oxidative capacity (Figure 2A–D). Of particular interest, in cord blood, HDL cholesterol efflux capacity and PON1 activity were significantly impaired, whereas HDL-C levels were unaltered (Figure 2A–C). Moreover, we observed an increased anti-oxidative capacity of cord serum (Figure 2D) ($p = 0.04$). Serum apoM levels showed a non-significant trend toward higher levels in women diagnosed with GHTN at all-time points of pregnancy but lower levels in offspring (Figure 2E).

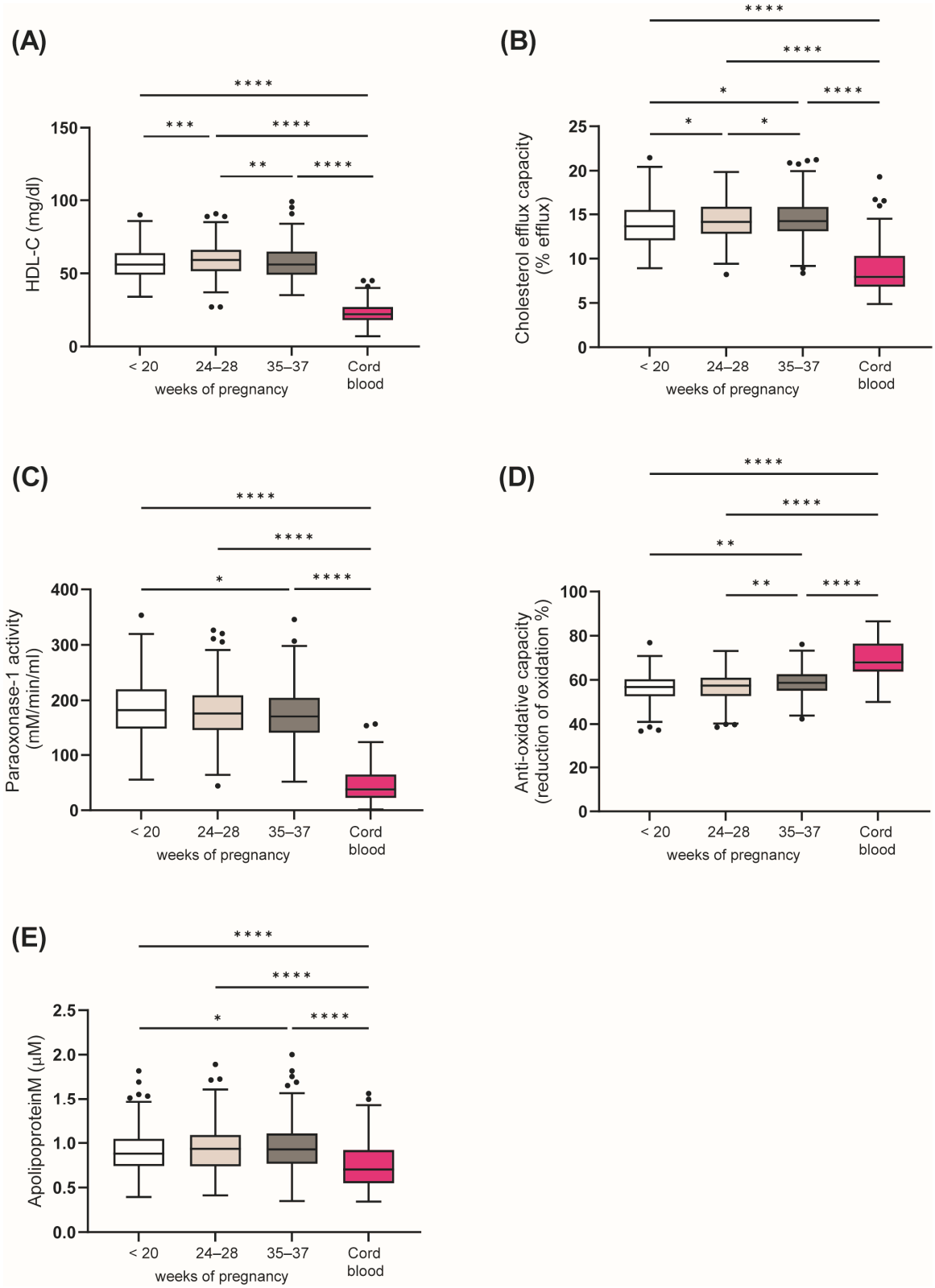

Figure 1. Changes in HDL-related parameters during pregnancy and comparisons with cord blood. HDL-C (**A**), the ability to promote cholesterol efflux (**B**) and activity of HDL-associated PON1 (**C**) were assessed. (**D**) Serum anti-oxidative capacity and (**E**) apolipoprotein M levels. Data are presented as Tukey-Boxplots, showing the median and interquartile ranges, as well as minimum, maximum values and outliers. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ based on paired *t*-test.

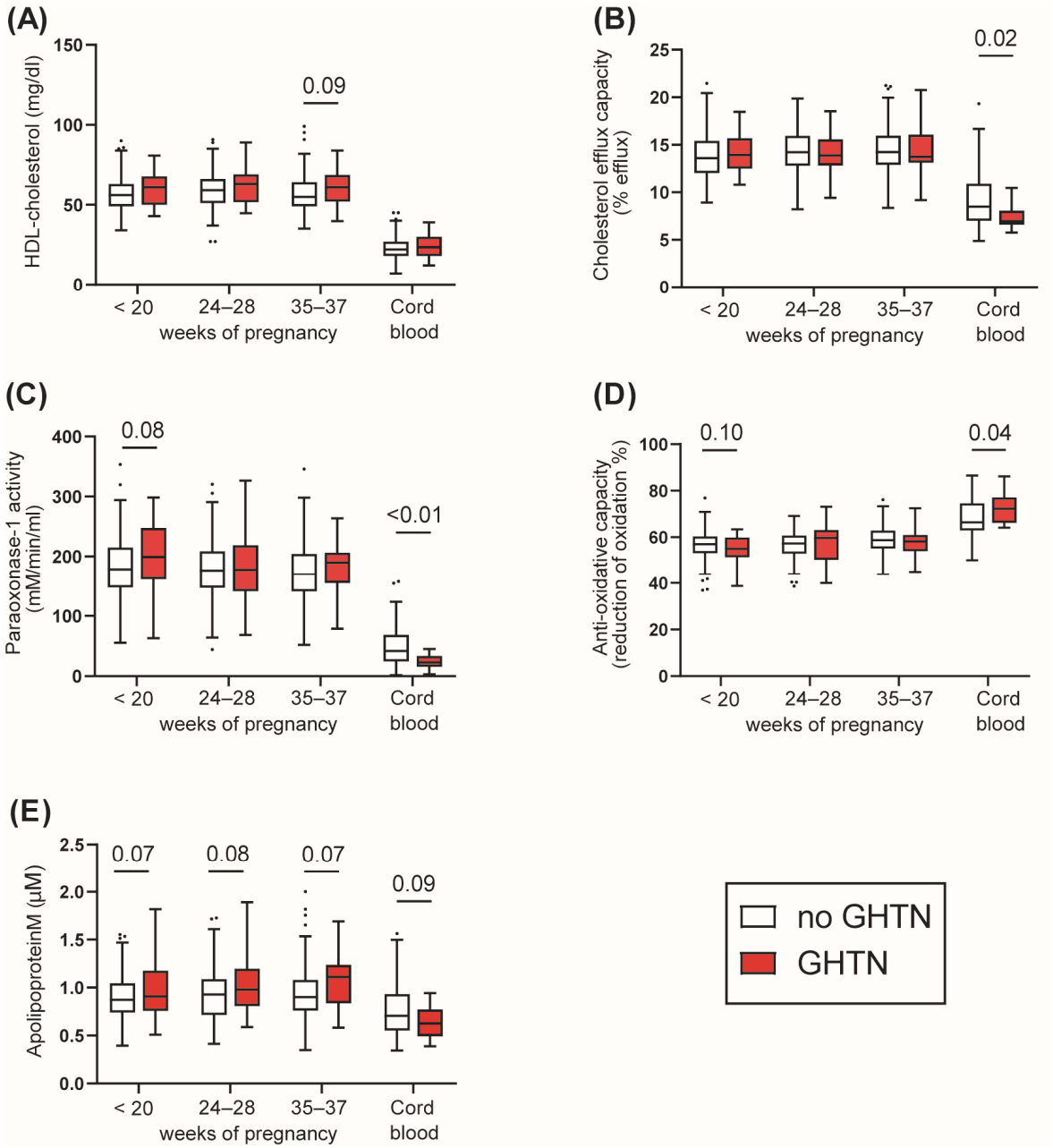

Figure 2. Differences in HDL-related parameters in women with GHTN and healthy controls. HDL-cholesterol (**A**), cholesterol efflux capacity (**B**) and the activity of HDL-associated PON1 (**C**) were assessed. (**D**) Serum anti-oxidative capacity and (**E**) apolipoprotein M levels. Data are presented as Tukey-Boxplots showing the median and interquartile ranges as well as minimum, maximum values and outliers. Significant ($p < 0.05$) and non-significant trends ($p < 0.10$) are indicated with p-value. Differences were analysed by student T-test (maternal samples) or Mann–Whitney U test (cord blood).

3.5. Association of HDL-Related Parameters with Pregnancy Outcome

We performed logistic regression analysis to identify possible associations between HDL-related parameters measured at baseline (<20 weeks) and GHTN (Figure 3). The models were adjusted for maternal age, BMI and HOMA-R. Interestingly, women having increased serum PON1 activity as well as increased apoM levels were at a higher risk of developing GHTN. Serum levels of HDL-C, cholesterol efflux capacity and the serum anti-oxidative capacity were not associated with GHTN incidence. Further adjustments for maternal smoking or interventions did not substantially change the results.

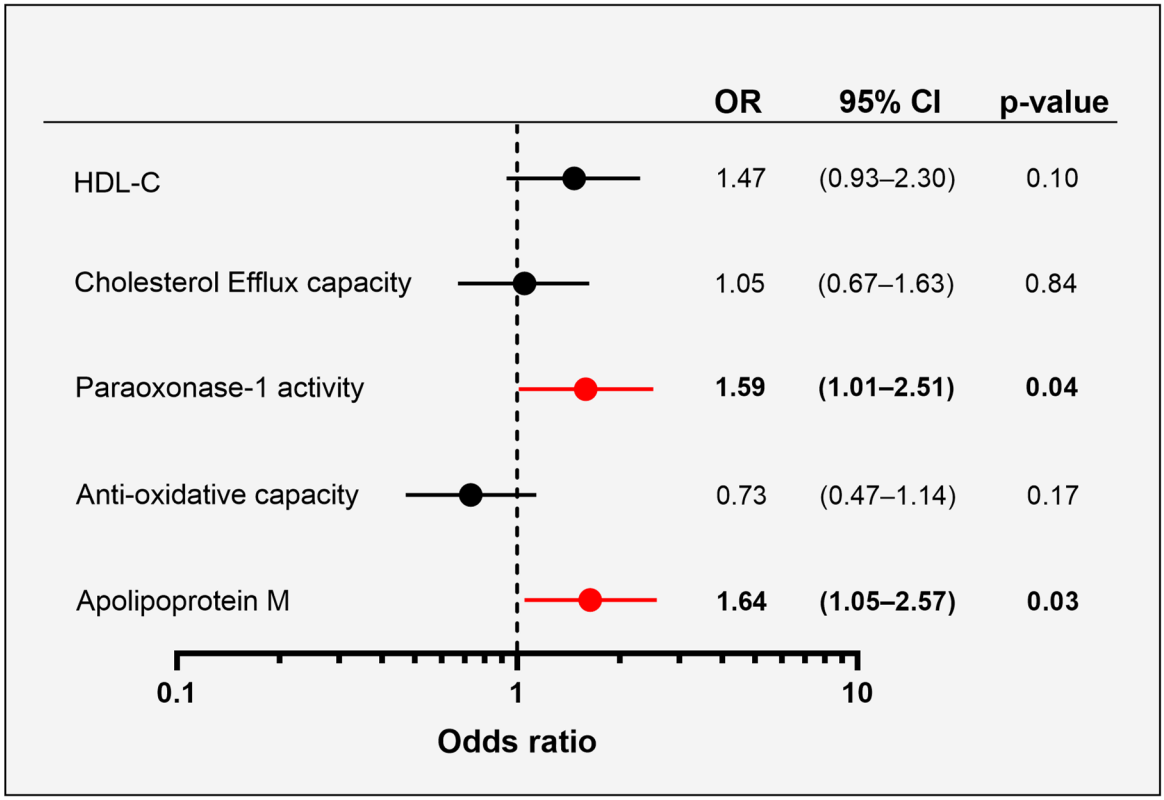

Figure 3. Forest plot with logistic regression analyses showing associations between HDL-related parameters (z-scores) at <20 weeks' gestation and the risk of developing GHTN in the second and third trimester of pregnancy. Odds ratio per 1 SD increase of the variables. Models were adjusted for maternal age, BMI and HOMA-R. Significant results are highlighted in red.

3.6. Consequences of Maternal Pregnancy Disorders on HDL-Related Parameters in Cord Blood

We then conducted linear regression analyses to investigate the relationship between GHTN and the functional parameters of HDL in cord blood (Table 2). Cord blood was available from 102 pregnancies, and the model was adjusted for maternal age, parity, and gestational age at birth. Of particular interest, maternal GHTN was associated with a decrease in PON1 activity and cholesterol efflux capacity and an increase in anti-oxidative capacity of cord serum. No association was found between GHTN and HDL-C levels. Further adjustment for maternal smoking, intervention or mode of delivery did not change the results.

Table 2. Linear regression models of the association of GHTN with cord blood HDL-parameters.

	HDL-C B (95% CI)	*p*	**Anti-Oxidative Capacity B (95% CI)**	*p*	**Paraoxonase-1 Activity B (95% CI)**	*p*	**Cholesterol Efflux Capacity B (95% CI)**	*p*	**ApoM B (95% CI)**	*p*
GHTN	0.04 (−0.06; 0.14)	0.44	5.29 (0.63; 9.95)	0.03	−24.88 (−41.87; −7.89)	0.005	−2.04 (−3.61; −0.47)	0.01	−0.14 (−0.28; 0.003)	0.055

Models adjusted for maternal age, parity and gestational age at birth.

4. Discussion

In the current study, we assessed GHTN-associated changes in HDL-related functional parameters in mothers and their offspring. We observed that GHTN was associated with marked changes in HDL function in the offspring, whereas HDL functionality was not

affected in mothers. Specifically, GHTN was associated with impaired HDL cholesterol efflux capacity and PON1 activity in cord blood, whereas serum anti-oxidative capacity was increased.

It is well known that during the course of pregnancy, HDL-C levels increase modestly in mothers [32–34], which was also seen in our study from <20 weeks to 24–28 weeks of gestation. We observed increased maternal HDL cholesterol efflux capacity, apoM levels and anti-oxidative capacity of serum during the course of pregnancy. However, after normalization of functional HDL parameters to HDL-C levels, no significant differences remained, suggesting that the functionality of individual HDL particles was not altered during the course of pregnancy.

Pregnancy induced changes in HDL function in gestational hypertension have remained poorly investigated. A few previous studies have focused on analysing changes of HDL structure and function in women with preeclampsia, a more severe form of GHTN. HDL of mothers diagnosed with preeclampsia showed an increased particle diameter and reduced PON1 activity and was less effective in reducing adhesion molecule expression on endothelial cells [35]. Mixed results were reported when HDL cholesterol efflux capacities were assessed in preeclampsia. One study reported increased maternal and fetal HDL cholesterol efflux capacities in preeclampsia [36], whereas another study reported that women with a history of preeclampsia (6 months postpartum) display decreased HDL cholesterol efflux capacity [37]. These data suggest that in more severe forms of GHTN, parameters of HDL function in maternal blood may also be affected.

A very interesting finding of our study was that maternal PON1 activity and apoM levels in early pregnancy were associated with the risk of developing GHTN. PON1 is capable of hydrolyzing a wide spectrum of substrates including oxidized lipids and is thought to play a role in the development of a large variety of diseases with an inflammatory component, including heart disease, diabetes, rheumatic diseases, neurological diseases and cancer [38,39]. Given this close relationship between PON1 activity and various diseases, further studies would be of great interest to investigate the extent to which PON1 activity can serve as an early marker for a wide variety of pregnancy-related diseases.

ApoM has gained attention in recent years, as studies have shown that apoM is important for the formation of preβ-HDL increasing cholesterol efflux capacity and promoting endothelial protective activities via its bound S1P [15,40,41]. Perinatal and long-term offspring morbidities are strongly dependent on the preservation of placental vascular homeostasis during pregnancy. Recent studies have shown that impaired S1P signalling in the endothelium indicates the health/disease state of the vasculature and is thought to contribute to the pathogenesis of preeclampsia [14,42]. Thus, a decrease in apoM in GHTN could have negative consequences for endothelial function in neonates with long-term pathological implications for the heart later in life.

An important finding of our study was that GHTN significantly impaired HDL cholesterol efflux capacity as well as HDL PON1 activity in cord blood. Both factors could increase long-term cardiovascular risk of the offspring. This notion is supported by recent studies showing that reduced HDL cholesterol efflux capacity predicts cardiovascular risk independent of HDL-C [9] and that low PON1 activity is linked to systemic oxidative stress and prospective cardiovascular risk [43].

On the other hand, surprisingly, GHTN was associated with increased antioxidant capacity of cord blood serum. This might indicate a compensatory mechanism in response to reduced blood flow and increased oxidative stress in the neonatal/placental circulation. It is important to note that the anti-oxidative capacity of serum is predominantly determined by serum albumin levels in addition to low-molecular-weight antioxidants with a minor contribution of HDL [44]. It is reasonable to speculate that decreased excretion of hydrophilic antioxidants may explain the increased antioxidant capacity of cord blood serum.

Some limitations of our study have to be acknowledged. A limitation is the small sample size of GHTN patients. Therefore, further larger studies are needed to confirm

our results and to draw firm conclusions. Because this study was originally designed as an intervention study for the prevention of GDM in obese women, no lean control group was available. Moreover, due to the small sample size of GHTN women, interactions with offspring sex were not assessed, and due to very low prevalence of women with preeclampsia, these participants were not included in our analyses.

Strengths of our study are that we assessed multiple functional parameters of HDL and its prospective and longitudinal study design. Maternal serum samples were collected at three time-points during pregnancy, which enabled us to study changes overtime. Moreover, paired mother-offspring blood samples available for all pregnancies included. To the best of our knowledge, this is the largest study on effects of obesity and pregnancy disorders on HDL-related parameters in mothers and offspring. Moreover, it is a pan-EU study, which is representative of pregnant Caucasian women with obesity in Europe, who are well phenotyped.

5. Conclusions

In this study, we demonstrated that HDL-C and functional parameters of HDL change over the duration of pregnancy. In addition, we showed that GHTN does not significantly alter maternal HDL-related parameters, but has profound effects on HDL-related parameters in the offspring. Follow-up studies are needed to clarify when and whether these changes normalize after birth or whether the changes contribute to long-term cardiovascular risk of the offspring. In addition, our results suggest that maternal PON1 activity and apoM levels are associated with the risk of developing GHTN.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox12010068/s1, additional methods; Figure S1: HDL-functionalities corrected for HDL-C levels in the DALI cohort; Table S1: Characteristics of women selected for analyses and those who were excluded. References [25,26,29] are cited in Supplementary Materials file.

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Informed Consent Statement: Informed written consent was obtained from all subjects involved in the study at baseline.

Data Availability Statement: The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, on request to the corresponding author.

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Preeclampsia Affects Lipid Metabolism and HDL Function in Mothers and Their Offspring

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Abstract: Preeclampsia (PE) is linked to an overall increased cardiovascular risk for both the mother and child. Functional impairment of high-density lipoproteins (HDL) may contribute to the excess cardiovascular risk associated with PE. In this study, we investigated the effects of PE on maternal and neonatal lipid metabolism, and the parameters of HDL composition and function. The study cohort included 32 normotensive pregnant women, 18 women diagnosed with early-onset PE, and 14 women with late-onset PE. In mothers, early- and late-onset PE was associated with atherogenic dyslipidemia, characterized by high plasma triglycerides and low HDL-cholesterol levels. We observed a shift from large HDL to smaller HDL subclasses in early-onset PE, which was associated with an increased plasma antioxidant capacity in mothers. PE was further associated with markedly increased levels of HDL-associated apolipoprotein (apo) C-II in mothers, and linked to the triglyceride content of HDL. In neonates of early-onset PE, total cholesterol levels were increased, whereas HDL cholesterol efflux capacity was markedly reduced in neonates from late-onset PE. In conclusion, early- and late-onset PE profoundly affect maternal lipid metabolism, potentially contributing to disease manifestation and increased cardiovascular risk later in life. PE is also associated with changes in neonatal HDL composition and function, demonstrating that complications of pregnancy affect neonatal lipoprotein metabolism.

Keywords: preeclampsia; HDL; cholesterol efflux; anti-oxidative capacity; HDL subclasses

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1. Introduction

Pre-eclampsia (PE) is a pregnancy complication that affects 4–5% of pregnancies worldwide and has serious consequences for mothers and their children [1,2]. This disorder is one of the main causes of maternal and fetal morbidity and mortality, particularly in low- and middle-income countries [3]. PE manifests after 20 weeks of gestation and is defined by the onset of hypertension (systolic $\geq$ 140 mm Hg, diastolic $\geq$ 90 mm Hg), combined with proteinuria ($\geq$300 mg/24 h) or, in the absence of proteinuria, one of the following diagnoses: thrombocytopenia, renal insufficiency, impaired liver function, cerebral or visual problems or pulmonary oedema [3,4]. Without intervention, PE can cause significant complications and can only be cured by the delivery of the baby [5,6]. After delivery, the symptoms of PE are typically ameliorated once the blood pressure returns to pre-pregnancy levels. However, this hypertensive pregnancy disorder is also considered a major risk factor for the development of cardiovascular disease later in life for both the mother [7,8] and her child [9–11].

The major characteristics of PE comprise endothelial dysfunction, increased oxidative stress in the maternal circulation and hyperlipidemia [12,13]. Specifically, high levels of triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C) and reduced

levels of high-density lipoprotein cholesterol (HDL-C) have been suggested to be associated with an increased risk of PE [14–16].

PE is thought to be driven by abnormal placentation caused by poor trophoblast differentiation and defective trophoblast invasion [17], leading to insufficient spiral artery remodeling [18]. This results in placental hypoperfusion, vascular dysfunction, and the release of anti-angiogenic factors, such as soluble fms-like tyrosine kinase 1, to the maternal circulation [19,20]. Consequently, this can lead to oxidative stress, hypertension, or even end-organ damage [6]. However, the underlying molecular mechanism leading to the development of abnormalities in the vasculature of the placenta remains poorly understood.

PE is usually classified into two different subtypes based on the development of the disease. Early-onset PE develops before gestational week 34 and is usually more severe, while late-onset PE is diagnosed after week 34 [21].

Recent studies have shown that certain subclasses of lipoproteins, especially HDL, are involved in a number of important physiological functions, many of which are important for a healthy pregnancy [22]. HDL is now valued for its role in regulating lipid metabolism, hemostasis, immune response, inflammation, complement activation and vitamin transport [23]. HDL particles exhibit cardio-protective properties [24], including the promotion of cholesterol efflux from peripheral cells and transport to the liver for excretion [25]. Further, HDL shows anti-inflammatory [26], anti-oxidative [27] and anti-thrombotic [28] effects, including regulation of endothelial functions by promoting nitric oxide production and maintaining endothelial integrity [28]. HDL particles comprise a considerable number of proteins and are structurally and functionally heterogenous [22,29]. Apolipoprotein (apo)A-I and apoA-II stabilize HDL particles, and depict anti-oxidant properties; whereas, HDL-associated apoC-II acts as a cofactor for lipoprotein lipase, thereby regulating the hydrolysis of triglycerides. Furthermore, several enzymes are associated with HDL, such as paraoxonase-1 (PON1), a hydrolytic enzyme with a wide range of substrates and partly responsible for the anti-oxidative and anti-inflammatory properties of HDL [30]. The HDL-associated enzyme lecithin-cholesterol acyltransferase (LCAT) participates in the remodeling and maturation of HDL [31]. Functionally, LCAT is the major source of plasma-derived cholesteryl esters, and is responsible for the conversion of nascent or lipid-poor HDL into spherical HDL [32]. Disturbances in the activity of LCAT lead to altered lipoprotein metabolism, which has been described to be present and to execute crucial roles in several diseases [33–35].

In the present study, we hypothesized that maternal, but also neonatal HDL composition and function are altered in PE-affected pregnancies. As early-onset and late-onset PE are assumed to have different pathophysiological origins, we aimed to investigate the effects of these pregnancy complications on HDL metabolism and function. We characterized lipoprotein subclass distribution, compared functional metrics of HDL and investigated differences in lipid protein and apolipoprotein composition from normotensive and PE pregnancies.

2. Materials and Methods

2.1. Recruitment and Group Characteristics

The study protocol was approved by the local ethical committee of the Medical University of Graz. Women without pregnancy complications and normal blood pressure levels (n = 32), early-onset PE (n = 18) and late-onset PE (n = 14) were recruited at the time of delivery, and gave informed written consent (26-333 ex 13/14). Women with multiple births were excluded from the study. PE was defined as new-onset hypertension above 140/90 mm Hg occurring after 20 weeks of gestation, and one or multiple of the following emerging conditions: proteinuria, renal insufficiency, thrombocytopenia, compromised liver function, pulmonary edema, uteroplacental dysfunction or neurologic complications. The occurrence of PE was defined as early-onset if it was detected before 34 weeks gestation, or late-onset if it was detected after 34 weeks of gestation [2]. Healthy participants were selected based on normal blood pressure and the absence of medical complications during

pregnancy, and were matched for age and pre-pregnancy BMI of the PE groups. Venous blood from pregnant women at term was collected before delivery, while corresponding umbilical cord blood was collected no later than 10 min after delivery. EDTA plasma was isolated by centrifugation at 3500 rpm for 10 min at 4 °C, and stored at −80 °C until further analysis. Blood pressure, C-reactive protein levels and platelet count were assessed among all participants, whereas the concentration of soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF) and liver markers were only measured in PE women.

2.2. ApoB-Depleted Plasma and Plasma Lipids

ApoB-depleted plasma was prepared by the addition of 40 µL polyethylenglycol (P1458, Sigma-Aldirch, Darmstadt, Germany) (20% in 200 mmol/L glycine buffer) to 100 µL plasma, followed by gentle mixing. Plasma samples were incubated for 20 min at room temperature. After centrifugation at 10,000 rpm for 30 min at 4 °C, the supernatant was collected and stored at −70 °C until use. Enzymatic photometric transmission measurement (Roche Diagnostics, Mannheim, Germany) was used to measure plasma lipids, such as total cholesterol, triglycerides and HDL-C. LDL-cholesterol concentrations were calculated using the Friedewald's formula [36].

2.3. HDL-Associated Apolipoproteins and Lipids

HDL-associated apoA-I, apoA-II, apoC-II, apoC-III and apoE were determined by immunoturbidimetry in apoB-depleted plasma [37]. HDL-associated lipids, including cholesterol, phospholipids and triglycerides, were determined by enzymatic techniques, as previously described [38]. Cholesteryl-ester levels were calculated as the difference between total cholesterol and free cholesterol, measured in apoB-depleted plasma. As previously described, all lipoprotein analyses were performed on an Olympus AU680 analyzer (Beckman Coulter, Brea, CA, USA) [37]. To determine HDL particle composition, apolipoproteins/lipids were corrected for total HDL protein.

2.4. HDL Subclass Distribution

HDL subfractions were determined using the Lipoprint© System (48-9002, Quantimetrix, CA, USA), according to the manufacturer's instructions. This system separates HDL subclasses from human plasma on the basis of size, using preloaded gel tubes for HDL determinations [39]. In brief, 25 µL of plasma of each patient was loaded into polyacrylamide gel tubes, along with 300 µL loading gel solution containing a lipophilic dye. The tubes were photopolymerized at room temperature for 30 min. Electrophoresis with tubes containing plasma samples, along with the manufacturer's quality controls, was performed at a constant of 3 mA/tube for 50 min. Subfraction bands were scanned and identified by their mobility (Rf) using very-low-density lipoprotein and low-density lipoprotein as the starting (Rf 0.0) and albumin as the ending (Rf 1.0) reference points. Ten HDL subfractions were identified and grouped into three major classes: large (HDL1 to HDL3), intermediate (HDL4 to HDL7) and small (HDL8 to HDL10) subfractions [39].

2.5. Cholesterol Efflux Capacity Assay of Apob-Depleted Plasma

Evaluation of cholesterol efflux capacity was performed as described elsewhere [40,41]. J774.2 cells (Sigma-Aldrich, Darmstadt, Germany) were cultured in DMEM (Life Technologies, Carlsbad, CA, USA), containing 10% fetal bovine serum and 1% penicillin/streptomycin. In each well, 300,000 cells were seeded on 48-well plates (Greiner Bio-One, Kremsmünster, Austria), cultured for 24 h and labeled with 0.5 µCi/mL radiolabeled [^{3}H]-cholesterol (ART0255, Hartmann Analytic, Braunschweig, Germany) in DMEM containing 2% BSA, in the presence of 0.3 mM 8-(4-chlorophenylthio)-cyclic adenosine monophosphate (c3912, Sigma-Aldrich, Darmstadt, Germany) overnight. Cyclic adenosine monophosphate was used for the upregulation of ATP-binding cassette transporter A1. After 18 h, the cells were rinsed with DMEM (serum-free) and equilibrated with DMEM (serum-free) containing 2 mg/mL bovine serum albumin (Sigma-Aldrich, Darmstadt, Germany) for 2 h. Cells

were then incubated with 2.8% apoB-depleted plasma for 3 h to determine [^{3}H]-cholesterol efflux. The cholesterol efflux capacity was expressed as the radioactivity in the medium in relation to the total radioactivity in medium and cells. All steps were performed in the presence of 2 µg/mL acyl-coenzyme A cholesterol acyltransferase inhibitor Sandoz 58-035 (Sigma-Aldrich, Darmstadt, Germany).

2.6. Arylesterase Activity of PON1

As described elsewhere, the arylesterase activity of HDL-associated paraoxonase was evaluated by a photometric assay, using phenylacetate (10873, Sigma-Aldrich, Darmstadt, Germany) as substrate [42].

2.7. Anti-Oxidative Capacity

Plasma anti-oxidative activity was determined using fluorometric assay, as previously described [43]. The ability of apoB-depleted plasma samples to inhibit dihydrorhodamine (Cay85100-5, Biomol, Hamburg, Germany) oxidation was monitored.

2.8. LCAT Activity

Plasma lecithin-cholesterol acyltransferase (LCAT) activity was determined using a commercially available kit (MAK107, Merck, Darmstadt, Germany), according to the manufacturer's instructions. Briefly, plasma samples were incubated with the LCAT substrate at 37 °C for a period of 4 h. The fluorescent substrate emits fluorescence at a wavelength of 470 nm. When the substrate is hydrolyzed by LCAT, a monomer is released, which emits fluorescence at 390 nm. LCAT activity was assessed over time and expressed as the change of 470/390 nm emission intensity.

2.9. VLDL and LDL Subclass Distribution

Plasma levels of very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and distribution of LDL subclasses were assessed using the Lipoprint© System (48-7002, Quantimetrix Corp., Redondo Beach, CA, USA) according to the manufacturer's instructions. Plasma samples were loaded on gel tubes and mixed with 200 µL of Lipoprint loading gel, containing a lipophilic dye. The dye proportionally binds to lipids in the plasma. Gel tubes were photopolymerized for 30 min. Electrophoresis was performed for 60 min at 3 mA per gel tube and 500 V maximum. Gel tubes were scanned and analyzed using the Lipoware software (Lipoware HDL Research LW03-v.16-134), after a rest period of 30 min.

2.10. Statistical Analysis

Differences between the two groups were analyzed using an ANOVA or Kruskal–Wallis test, followed by multiple comparisons corrected according to Bonferroni. Individual data are depicted on top of boxplots, showing the median and interquartile range, as well as the minimum and maximum values. Correlations were assessed using a Spearman's correlation coefficient rho, due to the skewed nature of many of the parameters. Statistical analyses were performed using GraphPad Prism (Version 9.5.0, GraphPad Software, San Diego, CA, USA) and SPSS Statistics (Version 26, Armonk, NY, USA: IBM Corp).

3. Results

3.1. Maternal and Fetal Characteristics of the Study Population

In this explorative study, we included 32 normal pregnancies, 18 early-onset PE and 14 late-onset PE cases, of which maternal and cord blood samples were collected. The control cohort had normal blood pressure levels and no incidence of pregnancy complications. Some of the risk factors for developing PE include nulliparity, high pre-pregnancy BMI, age, chronic hypertension, pre-gestational diabetes or renal disease [2]. As it has already been shown that BMI impacts HDL function in mothers and neonates [44], our participants

were matched for age and pre-pregnancy BMI to exclude possible confounding factors. Clinical characteristics are shown in Table 1.

Table 1. Maternal and fetal characteristics of the study population.

Maternal Characteristics	Normal Pregnancy	Early-Onset PE	*p*-Value	Late-Onset PE	*p*-Value
Number of matched samples	32	18		14	
Maternal age (years)	30 (27–33)	34 (28–37)	ns	31 (30–36)	ns
Pre-pregnancy BMI (kg/m^2)	22.5 (20.7–28.3)	25.4 (22.0–27.4)	ns	26.5 (22.8–29.3)	ns
Gestational age (weeks)	38.8 (38.1–39.1)	33.6 (31.7–34.3) †	<0.001	37.2 (35.5–37.9)	ns
Mode of delivery (% C-section)	88	100	ns	72	ns
Systolic blood pressure (mmHg)	117 (110–122)	163 (156–185)	<0.001	153 (143–169)	<0.001
Diastolic blood pressure (mmHg)	74 (66–79)	107 (100–115)	<0.001	101 (94–108)	<0.001
CRP (mg/mL)	4.8 (2.5–7.9)	5.1 (3.4–19.9)	ns	7.0 (2.5–17.1)	ns
Sflt-1 (pg/mL)	-	15,721 (12,184–21,500) †	-	9542 (8125–154,001)	-
PLGF (pg/mL)	-	44.5 (29.1–56.8) †	-	67.3 (36.9–86.6)	-
Platelets	212 (174–244)	202 (150–239)	ns	189 (140–220)	ns
Uric acid (mg/dL)	-	6.2 (5.5–7.0)	-	5.9 (5.1–7.1)	-
ALT (U/L)	-	30.0 (21.8–44.3)	-	24.5 (16.8–38.8)	-
AST (U/L)	-	25.0 (18.8–51.0) †	-	13.5 (8.8–45.8)	-
Fetal characteristics					
Sex (% female)	59	50	ns	57	ns
Weight at birth (g)	3230 (2951–3683)	1660 (1438–2021)	<0.001	2545 (2303–3185)	0.054
Placenta weight (g)	615 (563–665)	360 (320–455)	<0.001	495 (448–533)	0.005

Results are presented as the median (Q1–Q3) or as a relative abundance (%). Differences between normal pregnancy and early- as well as late-onset PE were calculated using the Kruskal–Wallis test, followed by multiple comparisons, corrected according to Bonferroni. BMI, body mass index; CRP, c-reactive protein; Sflt-1, soluble fms-like tyrosine kinase-1; PlGF, placental growth factor; ALT, alanine aminotransferase; AST, aspartate transaminase. † represents a significant difference between early-onset PE and late-onset PE.

As expected, the gestational age was lower in the early-onset PE group, compared to normal pregnancy, while it was not different in late-onset PE patients. Significant differences in both PE groups were observed in systolic and diastolic blood pressure levels compared to the control group. The study groups did not show differences in the levels of CRP, platelet count or mode of delivery. A significant difference in the levels of sflt-1 and PlGF was observed between early-onset and late-onset PE, whereas the concentration of uric acid and liver markers showed no differences.

Placental weight, as well as the weight of the neonate, differed significantly between normal pregnancy and PE pregnancies, while there was no difference in fetal sex.

3.2. Preeclampsia Is Associated with Altered Plasma Lipid Levels in Mothers and Offspring

One of the risk factors and characteristics of PE is maternal hyperlipidemia [45,46]. In our study cohort, we observed that early- and late-onset PE was associated with atherogenic dyslipidemia in mothers, characterized by high triglycerides and low HDL-C levels. Total cholesterol and non-HDL-C levels did not differ between the maternal study groups (Figure 1A). In contrast, in cord blood, total cholesterol and non-HDL-C levels were elevated in early-onset PE compared with offspring of normal pregnancies, whereas triglyceride and HDL-C levels were not significantly different (Figure 1B).

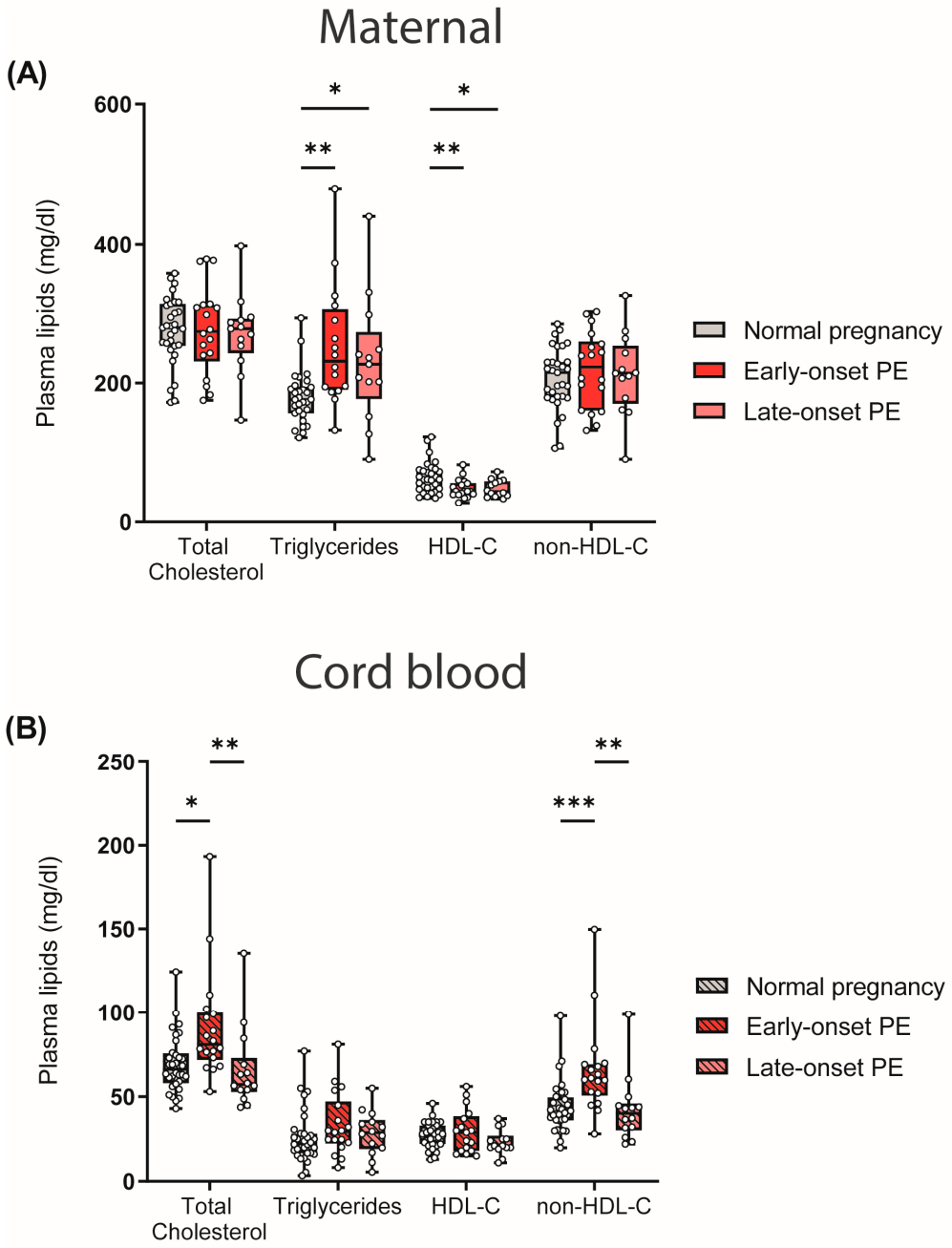

Figure 1. Differences between plasma lipid levels in women with normal pregnancy, early-onset PE and late-onset PE, and corresponding offspring: (**A**) Plasma total cholesterol levels, triglycerides, HDL-C and non-HDL-C of mothers and (**B**) neonates. Individual data are presented on top of boxplots, showing the median, interquartile range, and minimum and maximum values. Differences between the groups were analyzed by the Kruskal–Wallis test, followed by multiple comparisons, corrected according to Bonferroni. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (Maternal: early-onset PE, $n = 17$; late-onset PE, $n = 13$; normal pregnancy, $n = 31$; cord blood: early-onset PE, $n = 17$; late-onset PE, $n = 13$; normal pregnancy, $n = 32$).

3.3. Preeclampsia-Related Changes of HDL-Associated Apolipoprotein and Lipid Composition

The particle composition of HDL critically determines functionality [47]. Since we observed differences in the quantity of HDL-cholesterol levels, we determined the relative abundance of major apolipoproteins of HDL by calculating the ratio between apolipoproteins and total protein content of HDL. We observed a higher abundance of HDL-associated

apoC-II in both maternal PE groups (Figure 2A). ApoC-II is a cofactor of lipoprotein lipase, promoting the hydrolysis of triglycerides [48].

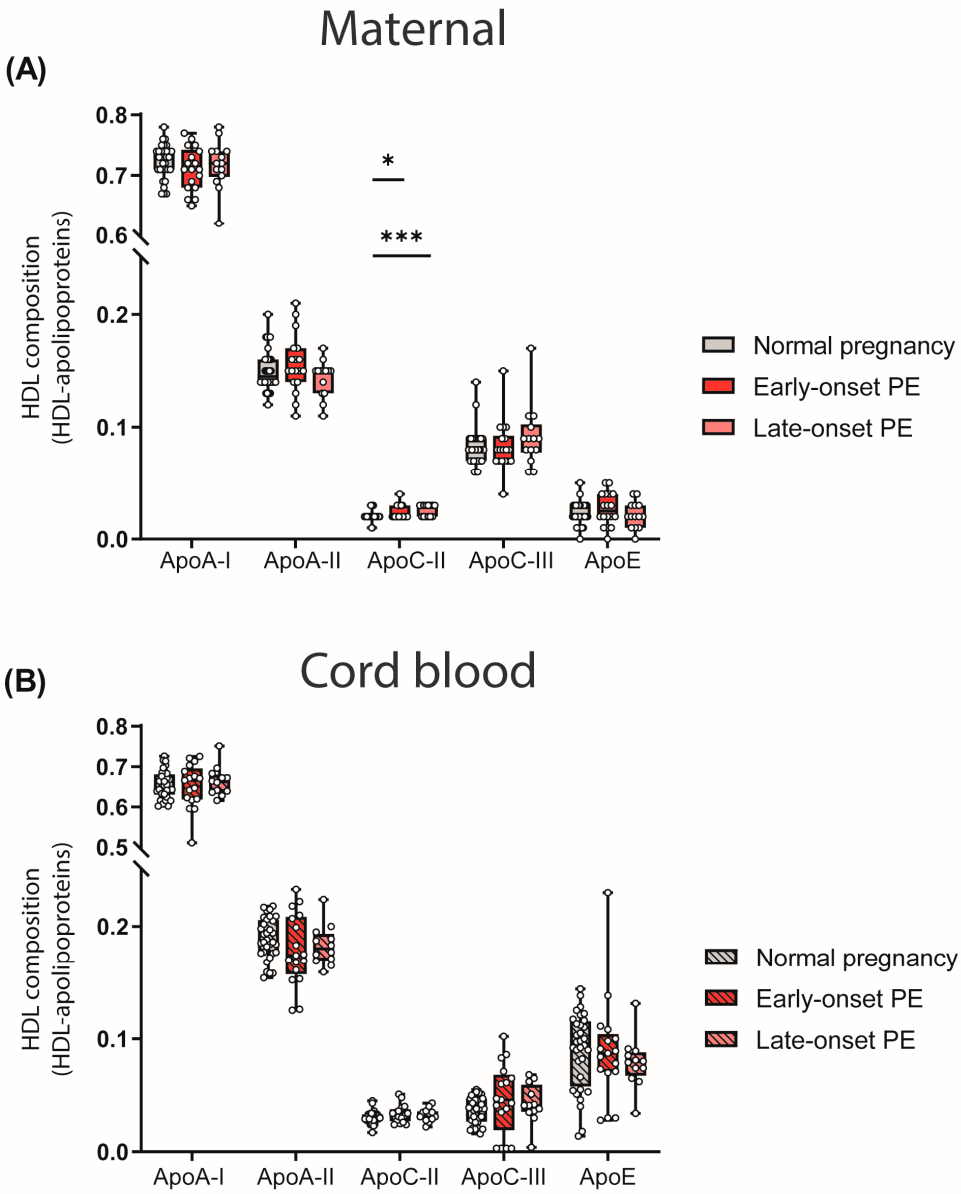

Figure 2. HDL composition: HDL-associated apolipoproteins in mothers with normal pregnancy, early-onset PE and late-onset PE (**A**) and in their offspring (**B**). Individual data are presented on top of boxplots, showing the median, interquartile range, and minimum and maximum values. Differences between the groups were analyzed by the Kruskal–Wallis test, followed by multiple comparisons, corrected according to Bonferroni. * $p < 0.05$, *** $p < 0.001$. (Maternal: early-onset PE, $n = 18$; late-onset PE, $n = 14$; normal pregnancy, $n = 32$; cord blood: early-onset PE, $n = 17$; late-onset PE, $n = 13$; normal pregnancy, $n = 32$).

No differences in the relative abundance of apolipoproteins between maternal normal pregnancy and PE were observed for apoA-I, apoA-II, apoC-III, apoE and cord blood apolipoproteins (Figure 2B).

We next assessed the abundance of major lipid constituents of HDL. To determine the HDL composition, lipid levels were measured in apoB-depleted plasma and corrected for the total protein content of HDL. While we did not find any differences between maternal

PE groups and the control (Figure 3A), a significant increase in HDL-associated triglycerides in the cord blood of early-onset PE was observed (Figure 3B).

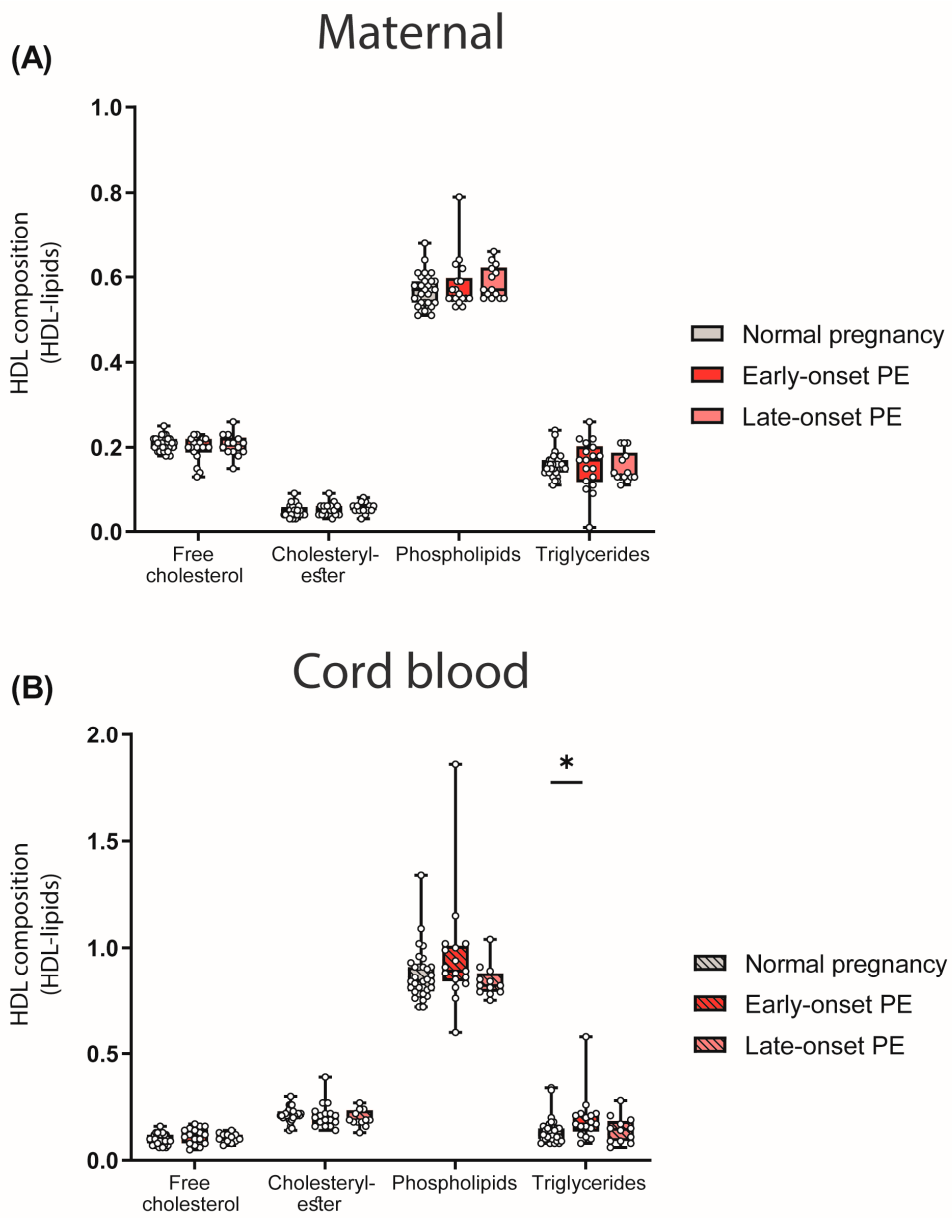

Figure 3. Lipid composition of HDL particles in mothers with normal pregnancy, early-onset PE and late-onset PE (**A**) and their offspring (**B**). Individual data are presented on top of boxplots, showing the median, interquartile range, and minimum and maximum values. Differences between the groups were analyzed by the Kruskal–Wallis test, followed by multiple comparisons, corrected according to Bonferroni. * $p < 0.05$. (Maternal: early-onset PE, $n = 18$; late-onset PE, $n = 14$; normal pregnancy, $n = 32$; cord blood: early-onset PE, $n = 17$; late-onset PE, $n = 13$; normal pregnancy, $n = 32$).

Analyses revealed that maternal HDL apoC-II levels were inversely correlated with HDL triglycerides ($r_S = -0.287$, $p = 0.026$), consistent with the concept that apoC-II is a cofactor of lipoprotein lipase [48].

3.4. Preeclampsia Affects Maternal HDL Subclass Distribution

HDL particles are heterogeneous in structure and composition, providing the basis for their functional variability. HDL particles can be divided into large and cholesterol-

rich HDLs, and protein-rich and denser small HDLs, which also vary in their protective properties [49,50].

We next determined HDL subclass distribution. By using the Quantimetrix Lipoprint© system, we determined 10 different HDL subclasses based on their size, which were then grouped into large, intermediate and small HDL subclasses. Of particular interest, maternal early-onset PE group showed reduced levels of large and cholesterol-rich HDL particles, whereas small HDLs were increased when compared to normotensive controls (Figure 4A). No differences in HDL subclass distribution were observed in the cord blood of PE pregnancies (Figure 4B).

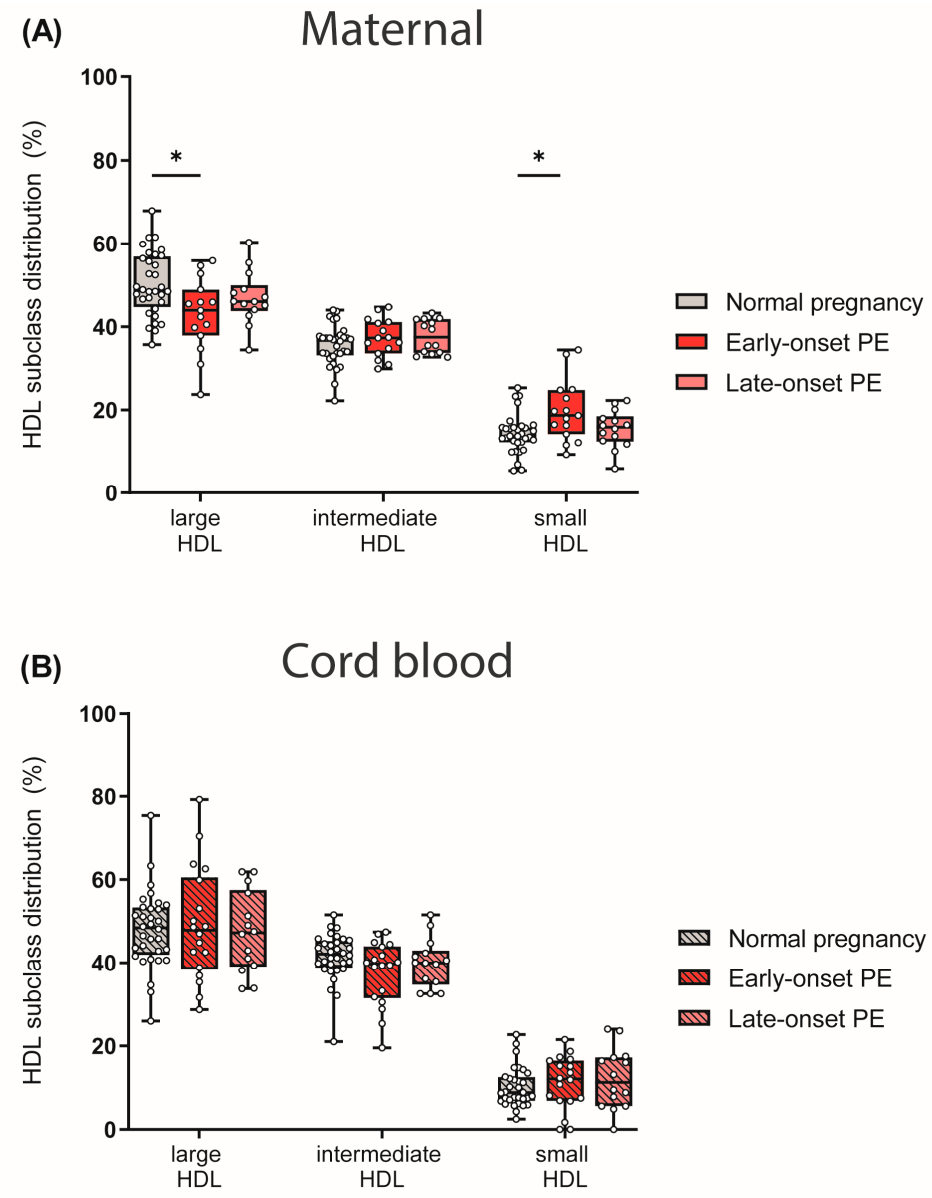

Figure 4. The distribution of HDL subclasses in mothers (**A**) with normal pregnancy, early-onset PE, and late-onset PE and in their offspring (**B**) was investigated. HDL subclasses were determined using the Quantimetrix Lipoprint© system. Individual data are presented on top of boxplots, showing the median, interquartile range, and minimum and maximum levels. Differences between the groups were analyzed by the Kruskal–Wallis test, followed by multiple comparisons, corrected according to Bonferroni. * $p < 0.05$. (Maternal: early-onset PE, $n = 17$; late-onset PE, $n = 14$; normal pregnancy, $n = 32$; cord blood: early-onset PE, $n = 18$; late-onset PE, $n = 14$; normal pregnancy, $n = 32$).

3.5. Effect of Preeclampsia on LCAT Activity in Mother and Child

LCAT is critically involved in HDL metabolism, catalyzing an important step in HDL maturation [49]. This enzyme esterifies free cholesterol to cholesteryl-ester, which converts nascent HDL into the mature and larger spherical form [51]. Given the observed differences in the distribution of HDL subclasses, we were interested in whether PE affects the enzyme activity of LCAT. Interestingly, we observed that PE had no effect on LCAT activity in maternal plasma, despite the revealed shift in HDL subclass distribution. However, we observed a reduction in LCAT activity in the cord blood plasma of early-onset PE neonates, although HDL subclass distribution in the cord blood was unaltered (Figure 5).

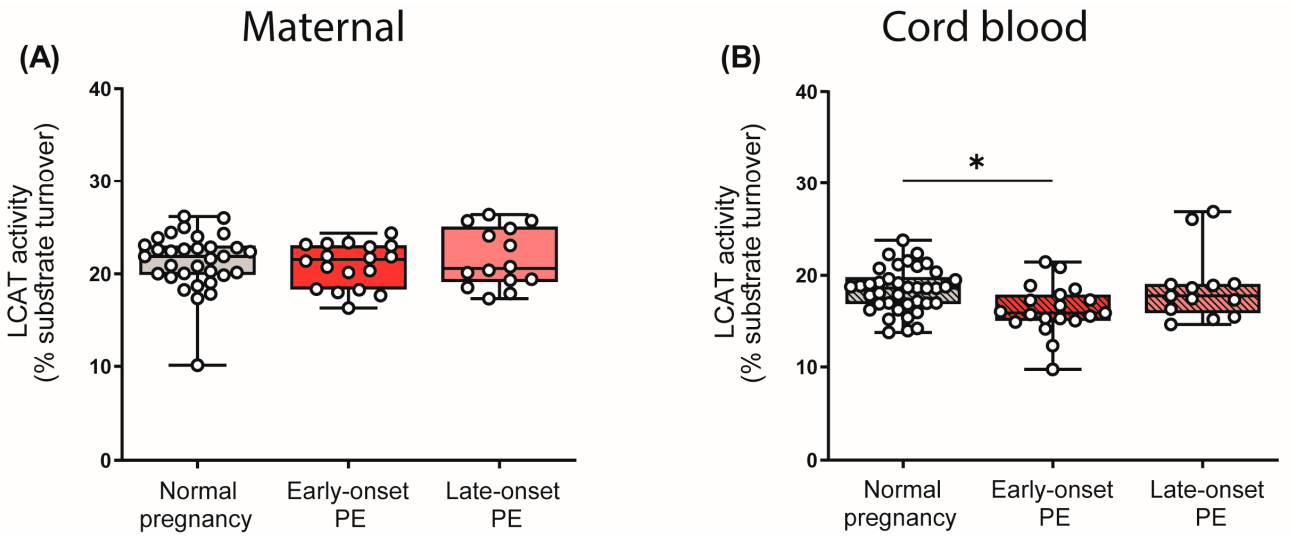

Figure 5. Enzyme activity of lecithin-cholesterol acyltransferase (LCAT) in the study cohort. The activity of LCAT was assessed in maternal plasma (**A**) and in the offspring (**B**). Individual data are presented on top of boxplots, showing the median, interquartile range, and minimum and maximum levels. Differences between the groups were analyzed by the Kruskal–Wallis test, followed by multiple comparisons, corrected according to Bonferroni. * $p < 0.05$. (Maternal: early-onset PE, $n = 17$; late-onset PE, $n = 14$; normal pregnancy, $n = 32$; cord blood: early-onset PE, $n = 18$; late-onset PE, $n = 14$; normal pregnancy, $n = 32$).

3.6. Effects of Preeclampsia on Parameters of HDL Function

HDL particles are well known to be athero-protective by promoting reverse cholesterol transport [41]. In addition, HDL particles show anti-inflammatory [26] and anti-oxidative properties [27]. In our experiments, apoB-depleted plasma (containing all HDL subclasses, but no apoB-containing lipoproteins) of the mothers and the cord blood was used to measure functional metrics of HDL. HDL cholesterol efflux capacity was determined using a well-established cell-based assay [25,52]. Of particular interest, HDL cholesterol efflux capacity was significantly reduced in the cord blood of the late-onset PE group (Figure 6B), while we observed no changes in maternal samples (Figure 6A). Paraoxonase 1 (PON1) is a HDL-associated antioxidant and an anti-inflammatory enzyme [30]. We did not detect PE-associated changes in the arylesterase activity of PON1 in either maternal or cord blood (Figure 6C,D).

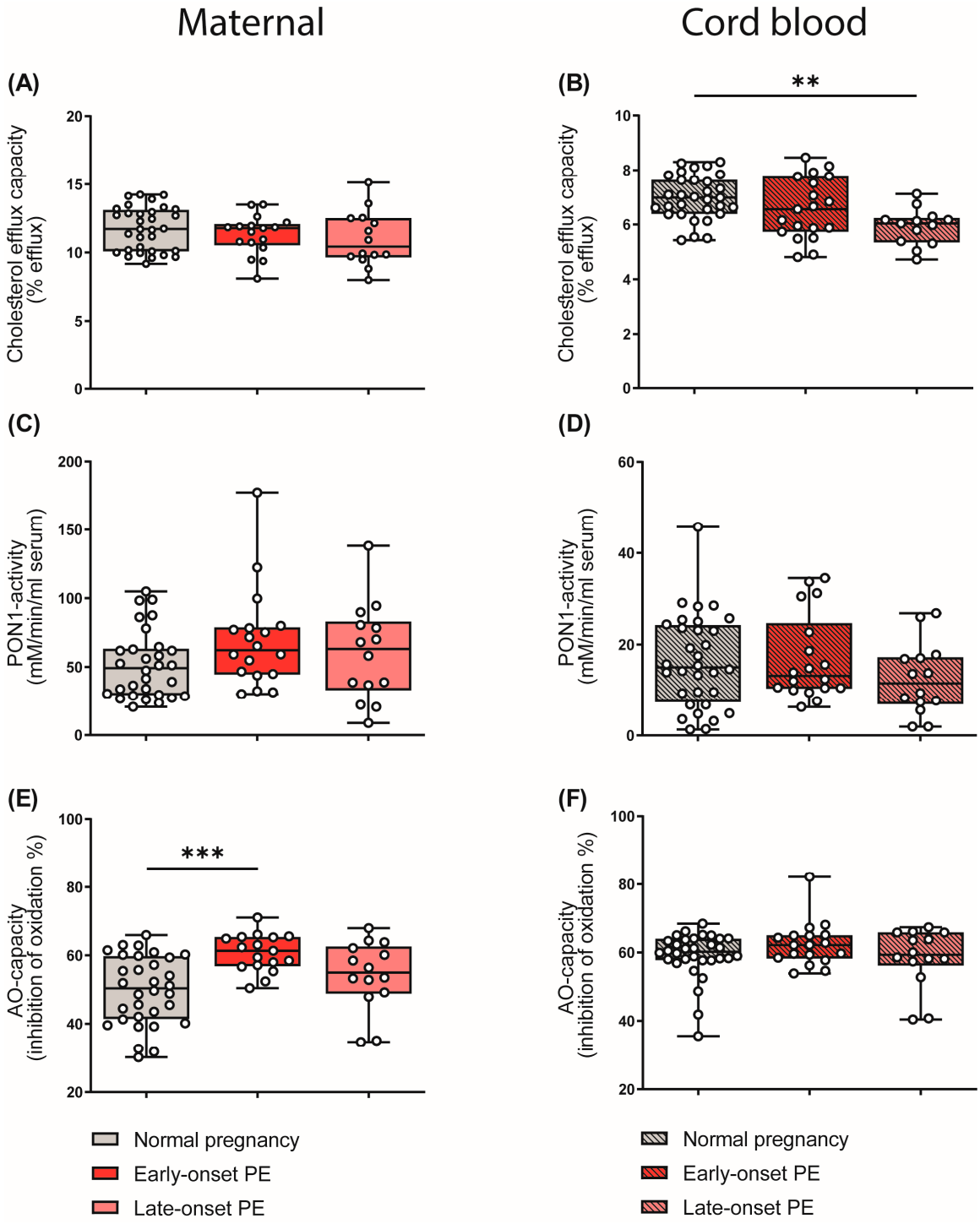

Figure 6. Differences in parameters of HDL function and plasma anti-oxidative capacity of mothers with normal pregnancy, early-onset PE, and late-onset PE, and their offspring. ApoB-depleted plasma was used to assess cholesterol efflux capacity (**A**,**B**) and the activity of HDL-associated paraoxonase-1 (PON1) (**C**,**D**) in mothers and offspring. The anti-oxidative capacity of plasma was assessed by measuring the inhibition of oxidation of a substrate (**E**,**F**). Individual data are presented on top of boxplots, showing the median, interquartile range, and minimum and maximum levels. Differences between the groups were analyzed by the Kruskal–Wallis test, followed by multiple comparisons, corrected according to Bonferroni. ** $p < 0.01$, *** $p < 0.001$. (Maternal: early-onset PE, $n = 18$; late-onset PE, $n = 14$; normal pregnancy, $n = 32$; cord blood: early-onset PE, $n = 17$; late-onset PE, $n = 13$; normal pregnancy, $n = 32$).

We next examined the total anti-oxidative capacity of apoB-depleted plasma by determining the ability of plasma to inhibit free-radical-induced oxidation of the fluorescent dye dihydrorhodamine [53]. Against the expectation, early-onset maternal PE was associated with increased plasma antioxidant capacity (Figure 6E), whereas the cord plasma antioxidant capacity was not altered (Figure 6F). Correlation analysis further revealed that the anti-oxidative capacity of plasma correlated with the shift in HDL subclass distribution to small HDL particles in maternal early-onset PE (r_S = 0.301, p = 0.010). Moreover, we found a significant correlation between plasma uric acid and anti-oxidative capacity (r_S = 0.448, p = 0.011). Plasma uric acid is commonly elevated in subjects with impaired kidney function [54] and is a powerful antioxidant [55].

3.7. Association of PE with Alterations in Subclasses of Triglyceride-Rich Lipoproteins

Women affected by PE during pregnancy have a profound increased risk for cardiovascular complications later in life [56]. Certain LDL subclasses are strongly associated with cardiovascular risk [57–59]. We next assessed the distribution of low-density lipoproteins by using the Quantimetrix Lipoprint© system. As the abundance of LDL is much lower in cord blood than in adults [60], we were not able assess LDL subclasses in offspring samples. Of particular interest, our analyses revealed a decrease of intermediate-density lipoprotein (IDL)-C in early-onset PE and a trend (p = 0.088) for reduced levels in late-onset PE, while IDL-A was significantly increased in both PE groups (Figure 7). Distribution of large LDL subclasses was higher in late-onset PE, while no differences were observed for intermediate or small LDL subclasses.

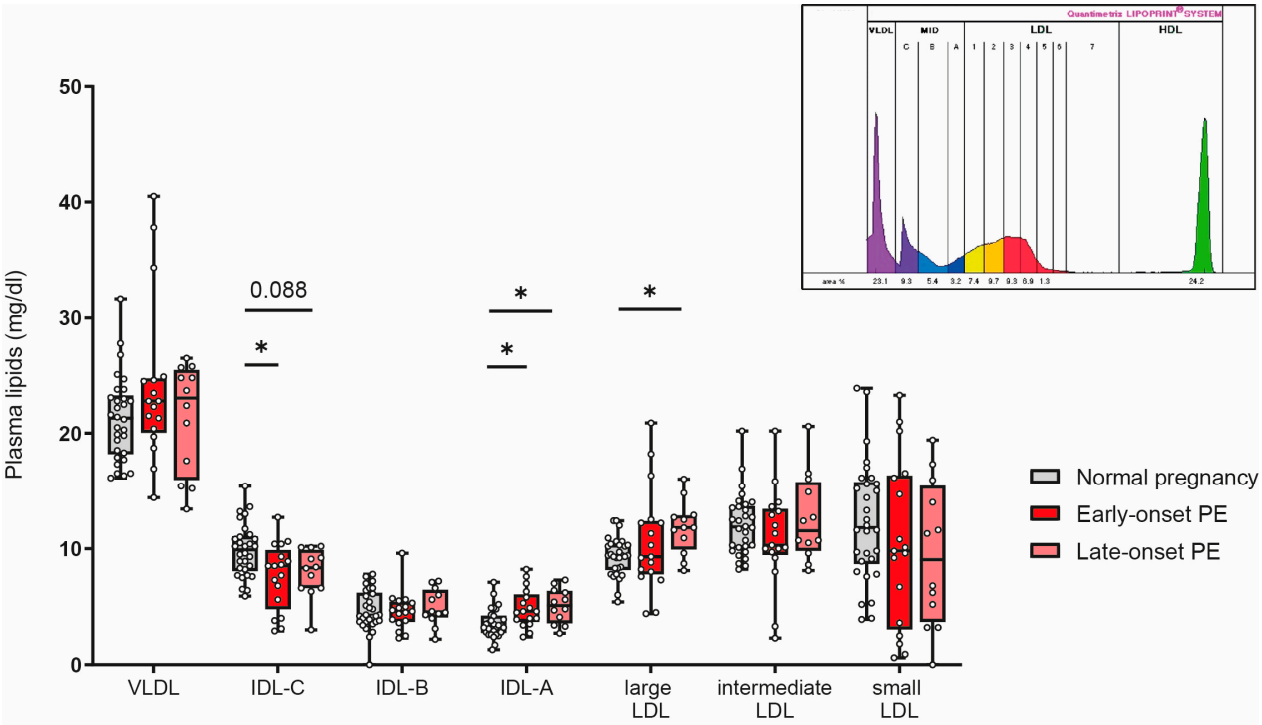

Figure 7. The distribution of low-density subclasses in mothers with normal pregnancy, early-onset PE, and late-onset PE was investigated. LDL subclasses were assessed using the Quantimetrix Lipoprint© system. An example of a graph of low-density lipoprotein subclass distribution in a pregnant women is shown in the insert. Individual data are presented on top of boxplots, showing the median, interquartile range, and minimum and maximum levels. Differences between the groups were analyzed by the Kruskal–Wallis test, followed by multiple comparisons, corrected according to Bonferroni. * $p < 0.05$. (Maternal: early-onset PE, n = 17; late-onset PE, n = 12; normal pregnancy, n = 30).

4. Discussion

Lipoproteins are most commonly studied for their role in transporting lipids and the maintenance of tissue lipid homeostasis, and are therefore usually defined by measures of triglyceride and cholesterol content. Recent studies have shown that primarily HDLs are involved in a number of critical physiological functions, many of which are involved in ensuring a healthy pregnancy [22]. In this exploratory study, we observed PE-associated changes in lipoprotein metabolism, affecting both the mothers and their offspring. These changes lead to altered biological activity of HDL particles and changes in triglyceride-rich lipoproteins, which could contribute to the manifestation of this pregnancy complication and increased morbidity and mortality.

4.1. PE-Associated Alterations in Lipid Metabolism and HDL Function in Mothers

A few studies have reported that maternal hyperlipidemia is associated with the risk of PE [46,61]. Moreover, a meta-analysis revealed that PE is linked to elevated total cholesterol, non-HDL-C and, particularly, triglycerides, regardless of gestational age at the time of blood sampling [46]. In this study, we confirmed the association of plasma triglycerides with an observed increase of about 30% in early-onset PE and late-onset PE, when compared with the normotensive control group. In line with our observed results, lower levels of HDL-C in the third trimester have also been previously reported in PE [46,62,63].

Strong evidence is accumulating that hypertriglyceridemia is associated with endothelial dysfunction. Hypertriglyceridemia is accompanied by an increase in free fatty acids, which are then increasingly taken up by endothelial cells and further esterified to triglycerides [64]. The accumulation of triglycerides can harm endothelial cells and may contribute to endothelial dysfunction in PE [65]. The combination of hyperlipidemia with increased oxidative stress found in preeclamptic mothers could lead to the accumulation of oxidized lipids in the arterial wall, promoting inflammation and cardiovascular disease.

Metabolic characteristics, besides hypertriglyceridemia in PE, are hyperuricemia, hyperinsulinemia and low levels of large HDL (HDL2) particles, which are similar to the main features of insulin resistance [66]. Consistent with these previous findings, we observed lower HDL-cholesterol levels in mothers and a shift in HDL subclass distribution from large to small subclasses in early-onset PE. These changes in HDL subclass distribution might be explained by a reported increased activity of hepatic lipase in PE when compared to normotensive pregnancy [67]. Additionally in good agreement with previous reports, we observed that early- and late-onset PE were associated with markedly increased plasma triglyceride levels.

To determine whether PE is associated with alterations in HDL composition, which strongly determines HDL-protective functions [23,68–70], we evaluated the distribution of the most abundant apolipoproteins and the concentration of HDL-associated lipids.

In preeclamptic mothers, the HDL content of apoC-II was increased. Moreover, the HDL content of apoC-II was inversely correlated with concentration of HDL triglycerides, consistent with the fact that apoC-II is a cofactor of lipoprotein lipase, the main enzyme promoting hydrolysis of triglycerides in plasma [71].

Of particular interest, analyses of LDL subclass distribution revealed a lower percentage of IDL-C, the first intermediate particle formed after hydrolysis of VLDL in PE, while IDL-A and the large LDL subclasses were increased.

In contrast to our results, earlier studies have shown an increase in small atherogenic LDL particles and a decrease in the large buoyant LDL [67]. However, we detected no differences in small LDL, similar to a previous study [72].

One major characteristic of PE is increased oxidative stress in the maternal circulation [5,73], therefore, we investigated the effects on plasma anti-oxidative capacity. We observed that maternal anti-oxidative activity of plasma in the early-onset PE group was markedly increased. The higher anti-oxidative capacity related to hypertension during pregnancy might indicate a compensatory mechanism in response to increased oxidative stress in the circulation. Interestingly, we observed that plasma anti-oxidative capacity cor-

related with the PE-associated shift from large HDL to small HDL subclasses in early-onset PE. This might be explained, at least in part, by the fact that small, dense HDLs are known to exhibit potent anti-oxidant activity, which may arise from synergy in the inactivation of oxidized lipids by enzymatic and nonenzymatic mechanisms [74]. Moreover, we found a significant correlation between uric acid [54] and anti-oxidative capacity. Plasma uric acid is commonly elevated in subjects with impaired kidney function [54] and is a powerful antioxidant and scavenger of singlet oxygen and radicals [55,75]. Uric acid and other hydrophilic antioxidants could explain the relationship between plasma antioxidant capacity and markers of renal dysfunction [76]. However further studies are warranted to underline this, so far speculative, hypothesis. We further examined the activity of the HDL-associated PON1, an antioxidant and anti-inflammatory enzyme [30]. We found that PE did not affect PON1 activity. This is in contrast to some previous studies reporting decreased PON1 activity in mothers diagnosed with PE [77,78], but these studies used methods of measuring PON1 activity other than assessing arylesterase activity in apoB-depleted plasma.

4.2. PE-Related Alterations in Lipid Metabolism and HDL Function in Neonates

In contrast to previous studies that focused mainly on PE-affected mothers, we also emphasized measurements of HDL metabolism, composition and function in the corresponding cord blood of the offspring. The availability of many substrates for the fetus depends on their concentration in the maternal circulation and the extent to which they are transported across the placenta [79,80]. Therefore, it is also reasonable to assume that lipoprotein metabolism in PE is altered not only in the affected mothers, but also in the offspring.

We observed that total cholesterol, as well as non-HDL-C, were profoundly increased in neonates of early-onset PE, while triglyceride levels and HDL-C were unaltered. These results of lipid measurements in PE cord blood are in line with a previous study [81]. However, it has to be noted that in our study, these changes were only seen in early-onset PE, which represents the more severe type. Interestingly, we observed no differences in the composition of apolipoproteins of cord-blood-derived HDL. In line with previous reports [82], we observed that apoE levels in HDL from neonates were more than three times higher than in HDL from adults. Since apoE binds with high affinity to the LDL receptor, it appears that the primary function of apoE-enriched neonatal HDL may be cholesterol transport to tissues, as is carried out in adults by LDL [82].

We were further interested in whether PE is associated with changes in protective functions of HDL in neonates. ApoB-depleted plasma was used to assess the cholesterol efflux capacity of HDL, an anti-atherogenic property of HDL, which has been shown to be inversely correlated with coronary artery disease, independent of HDL-cholesterol concentrations [41]. While our analyses did not reveal PE-associated changes in maternal samples, cholesterol efflux capacity of HDL in neonates of late-onset PE pregnancies was profoundly reduced. Of particular interest, we have previously observed a reduction in HDL cholesterol efflux capacity in neonates of pregnancies affected by gestational hypertension, whereas no significant changes were seen in mothers [83]. Similar to our results, a previous study reported a PE-associated decrease in ABCA1-mediated HDL cholesterol efflux capacity in neonates; however, in that study, an increased total cholesterol efflux capacity of maternal plasma was observed [84].

We observed that neonates with early-onset PE depicted reduced LCAT activity, but this was not associated with changes in HDL apolipoprotein levels or HDL subclass distribution. LCAT is a key enzyme involved in the remodelling of HDL by esterifying free cholesterol on HDL surface, which leads to particle maturation [85].

Moreover, we observed that in neonates the anti-oxidative activity of plasma, as well as PON1 activity, were not significantly altered, although maternal plasma anti-oxidant activity was significantly increased in the early-onset PE group.

Some limitations should be mentioned. The control group with normal pregnancy differed from the group with early-onset PE in gestational age, accompanied by lower

placental and fetal weights. However, the group with late-onset PE did not differ in gestational age, but showed alterations similar to those of the group with early-onset PE. Nevertheless, we cannot exclude the possibility that premature birth affected the observed changes. Moreover, our study is exploratory and the design is limited to being correlative in nature, not permitting causal inference. Further studies in larger cohorts are needed to confirm our results and draw firm conclusions.

5. Conclusions

In this study, we demonstrated that PE is associated with marked changes in maternal lipid metabolism. Of particular interest, PE also was associated with changes in neonatal HDL composition and function, demonstrating that complications of pregnancy affect neonatal lipoprotein metabolism. Despite the presumed different origin of early- and late-onset PE, we observed similar differences in maternal plasma lipid levels and HDL composition.

Notably, early-onset PE led to a shift in HDL subclass distribution from large to smaller particles, associated with an increase in plasma anti-oxidative capacity. Moreover, neonates of mothers affected by late-onset PE showed markedly reduced HDL cholesterol efflux capacity, whereas early-onset PE depicted a decreased LCAT activity. In conclusion, our results suggest that early-onset, as well as late-onset, PE affect maternal and neonatal lipid metabolism, potentially contributing to disease manifestation and increased cardiovascular risk later in life. However, larger studies are needed to confirm our results and investigate whether these changes persist after the birth of the child.

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Institutional Review Board Statement: The study protocol was approved by the local ethical committee of the Medical University of Graz (26-333 ex 13/14) and performed according to the Declaration of Helsinki.

Informed Consent Statement: Informed written consent was obtained from all study participants.

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Review

Fetal High-Density Lipoproteins: Current Knowledge on Particle Metabolism, Composition and Function in Health and Disease

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Abstract: Cholesterol and other lipids carried by lipoproteins play an indispensable role in fetal development. Recent evidence suggests that maternally derived high-density lipoprotein (HDL) differs from fetal HDL with respect to its proteome, size, and function. Compared to the HDL of adults, fetal HDL is the major carrier of cholesterol and has a unique composition that implies other physiological functions. Fetal HDL is enriched in apolipoprotein E, which binds with high affinity to the low-density lipoprotein receptor. Thus, it appears that a primary function of fetal HDL is the transport of cholesterol to tissues as is accomplished by low-density lipoproteins in adults. The fetal HDL-associated bioactive sphingolipid sphingosine-1-phosphate shows strong vasoprotective effects at the fetoplacental vasculature. Moreover, lipoprotein-associated phospholipase A2 carried by fetal-HDL exerts anti-oxidative and athero-protective functions on the fetoplacental endothelium. Notably, the mass and activity of HDL-associated paraoxonase 1 are about 5-fold lower in the fetus, accompanied by an attenuation of anti-oxidative activity of fetal HDL. Cholesteryl ester transfer protein activity is reduced in fetal circulation despite similar amounts of the enzyme in maternal and fetal serum. This review summarizes the current knowledge on fetal HDL as a potential vasoprotective lipoprotein during fetal development. We also provide an overview of whether and how the protective functionalities of HDL are impaired in pregnancy-related syndromes such as pre-eclampsia or gestational diabetes mellitus.

Keywords: HDL; fetal development; pregnancy; sphingosine-1-phosphate; $LpPLA_2$; gestational diabetes mellitus; preeclampsia

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1. Introduction

Cholesterol is an essential constituent in fetal development [1,2]. It has long been assumed that the fetus synthesizes most of its cholesterol requirements for growth de novo. However, in vitro and in vivo studies have shown that maternal circulating cholesterol can influence fetal metabolism [3,4]. Maternal dyslipidemia which is reflected either by an excess-, or also by limited cholesterol in the fetal circulation can affect fetal growth and health [5]. Maternally derived lipoproteins carrying cholesterol bind to their respective receptors expressed on the syncytiotrophoblast of the placental villi [6–8]. After cholesterol is taken up into the syncytium it is transported across the placental stroma to the fetal side. The exact transport mechanism is still elusive. At the endothelium of the fetoplacental vasculature, cholesterol is then transported via ATP-binding cassette G1 (ABCG1) or ATP-binding cassette A1 (ABCA1) to acceptors such as fetal HDL or lipid-poor apolipoproteins [4,9]. Interestingly, cholesterol is mainly carried by HDL in the fetal circulation, whereas in adults the majority of cholesterol is carried by low-density lipoproteins (LDL).

Fetal HDL shows a unique composition and is suggested to exert different functions as in adults [10]. The current review focuses on the role of lipids carried by HDL in fetal circulation and the importance of exogenous cholesterol supply by the mother. Here, we summarize the proteomic composition of fetal HDL and highlight conspicuous changes compared to adult HDL. Further, we discuss the relationship of fetal HDL and sphingosine-1-phosphate (S1P) and the importance of S1P signaling at the fetoplacental vasculature in maintaining vascular integrity. Finally, the impact of pregnancy-associated disorders, such as preeclampsia (PE) and gestational diabetes mellitus (GDM) on HDL metabolism and function is comprehensively discussed.

2. Changes in Maternal Lipid Metabolism during a Normal Pregnancy

During pregnancy, multiple physiological changes occur that strongly influence maternal lipid metabolism. In the first two trimesters of pregnancy, maternal lipid metabolism is primarily anabolic and characterized by several factors that increase lipid accumulation in maternal tissues in preparation for the exponential increase in fetal energy requirements later in pregnancy [11,12]. These factors include maternal hyperphagia, to increase the availability of exogenous metabolic substrates [11,13] and an increase in insulin sensitivity which results in increased lipogenesis [14]. In the anabolic phase, hormonal and metabolic changes occur such as cortisol and leptin stimulation, and increased progesterone levels contribute to the accumulation of maternal fat depot [14].

During the last trimester of pregnancy, the lipid metabolism changes to the catabolic phase with a decline of fat accumulation [15]. This phase is characterized by increased lipolysis and mobilization of triglycerides from adipocytes. Furthermore, these changes are accompanied by a decrease of lipoprotein lipase (LPL) activity, leading to inefficient clearance of triglyceride-rich lipoproteins [16,17]. Maternal hyperlipidemia in late pregnancy coincides with changes in insulin sensitivity, which consistently decreases during this phase [18]. This decline is mediated by several factors, including increased levels of estrogen, placental lactogen, and progesterone [19].

During pregnancy, the lipid profile of mothers shows a 2.5-fold increase in very-low-density lipoprotein (VLDL) triglycerides and cholesterol and a 1.6-fold increase in LDL-cholesterol compared to non-pregnant women [20]. Plasma levels of VLDL and LDL steadily increase during gestation, while HDL levels show the highest rise in midgestation (45% above baseline) followed by a decline at term to about 15% [20]. HDL subclass analysis showed that levels of the triglyceride-rich HDL2 rise, while the smaller, lipid-poor HDL3 becomes less abundant [17]. These well-described alterations in lipoproteins, which are responsible for respective changes in maternal lipid profile during gestation are explained by several mechanisms: The increase of insulin resistance in late pregnancy mediates the elevated lipolytic activity in adipocytes, resulting in increased accessibility of substrates for triglyceride production in the liver [21,22]. Together with the decreased activity of LPL [17] and the stimulative effect of estrogen [23], these metabolic adaptions lead to an increased hepatic production of VLDL. The increased activity of the cholesteryl-ester transfer protein (CETP), which mediates the transfer of triglycerides on lipoproteins with higher density, contributes to the enrichment of triglycerides in HDL and LDL [17,24]. Another factor, contributing to the increase of triglyceride-rich HDL, is the reduced hepatic lipase activity, which reduces the clearance of HDL2 to smaller HDL3 [25].

Maternal hyperlipidemia during pregnancy is a prerequisite for delivering sufficient lipids of lipoproteins to the fetus. However, reduced or too high cholesterol supply to the fetus may lead to long-term consequences to the fetus [26].

3. Importance of Cholesterol in Fetal Development

Cholesterol is an essential constituent in embryonic and fetal development. It is a crucial component of cell membranes by defining fluidity and permeability. Further, cholesterol is an integral part of membrane microdomains, such as lipid rafts, which are essential for plasma-membrane-dependent signaling cascades. Cholesterol is a precursor

of steroid hormones, including progesterone, and of its oxidative derivate oxysterol, which plays an important role in several metabolic processes [27].

The high requirements of cholesterol for the developing fetus have been described with 1.5–2.0 g of accumulated cholesterol per kg of added tissue [28]. The endogenous cholesterol originates from either de novo biosynthesis or hydrolysis of intracellular cholesteryl deposits by cholesterol esterases [29]. The fetus additionally possesses the capability to cover its demand of cholesterol from exogenous deposits. Yolk sac in early pregnancy and later the placenta has the same property to store maternally derived cholesterol [13]. The fact that the fetus does not rely on its own endogenously cholesterol was demonstrated in fetuses with the Smith-Lemli-Opitz syndrome, a condition with an inborn error of cholesterol synthesis. Fetuses affected by this syndrome harbor a nonsense mutation in the 7-dehydrocholesterol reductase, an enzyme that catalyzes the conversion of 7-dehydrocholesterol to cholesterol. Fetuses with this congenital condition are capable of developing to term, thereby demonstrating that maternal cholesterol needs to be transported across the placenta to maintain the demands of the fetus [13,30].

The human placenta is a unique organ, which is composed of several specialized cell types and mediates many metabolic exchange mechanisms between mother and fetus. To fulfill the demands of the fetus, nutrients and oxygen diffuse from maternal to fetal circulation by crossing directly into different cell layers. The first physical barrier, which limits nutrient transfer across the placenta is build up by the syncytiotrophoblast, a layer of multinucleated trophoblasts localized by the microvillous and basal membrane faced to the maternal and fetal side, respectively [31,32].

The first step of cholesterol transport from the mother to the fetus is the uptake on the apical, maternal side. Human placental trophoblasts express lipoprotein receptors such as scavenger receptor BI (SR-BI), LDL-receptor (LDL-R), and LDL receptor-related protein 1 (LRP1) (Figure 1) [4,6–8]. These receptors mediate the uptake of cholesterol and cholesteryl-esters from maternally derived lipoproteins [29,33]. After receptor-mediated endocytosis, the lipoprotein-associated cholesteryl-esters are intracellularly hydrolyzed [4]. Via Niemann-Pick C1 and/or other sparsely described cholesterol transporter proteins, free cholesterol is trafficked across the cell to membranes or metabolically active pools [4,34]. SR-BI mediates the selective uptake of cholesteryl-esters primarily from HDL, which are hydrolyzed by cytosolic cholesterol esterases and transported by potential carrier proteins to the basal membrane [4]. However, the exact pathway of transcellular cholesterol transport is still not known, but several transporters are thought to be involved, such as Niemann-Pick C1, Niemann-Pick C1-like protein 1, sterol carrier protein-x/2 and ABCA2. All these receptors are expressed in the human placenta [35]. To enter the fetal circulation, placental cholesterol needs to cross the endothelium at the fetoplacental vasculature. By using endothelial cells isolated from human term placentas, a study demonstrated efflux/secretion of exogenous cholesterol through ABCA1 and ABCG1 [9]. Acceptors of cholesterol in the cord blood are poorly lipidated apolipoprotein (apo) A-I (the major HDL associated apolipoprotein in adults), apoE, and HDL, with apoE-enriched HDL, was shown to be most efficient [9,35].

Maternally supplied cholesterol appears to be of great importance for fetal growth. Although there is no direct link between maternal and fetal lipoprotein metabolism, maternal serum cholesterol levels during pregnancy are directly related to infant birth weight. Low maternal serum cholesterol levels during pregnancy appear to increase the risk of microencephaly, while high maternal cholesterol levels promote the early incidence of atherogenicity [13,36]. Various further studies demonstrated a link between very high maternal cholesterol levels with prematurity and impaired fetal growth [37–39]. Dysregulated maternal cholesterol homeostasis during pregnancy has also been associated with disorders such as pregnancy-induced hypertension and preeclampsia [37,40,41].

Concluding, cholesterol plays an essential role in human fetal development and maternal hypocholesterolemia, as well as hypercholesterolemia, can affect fetal health and growth.

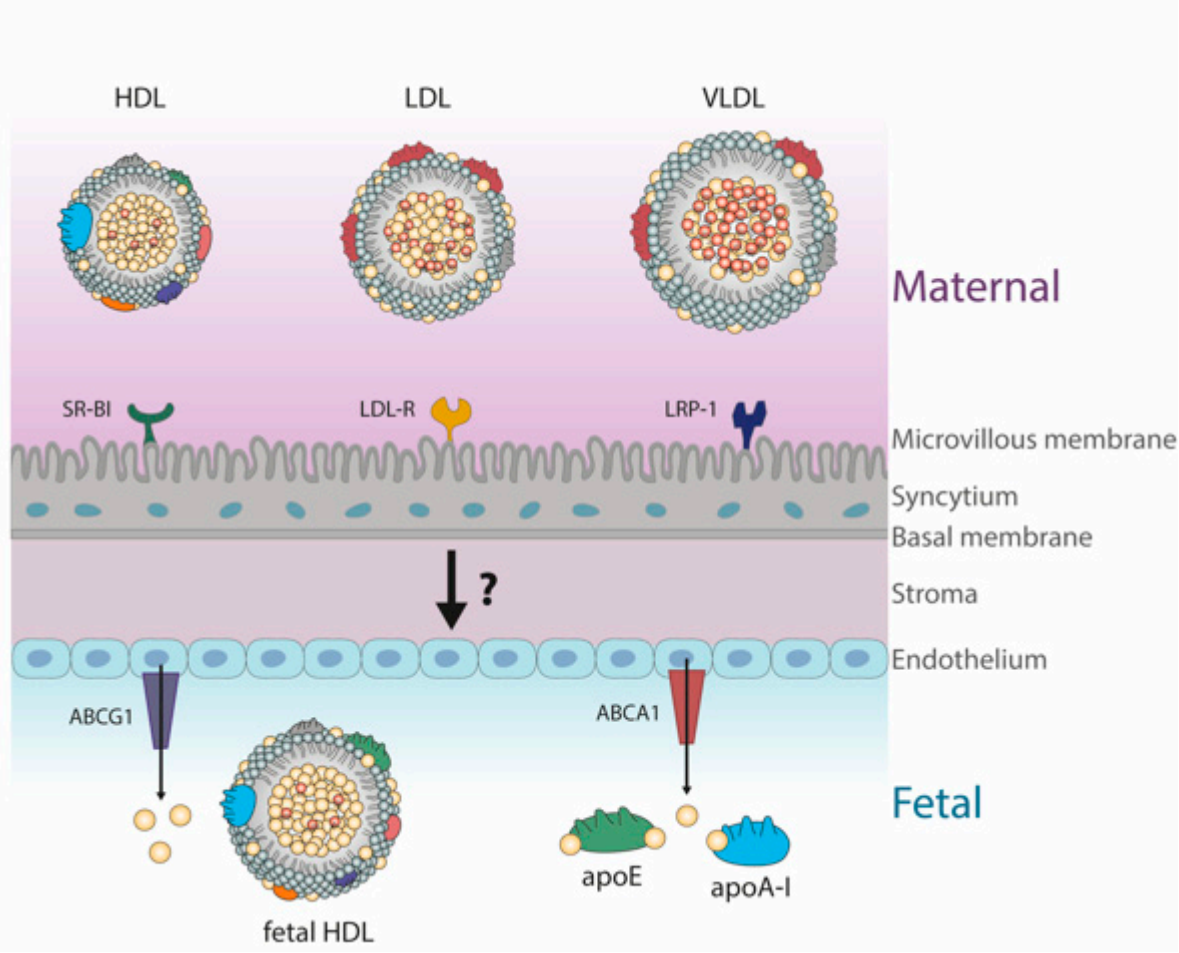

Figure 1. Described routes how maternal cholesterol is transported across the human placenta. First, maternally derived lipoproteins interact with respective receptors at the microvillous membrane of the syncytium. After uptake of cholesterol in the syncytium, it is secreted/effluxed to lipid-poor acceptor apolipoproteins of fetal HDL. How stroma transfers cholesterol to the fetoplacental endothelium remains elusive. High-density lipoprotein; SR-BI; scavenger receptor BI; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; LDL-R, low-density lipoprotein receptor; LRP-1, LDL receptor-related protein 1; ABCA1, ATP-binding cassette A1; ABCG1, ATP-binding cassette G1.

4. HDL Composition

HDLs are a group of highly heterogeneous lipoproteins, which are considered to have a high cardiovascular protective potential [42–44]. The heterogeneity of these particles depends on their size, shape, and compositional structure [45].

The major apolipoprotein in HDL is apoA-I, which accounts for around 70% of the total protein amount [46]. The second major apolipoprotein is apoA-II, which represents approximately 15–20% of total protein content [47]. The residual protein mass of HDL is composed of minor apolipoproteins, such as apoCs and apoA-IV, having an important enzyme regulatory function. ApoM is another crucial protein component on HDL, as it binds hydrophobic molecules, primarily sphingosine-1-phosphate (S1P) [48,49]. ApoE, apoD, apoF, apoJ, and apoL-I are further distinctly identified proteins on HDL whose exact roles have partly been identified. In addition, serum amyloid A (SAA), which is predominantly produced by the liver in the acute phase after an inflammatory stimulus, is mainly carried by HDL [50]. Furthermore, several enzymes are associated with HDL, including paraoxonase 1 (PON1), which has anti-inflammatory and antioxidative properties [51]. Direct binding of the enzyme to apoA-I on HDL stabilizes the protein and also stimulates PON1 lactonase activity [52]. Other HDL-associated enzymes are the lipoprotein-associated phospholipase A2 (LpPLA2) and lecithin-cholesterol-acyltransferase (LCAT). Addition-

ally, enzymes with lipid transfer activity are important in HDL metabolism, including cholesterol ester transfer protein (CETP) and phospholipid transfer protein.

The most abundant lipids in HDL are phospholipids. Phospholipids and sphingolipids make up about 40–60% of the HDL lipidome, whereas cholesteryl-ester (30–40%), free cholesterol (5–10%), and triglycerides (5–12%) are not as prominent [53]. Like HDL-associated proteins, lipids of HDL also fulfill important structural functions. The ability of HDL to mediate cholesterol efflux is markedly modulated by the characteristics of its surface lipids. Therefore, phospholipids, which compose the surface lipid monolayer of HDL are an important determinant of its ability to accept cholesterol [53]. Moreover, both, the phospholipid content [53,54] and lysophospholipid content of HDL [55–58] markedly affect its anti-inflammatory properties. Sphingosine-1-phosphate (S1P) plays an important role in maintaining vascular homeostasis, which will be discussed in more detail in 7.2. Altogether, a total of 200 lipids and 80 proteins make up the diversity of different HDL subclasses [59–61].

5. HDL Functionality

5.1. Cholesterol Efflux Capacity

The best-studied property of HDL, which is also considered as the most clinically relevant atheroprotective function of HDL, is its ability to promote reverse cholesterol transport [62]. The uptake of excessive and accumulated cholesterol from peripheral cells is the first step of reverse cholesterol transport to the liver for catabolism. Given the heterogeneity of HDL particles in terms of structure and lipidomic/proteomic composition, steady-state HDL-cholesterol (HDL-C) levels suffer from the limitations inherent in their mass-based and static measurement. As a snapshot of the steady-state cholesterol pool, HDL-cholesterol levels do not provide direct information on the rate of cholesterol flux from vascular macrophages to the liver, which is influenced by many factors beyond the mass of HDL-C. Recent evidence clearly suggests that the cholesterol efflux capacity of HDL better reflects cardiovascular disease risk than HDL-C [63,64].

The reverse cholesterol transport starts with the release of lipid poor apoA-I from the liver and intestine, which circulates to peripheral cells to take up excess cholesterol, forming nascent HDL. ApoA-I is preferentially lipidated via ABCA1 [65], while cholesterol efflux to larger HDL subclasses is stimulated by ABCG1 [66,67]. Collectively, cholesterol can be actively transferred by SR-BI, ABCA1, and ABCG1, but also via passive diffusion [68–70]. After absorption from cells, cholesterol is esterified, catalyzed by LCAT, and large and mature HDL is formed. The HDL-associated cholesteryl-esters can be further transferred to LDL/VLDL by CETP. Thus, the transport of cholesterol from peripheral cells to the liver occurs via two pathways: Direct uptake by SR-BI and indirectly through HDL-LDL/VLDL interactions [71]. Reaching the liver, cholesteryl-esters are hydrolyzed and free cholesterol is either converted into bile acids, reused for the production of VLDL, or transferred by ABCG5/G8 into the bile.

5.2. Anti-Inflammatory and Antioxidative Capacities

Circulating HDL cholesterol concentrations do not provide information about the anti-inflammatory, antioxidant, antithrombotic, and endothelial function-promoting activities of HDL. In addition to its important role in reverse cholesterol transport, HDL can inhibit the transmigration of monocytes through endothelial and smooth muscle cell co-cultures [72]. HDL inhibits the expression of adhesion molecules, including vascular cell adhesion molecule, intercellular cell adhesion molecule, and E-selectin [73–75]. Through modulation of NF-κB and PPAR gamma, HDL further decreases the production of chemokines and chemokine receptors in vivo and in vitro [76]. Because of these properties, HDL diminishes the recruitment of monocytes, lymphocytes, and basophils to the vascular endothelium, thus slowing downstream processes of inflammatory response.

In addition to its numerous anti-inflammatory effects, HDL also possesses antioxidative properties. HDL protects LDL and other lipoproteins from oxidative damage

induced by several oxidants, thereby reducing atherogenicity. ApoA-I plays a crucial role in the anti-oxidative capacity of HDL through the reduction of lipid hydroperoxides by their methionine residues [77,78]. The enzyme PON1 is associated with HDL and also contributes to the HDL-mediated antioxidative activity by reducing lipid peroxidation of LDL and HDL through a specific cysteine residue [79]. Other HDL-associated enzymes and apolipoprotein components, including $LpPLA_2$, LCAT, apoA-II, apoE, and apoJ also contribute to HDL's antioxidant properties [80–82]. Furthermore, HDL inhibits the formation of reactive oxygen species and reduces intracellular oxidative stress [83–85]. The attenuated cellular generation of ROS may be implicated in the antioxidative effect of HDL on endothelial cells [42,86].

5.3. Vasodilatory Activites

One of the most important functions of HDL is its vasodilatory effect, which is mainly seen in the increase in the availability of nitric oxide (NO) in the endothelial cells [87,88] and stimulating the generation and release of prostacyclin [89]. The initial step in the activation of NO production involves the binding of HDL to SR-BI, which initiates signaling in the endothelium [90]. The following intracellular events are facilitated by endothelial protein kinase B and intracellular Ca^{2+} mobilization, an increase in ceramide levels, and phosphorylation of endothelial NO synthase (eNOs) [42,87,91,92].

In addition, HDL, by its anti-oxidant activity, decreases the activity of nicotinamide adenine dinucleotide phosphate oxidase in the endothelium and decreases the formation of superoxide anions, which are potent inactivators of NO. Thereby, the bioavailability of NO is increased [93]. Vasodilatory actions of HDL also comprise the ABCG1 mediated efflux of cholesterol and 7-oxysterols, enhancing eNOs dimerization, leading to decreased production of reactive oxygen species [94].

6. Fetal Lipoproteins Show Altered Concentrations and Unique Composition

In cord blood, the concentration and composition of plasma lipoproteins are unique, suggesting that these particles may have an altered function in the developing fetus. While LDL represents the major class of lipoproteins in adult serum, HDL carries more than 50% of the cholesterol in fetal circulation. Although LDL and VLDL are detectable in the fetal circuit, but at low concentrations [95–99]. In the fetus, lipoproteins differ not only in concentrations but also in compositions, when compared with lipoproteins in adult plasma. In particular, the proteome of HDL has been shown to differ substantially from that in adults [10,99]. All studies investigating differences between maternal and fetal HDL found that only ApoE was present in higher concentrations compared to adult HDL, while all other apolipoproteins such as ApoA-I, ApoC-II, ApoC-III, and ApoD were lower (Figure 2) [10,95,100,101]. ApoA-I exerts a variety of important functions, such as interaction with cellular receptors, activation of LCAT, and anti-atherogenic activities [101–103]. ApoA-I further contributes to the anti-oxidative capacity of HDL, therefore lower levels in fetal HDL indicate diminished anti-oxidant function [10,95,101]. ApoE, which shows higher abundance on fetal HDL, plays an important role in cholesterol transport function by redistributing excess cholesterol from cells, to cells requiring it for metabolic processes such as membrane biosynthesis for cell proliferation or repair [104,105]. Large apoE enriched HDL particles are involved in the reverse cholesterol transport as ligands of SR-BI [9] and ABCG1 [105]. Further, apoE facilitates HDL binding to receptors of the LDL-receptor family [106]. In addition, apoE induces serum PON1 activity and stability comparable to apoA-I [107] and is reported as a major physiological activator of the lecithin-cholesterol acyltransferase (LCAT) [108]. Therefore, it appears that one function of HDL in the fetus is the transport of cholesterol to tissues as is accomplished by LDL in the adult [10,95]. Furthermore, studies on fetal HDL reported 5-fold lower PON1 mass and activity levels than in adults, which may be linked with a reduced anti-oxidative capacity and reduced defense against oxidative stress [10,109–111].

Although the proteomic differences between adult and cord blood HDL have been well described, there is currently no literature on the sphingolipid content of fetal HDL. The most abundant sphingolipid in HDL is sphingomyelin, which plays an important role in HDL functionality, by regulating fluidity and cholesterol efflux from different cells [112]. Furthermore, sphingomyelin affects the activity of enzymes involved in HDL metabolism and modulates the anti-oxidative properties of HDL [53,113]. Therefore, a highly interesting aspect for future studies would be to analyze the sphingolipidome of cord blood HDL, which could help to improve our understanding of the role and function of fetal HDL.

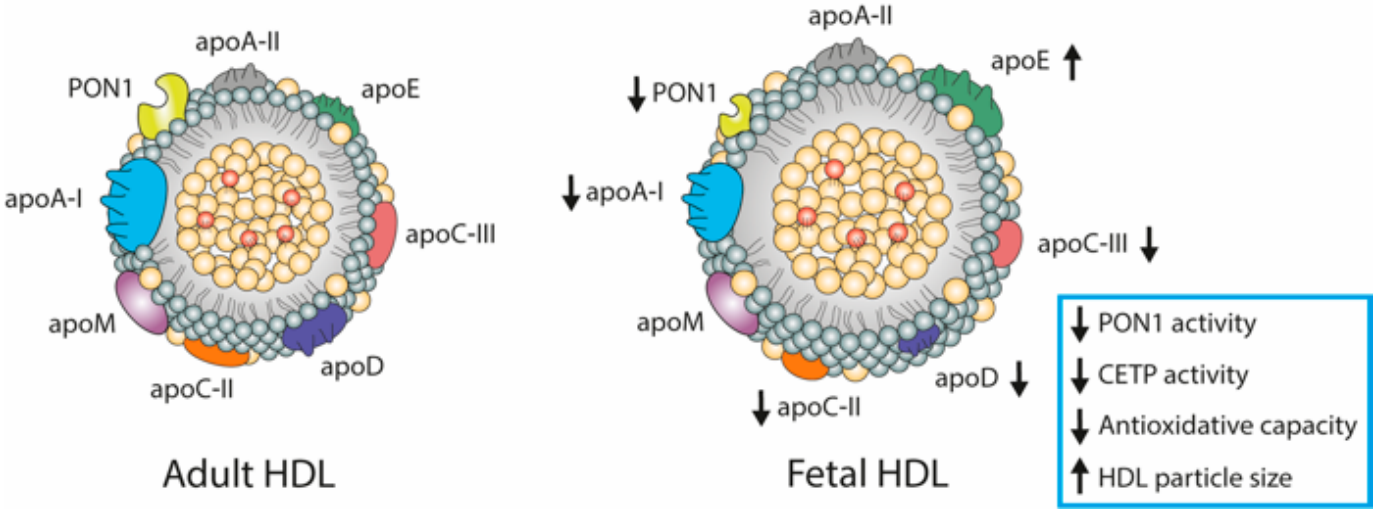

Figure 2. Schematic representation of differences between adult- and fetal HDL composition and function (indicated with black arrows). Cord blood-derived HDL exhibits several alterations in the apolipoprotein composition, such as decreased levels of apoA-I, apoC-III, apoD, and apoC-II and higher levels of apoE. In the fetus, the activity of CETP is decreased, while the mass and activity of PON1 and the antioxidative capacity are decreased. Fetal HDL is characterized by increased HDL particle size. HDL, high-density lipoprotein; apo, apolipoprotein; PON1, paraoxonase 1; CETP, cholesteryl-ester transfer protein.

HDL Metabolism in Cord Blood

Since there are strong differences in the fetal HDL composition, it is not surprising that HDL metabolism also significantly differs in fetal circulation. Sreckovic et al. showed that the activity of CETP was 55% lower in cord-compared to maternal serum, whereas LCAT activity did not differ [10]. Interestingly, it has been shown that CETP inhibition enhances the capacity of SR-BI and ABCG1 dependent efflux to the large HDL2 particles [114]. The decreased CETP activity and enrichment of HDL particles with apoE suggest a highly altered metabolism of HDL particles in fetal circulation [10]. Furthermore, analyses of subclass distribution revealed a shift in HDL subclasses, with a higher content of very large HDL particles, further supporting the hypothesis of a different physiological role of fetal HDL than in adults [109].

Fetal HDL is unique in every way, whether in composition or metabolism. However, there is not much literature on how these differences affect the function of HDL in the fetus and what specific physiological roles it may trigger.

7. The Role of Cord Blood-Derived HDL in Maintaining Fetoplacental Vascular Integrity

7.1. The Feto-Placental Endothelium

Understanding the mechanisms that underlie placental cholesterol transfer lies, at least in part, in the fetoplacental endothelium. The fetoplacental vasculature is unique in its lack of innervation, singular in being independent of the autonomic regulation to which other vascular beds are subject [115]. Therefore, locally produced vasoactive mediators such

as NO, endothelin-1, and angiotensin II regulate placental vascular resistance [115–117]. Moreover, the placental vasculature responds differently to humoral factors than vessels in other vascular beds. For example, the placental vasculature is the only vascular bed that has been reported to constrict rather than dilate in response to prostaglandin E2. It also demonstrates blunted responses to other vascular mediators including acetylcholine, bradykinin, and angiotensin II [118–120]. Interestingly it has been shown that inhibited and impaired angiogenesis further contribute to placental vascular resistance in fetal growth-restricted pregnancies, creating structural changes that restrict blood flow [118]. This study underpins the importance of an adequate perfusion of the placental tissue for peri- and postnatal health of the offspring.

The fetoplacental circulation allows the villous arteries to carry deoxygenated and nutrient-depleted fetal blood via the cord from the fetus to the placenta. After the exchange of oxygen and nutrients in the tissue, the villous veins carry fresh oxygenated and nutrient-rich blood circulating back to the fetal systemic circulation [121].

Studies have shown that an imbalance in the production of these vasoactive agents in the placenta is associated with the incidence of pregnancy disorders [122,123].

7.2. HDL-Sphingosine-1-Phosphate (S1P) as an Important Regulator of the Feto-Placental Vasculature

S1P is a bioactive lipid and is involved in the regulation of the vasomotor tone through induction of NO and prostacyclin synthesis [87,124]. In the circulation, this sphingolipid is mainly produced by erythrocytes, platelets, and vascular endothelial cells [125,126]. Once released from these cells into the bloodstream, S1P mainly binds to HDL via binding to apoM, while a small fraction is transported by albumin or other lipoproteins [49]. It has been shown that the half-life of HDL-associated S1P is 4-fold increased, when compared to S1P linked to albumin, indicating the importance of the carrier protein [127]. S1P is a ligand for five different G protein-coupled receptors, named S1P receptors 1-5 (S1PR1-5) [128]. On endothelial cells, S1PR1-3 are expressed, with S1PR1 showing the highest abundance. Through interaction with S1PR1, S1P can activate several signal cascades, which play a key role in vascular homeostasis. Mice lacking the endothelial S1PR1 exhibit a pro-inflammatory phenotype, showing the significance of S1P-S1PR1 signal transduction on vascular protection [129]. Several studies suggest that S1P signaling is responsible for many of the cardio-protective properties of HDL, including the enhancement of endothelial barrier function and the induced vasodilator production [87,124,130]. Interestingly, during disorders such as cardiovascular disease or diabetes, the functionality of HDL-S1P has been shown to be reduced [131–133]. However, there are only a few available studies on the influence of HDL-S1P on the fetus and the fetoplacental unit.

In a study examining S1P in cord blood-derived HDL, S1P was shown to be present on fetal HDL and also bound to apoM, as is the case in the maternal circulation [134]. Further, S1PR1 was found as the predominant receptor expressed on the fetoplacental vasculature [134]. Ligation of S1P with its receptors elicits cell-type-specific cytoskeletal rearrangements [49,135,136]. Experiments on the effect of fetal HDL on cytoskeletal remodeling revealed that S1P-HDL isolated from cord blood triggers reorganization of actin filaments, resulting in an enhanced placental barrier function [134]. Using human umbilical vein endothelial cells, Wilkerson et al. also showed that HDL-associated S1P strengthens the endothelial barrier more persistently than albumin-bound S1P [137]. Moreover, Del Gaudio and colleagues observed that fetal HDL induces vasorelaxation of precontracted placental chorionic arteries [134]. The same authors further investigated the role of cord blood-derived HDL and S1P on the fetoplacental endothelium [138]. Primary fetal placental endothelial cells were approached by and challenged with TNFα to induce inflammation. They showed that incubation with fetal HDL-S1P complex from healthy donors diminished the ability of TNFα to activate signaling of NF-κB and expression of pro-inflammatory markers [138]. Angiotensin II is a stimulator of NADPH oxidase, which produces reactive oxygen species, leading to a vascular inflammatory response [139]. After treatment of primary fetal placental endothelial cells with angiotensin II, the production of reactive oxygen

species was blunted in the presence of fetal HDL-S1P, whereas it was preserved when pre-incubated with an S1P receptor antagonist, suggesting that S1P signaling accounts for some of the vasculoprotective functions of HDL at the fetoplacental endothelium [138].

7.3. Protective Functions of Lipoprotein Associated Phospholipase A2 (LpPLA$_2$) on the Feto-Placental Endothelium

The enzyme LpPLA$_2$ is mainly produced by macrophages and binds to lipoproteins when secreted into circulation. In adults, LpPLA$_2$ is mainly bound to LDL (80%), while the remainder is bound to HDL [140]. The preferred substrate for hydrolysis of LpPLA$_2$ represents the platelet-activating factor (PAF), which is an important mediator of inflammation [141]. Activity and mass of LpPLA$_2$ are altered in several pathologies such as hypercholesterolemia, diabetes, essential hypertension, and atherosclerosis and have therefore been the target of many clinical studies [142–145].

In a study focusing on LpPLA$_2$ in the fetal circulation, HDL was identified as the major carrier, which is in contrast to adults [146]. In addition, this study reported that placental macrophages express LpPLA$_2$, whose activity was increased by insulin, pro-inflammatory cytokines, and leptin [146]. Fetal HDL- LpPLA$_2$ was shown to have a beneficial effect on endothelial barrier function, which was abrogated with a specific LpPLA$_2$ inhibitor [146]. Interestingly, LpPLA$_2$ levels in cord blood were inversely correlated with markers of oxidative stress [146]. These results suggest an important role of LpPLA$_2$ on the placental endothelium and the fetus through athero-protective and anti-oxidative actions.

8. Pregnancy-Related Diseases Affects HDL Metabolism and Function

Severe changes in HDL metabolism as well as in parameters of HDL function have been reported in several inflammatory conditions including obesity [147–149], diabetes [150–152], cardiovascular disease [153,154], chronic kidney disease [155–157] or liver disease [158,159]. Impairment of HDL function may have pro-atherogenic properties and promote the inflammatory state. Changes in HDL functionalities have also been demonstrated in pregnancy-related diseases such as preeclampsia and gestational diabetes mellitus, which we will briefly summarize in the following chapter.

8.1. Preeclampsia Associated Changes in HDL Composition and Function

Preeclampsia (PE) is a hypertensive pregnancy-associated disorder, which develops usually after 20 weeks of gestation. This syndrome affects 2–8% of pregnancies worldwide and is a leading cause of maternal and fetal mortality [160,161]. This multiorgan disorder is defined as *de novo* hypertension (systolic blood pressure $\geq$ 140 mm Hg, diastolic blood pressure $\geq$ 90 mm Hg) and proteinuria ($\geq$300 mg/24 h) [162]. Risk factors for the development of PE are pre-pregnancy body mass index, age, ethnicity (black women are at higher risk), primiparity, multiple pregnancies, and history of certain diseases before pregnancy such as chronic hypertension, diabetes mellitus, or renal disease [163]. In countries with low- and middle-income, PE and its convulsive form eclampsia account for 10–15% of direct maternal deaths [164,165]. This disorder is also associated with profound risks for the fetus including preterm birth, growth retardation, and death [166]. Mothers, affected by PE, but also their infants have a higher risk to develop cardiovascular disease later in life [167,168]. Nowadays, the only definitive treatment for PE is the management of clinical symptoms and delivery of the baby, which in turn increases the rate of preterm birth [165,169]. As the primary cause of PE, it has been suggested that impaired placentation and the subsequent systemic activation of the endothelium results in clinical manifestations [164].

During a healthy pregnancy, the vascular function has been shown to improve with gestational age [170], whereas obesity, a risk factor for PE, reduces endothelium-dependent and –independent vasodilation in mothers [171]. Interestingly, a study reported flow-induced dilatation in isolated vessels from healthy pregnant women, but not in arteries isolated from women diagnosed with PE [172]. These results suggest that enhanced responses to shear stress in the maternal circulation during pregnancy are important and, when absent as in PE, may contribute to the increase in maternal blood pressure [172].

Dyslipidemia in mothers diagnosed with PE has been reported in several studies, characterized by higher levels of total cholesterol, non-HDL-C, and triglycerides, but lower levels of HDL-C during the third trimester [173]. Due to the cardioprotective properties of HDL, changes in its function may contribute to the increased risk of cardiovascular events later in life in mothers, but also in children [174,175].

Einbinder et al. observed a decrease in PON1 lactonase activity in mothers affected by PE, indicating a decreased anti-oxidative and anti-inflammatory activity of HDL [176] (Figure 3). Moreover, they observed lower expression of endothelial NO synthase and an increased expression of the adhesion molecule VCAM-1 after preincubating human umbilical vein endothelial cells with isolated HDL from PE mothers [176]. Other studies focusing on structures of HDL and LDL in PE reported marked oxidative modifications, such as malondialdehyde and lipohydroperoxides in lipids and proteins of the isolated particles [177,178]. These results indicate that the markedly altered lipoprotein profile is due to PE-driven oxidative stress in the maternal systemic circulation. Other studies confirmed the reduction of PON1 activity in mothers suffering from PE, possibly due to PE-associated increased oxidative stress [177,179–181].

Of particular interest, the PE-associated oxidative modifications of lipids in HDL and LDL were also found in fetal lipoproteins, showing that also the infants are affected by increased oxidative stress and clear transplacental transmission of these effects in PE [182]. Similar to the results in PE mothers [177], PON1 activity was also shown to be decreased in the cord blood of the newborns [182]. In another study, HDL isolated from cord blood of PE pregnancies was reported to be linked with significantly reduced levels of apoM [138]. Given that levels of S1P are usually highly correlated with apoM levels, these results suggest less endothelial protection by this bioactive lipid [138]. Additionally, HDL from PE mothers has been shown to be depleted in apoM as well as S1P, accompanied with less anti-oxidative capacity [183].

Interestingly, another study reported an increased total- and HDL-mediated cholesterol efflux capacity of maternal and fetal PE sera, whereas ABCA1-mediated cholesterol efflux was decreased. This was partially explained by the increased concentration of apoE in maternal and fetal circulation. The authors proposed that the increased cholesterol efflux might be a rescue mechanism to remove excess cholesterol from cells to reduce lipid peroxidation [184].

Studies reported increased levels of $LpPLA_2$ in maternal PE plasma and the placenta [185,186] as well as in the fetus [187], which may represent a compensatory mechanism to control PAF and inflammatory responses.

Alterations in HDL function and composition may contribute to the endothelial dysfunction observed in mothers affected by PE. Whether this impairment also contributes to the increased cardiovascular morbidity of these women and children later in life remains to be elucidated.

8.2. HDL in Gestational Diabetes Mellitus (GDM)

GDM is a condition in which women without a history of diabetes develop hyperglycemia during pregnancy. GDM is the most common disorder during pregnancy, affecting up to 22% of all pregnancies, with increasing prevalence worldwide [188,189]. Women diagnosed with GDM, have an increased risk of developing diabetes, hyperlipidemia, hypertension, and coronary heart disease later in life [190–192]. Therefore, lifelong health monitoring of these women is meanwhile recommended. Similar to PE, risk factors for GDM also include age, ethnicity, and obesity [193]. However, GDM not only affects the health of the mother but also fetal growth and the long-term health of the offspring. The most prominent adverse outcome of GDM complicated pregnancies represents macrosomia with complications including metabolic abnormalities, impaired immune system, degraded antioxidant status, and potential metabolic syndrome in adulthood [194].

In general, diabetes mellitus is associated with an altered lipid profile with increased levels of triglycerides, elevated LDL, and reduced levels of HDL [150]. Diabetic dyslipi-

demia is not only characterized by changed levels, but also by different structures, function, and metabolism of lipoproteins. Studies reported decreased levels of HDL in type 2 diabetes mellitus (T2DM), with predominance of the small, protein-rich particles, which can undergo rapid catabolism [150,195]. The changes in HDL subclass distribution result from the increased transfer of triglycerides on HDL, mediated by CETP [196] and the increased activity of lipolytic enzymes such as hepatic lipase [197–199]. There is increasing evidence that low HDL levels may have a direct impact on plasma glucose and thereby contribute to the pathophysiology of T2DM [200]. Several experimental and clinical studies have suggested that HDL lowers blood glucose levels, by increased uptake of glucose from skeletal muscle via activation of the AMP-activated kinase pathway [200–202] and further through stimulation of pancreatic β-cell insulin secretion [201,203,204]. Other properties of HDL, such as its pivotal role in reverse cholesterol transport, as well as its anti-inflammatory capabilities in immune cells and metabolic tissues, may contribute to enhanced insulin sensitivity [200].

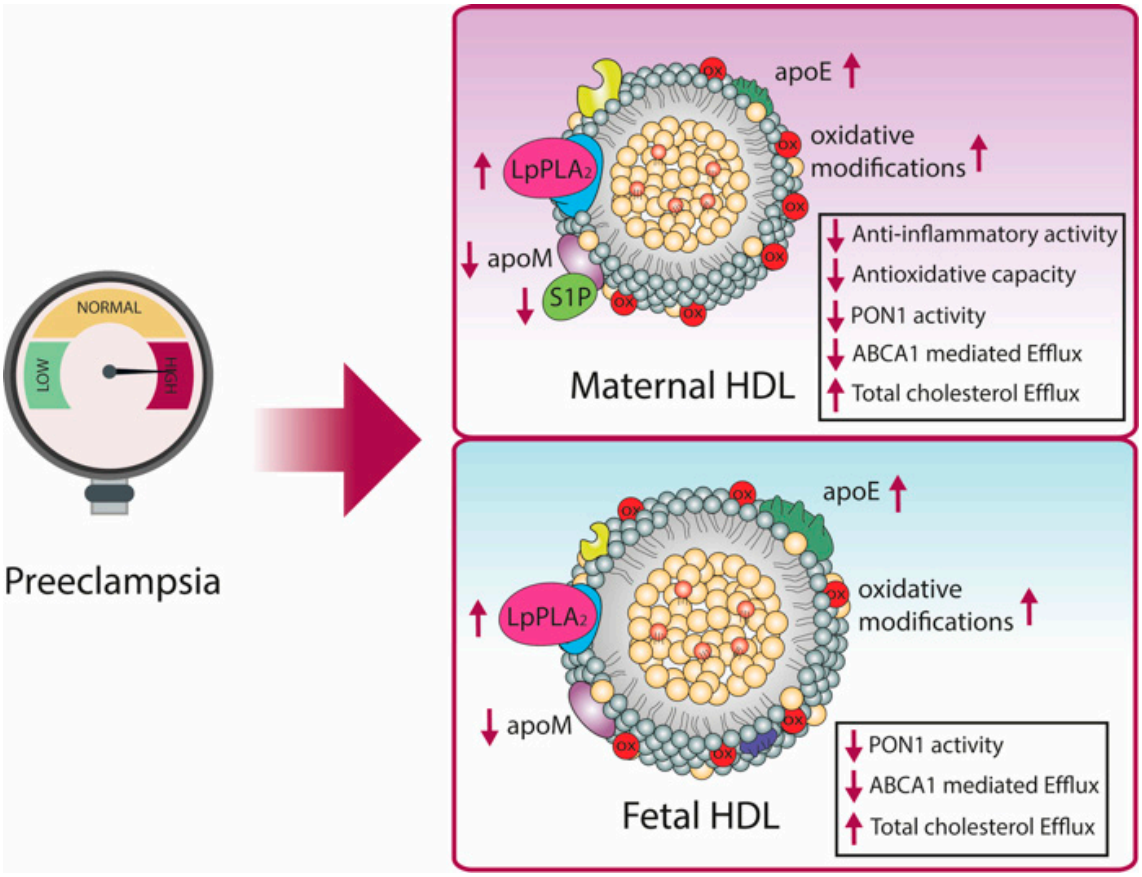

Figure 3. PE affects maternal and fetal HDL composition and function. Changes are indicated with purple arrows. In maternal HDL, a decrease in PON1 activity, apoM, and S1P content was observed, whereas apoE and $LpPLA_2$ were increased. These changes in HDL composition were associated with reduced anti-inflammatory and anti-oxidative activity, but increased cholesterol efflux capacity. Fetal HDL of PE pregnancies showed similar changes, with reduced PON1 activity and apoM, but increased $LpPLA_2$ and apoE, accompanied by increased cholesterol efflux capacity. Oxidative modifications of lipids were detected in both maternal and fetal HDL. HDL, high-density lipoprotein; apo, apolipoprotein; PON1, paraoxonase 1; $LpPLA_2$, lipoprotein-associated phospholipase A2.

In women with GDM, levels of triglycerides are markedly increased during pregnancy, while levels of HDL-C are decreased in the second and third trimesters [173]. Recent research on HDL subclass distribution in GDM revealed that small HDL particles are associated with GDM and provide a potential screening tool for early identification [205,206].

Mokkala et al. showed that women developing GDM have a distinct lipid profile in early pregnancy, with small-sized HDL particles being the strongest predictors for GDM [206].

In a study by Sreckovic et al., GDM-associated changes of HDL function and composition were examined in maternal as well as fetal HDL [207]. Shotgun proteomics of isolated HDL revealed lower levels of apoM and increased levels of the acute-phase reactant SAA on both, maternal and fetal GDM HDL [207] (Figure 4). Since apoM represents the main carrier of the vasoprotective S1P, the reduction of apoM on GDM HDL might contribute to endothelial dysfunction observed in GDM [208]. This was supported by another group using a migration assay with human umbilical vein endothelial cells [209]. Maternal GDM HDL showed less closure of cell migration, which was induced by TNF α, than control HDL [209]. Levels of apoA-I as well as mass and activity of PON1 were significantly decreased in maternal GDM HDL [207], similar to another study [209]. ApoA-I, as well as PON1, are important anti-oxidant components of HDL, therefore these results suggest decreased anti-oxidative protection [78,210]. On fetal GDM HDL, the abundance of PON1 was only barely detected, while activity was found to be reduced [207]. Interestingly, also HDL remodeling is altered during GDM. Both, maternal and fetal GDM HDL showed larger particle size than controls. Further, cholesterol efflux capacity was reduced in maternal as well as fetal GDM HDL [207]. Studies on $LpPLA_2$ in GDM revealed higher activity on maternal as well as fetal HDL, which might be relevant to exert protective activities against oxidative stress [146].

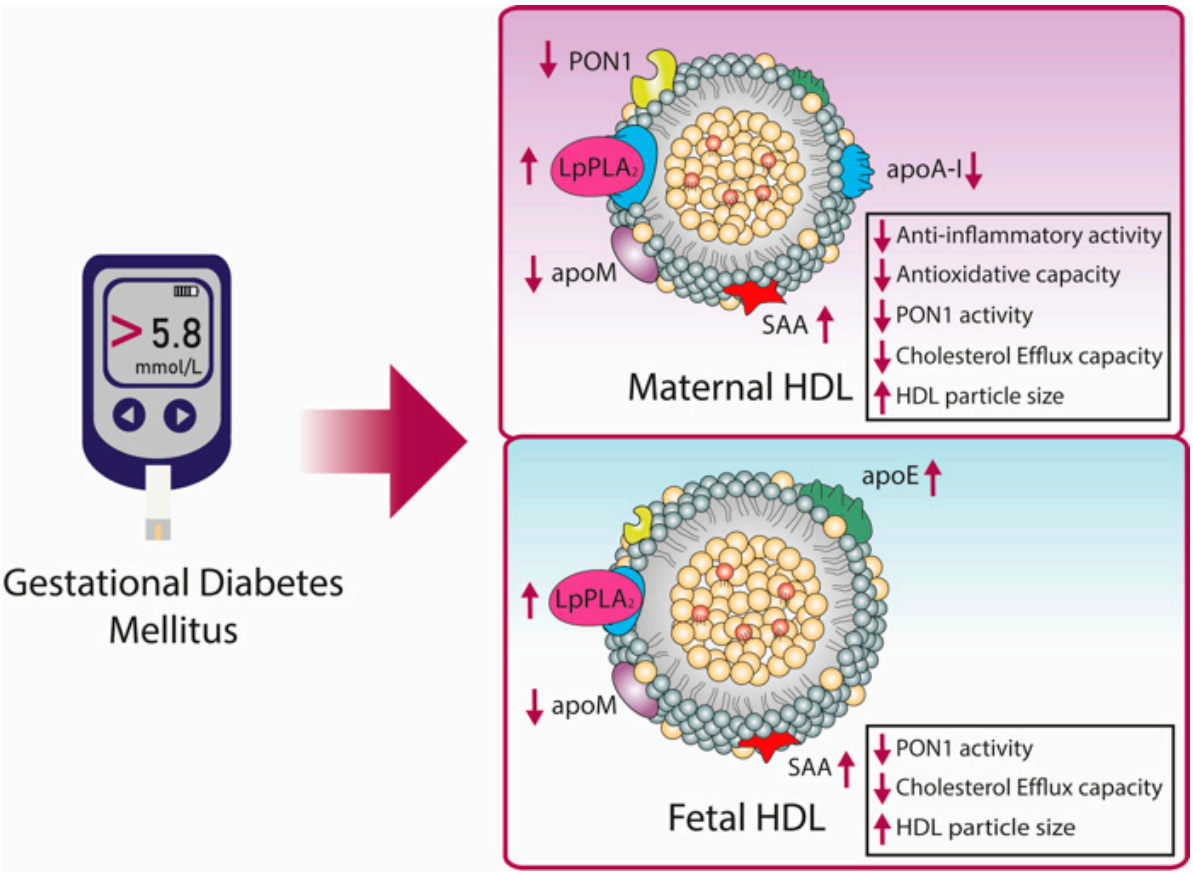

Figure 4. GDM affects maternal and fetal HDL composition and function. Changes are indicated with purple arrows. In maternal HDL, a decrease in PON1 activity and mass, apoM, and apoA-I content were observed, whereas SAA and $LpPLA_2$ were increased. These changes in HDL composition were associated with reduced anti-inflammatory and anti-oxidative activity and reduced cholesterol efflux capacity. Fetal GDM-HDL showed the same alterations in PON1 and $LpPLA_2$ activity, apoM and SAA content, and further increased apoE content. GDM was also accompanied by the increased particle size of both maternal and fetal HDL. HDL, high-density lipoprotein; apo, apolipoprotein; PON1, paraoxonase 1; $LpPLA_2$, lipoprotein-associated phospholipase A2; SAA, serum amyloid A.

Concluding, HDL proteome and size are markedly altered in GDM in both, maternal and fetal circulation. However, how these alterations affect the protective properties of fetal HDL and whether these alterations persist and are involved in the higher risk of becoming vascular diseases in offspring of GDM pregnancies later in life requires further studies.

9. Conclusions

Endogenous, as well as maternally-provided cholesterol, are important for fetal development. Although lipoprotein metabolism is separated between mother and fetus, maternal hyper- and hypocholesterolemia affect infant health and growth. Transplacental cholesterol transport from maternal lipoproteins to the fetal side involves receptor-mediated uptake of cholesterol from the syncytium and transport through the stroma. Cholesterol is then secreted/effluxed from the fetal endothelium to acceptors such as lipid-poor apolipoproteins and HDL.

With its unique apolipoprotein composition with high levels of apoE, fetal HDL seems to have an important cholesterol transport function that is accomplished by LDL in adults. Due to its distinct composition, it may also have an important role in atheroprotection. However, research should focus on elucidating the physiological function of fetal HDL and how this is developing with aging of the newborns.

Maintaining the vascular integrity of the fetoplacental vasculature is important for an adequate supply of oxygen and nutrients to the fetus and therefore crucial for fetal well-being. It has been shown that HDL-associated S1P is an important regulator of placental vascular inflammation, but also improves endothelial barrier function and induces vasorelaxation, thus playing an important role in maintaining vascular integrity. Further, $LpPLA_2$ has been suggested to act anti-inflammatory and to improve vascular barrier function in the placental endothelium.

HDL composition and function have been shown to be altered in pregnancy disorders such as PE and GDM. Of importance, these changes were also observed in the fetus of complicated pregnancies, therefore suggesting placental transmission of these effects. Disease-induced alterations of HDL composition and function might contribute to the pathophysiology of PE and GDM. Long-term follow-up studies are needed to clarify whether alterations in HDL composition and function (i) persist into adulthood and (ii) whether these changes are related to the increased risk of vascular pathologies later in life.

This review summarizes the current literature on the composition and function of fetal HDL in health and disease. Extensive future research is needed to further understand the physiological role of HDL in the fetus.

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Abbreviations

ABCA1	ATP-binding cassette A1
ABCG1	ATP-binding cassette G1
apo	Apolipoprotein
CETP	Cholesteryl-ester transfer protein
eNOs	Endothelial nitric oxide synthase
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol

GDM	Gestational diabetes mellitus
NO	Nitric oxide
LCAT	Lecithin-cholesteryl acyltransferase
LDL	Low-density lipoprotein
LDL-R	Low-density lipoprotein receptor
LpPLA2	Lipoprotein associated phospholipase A2
LR-P1	LDL receptor related protein 1
LPL	Lipoprotein lipase
PAF	Platelet activating factor
PON1	Paraoxonase 1
SAA	Serum amyloid A
SR-BI	Scavenger receptor BI
S1P	Sphingosine-1-phosphate
VLDL	Very low-density lipoprotein

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