HDL across the lifespan: their role in children, pregnancy, and Alzheimer's disease patients

By

BRIAN HONG
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Nutritional Biology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Angela M. Zivkovic, Chair

Carlito B. Lebrilla

Danielle J. Harvey

Committee in Charge

2022

**Acknowledgment**

My time in graduate school would not have been possible without the endless support and guidance from my mentor, Dr. Angela Zivkovic. Thank you, Angela, for warmly inviting me to your lab, for helping me develop the skills to succeed, and for well preparing me as a scientist outside of academia. I would like to thank my colleagues in the Zivkovic's lab, Jack Zheng, Joanne Agus, Cynthia Tang, Oscar Munoz, Eddie Romo, and previous lab members Jea Woo Kang, Trevor Zhu, and Chris Rhodes for their training and contribution to this dissertation. The completion of this doctorate is certainly not done single-handedly, and I am genuinely grateful to have a kind, reliable, team-oriented lab family who made my graduate journey nothing but positive.

I would like to sincerely thank my dissertation committee members, Dr. Carlito Lebrilla and Dr. Danielle Harvey, for their support of this dissertation. I would also like to acknowledge our collaborators from our Alzheimer's disease group, Dr. Lee-Way Jin, Dr. Izumi Maezawa, Dr, Carlito Lebrilla and his team, and Dr. Harvey, as well as our collaborators from the iLiNS-DYAD Ghana project, Dr. Kathryn Dewey and her team for providing the resources and expertise to complete this dissertation.

Lastly, I would like to thank my family, especially my mom Chantha Ouk who is a refugee from Cambodia, and my partner Padee Vang for all the years of support, both emotionally and financially. Thank you for encouraging my decision to pursue a doctorate.

# Table of Contents

## ABSTRACT

The purpose of this dissertation is to highlight the role of high-density lipoprotein (HDL) function, structure, and composition across different life stages and how HDL influences health and disease outcomes, from early childhood, to pregnancy, to old age and Alzheimer's disease (AD) dementia. The dissertation is divided into four chapters with the corresponding objectives:

1. The first chapter is a literature review on the role of HDL across the lifespan, particularly in children, during pregnancy, and in the elderly. These populations are often less studied in the context of HDL and health outcomes.
2. In the second chapter we determine whether small-quantity lipid-based nutrient supplements (SQ-LNS) provided to mothers during the second trimester (≤ 20 weeks), postpartum (0 – 6 months), and their children (6-18 months) improved HDL cholesterol efflux capacity (CEC) compared to children of mothers supplemented with the standard iron and folic acid (IFA) supplement. We further explored whether SQ-LNS altered HDL lipidomic and glycoproteomic composition. We found that children in the SQ-LNS group had improved HDL CEC and altered HDL protein glycosylation. CEC was also found to be associated with site-specific glycans on HDL.
3. The third chapter evaluates whether SQ-LNS altered HDL CEC and plasma enzyme activities involved in HDL metabolism of pregnant mothers at 36 weeks gestation and whether these functional metrics are associated with pregnancy outcomes. SQ-LNS did not alter HDL CEC or plasma enzyme activities in mothers, but these metrics were heavily influenced by factors mediated by season at the time of blood draw.
4. In the fourth chapter we determine whether the function and structure of HDL are altered in AD patients compared to non-demented controls and whether these alterations are

influenced by apolipoprotein E (*APOE*) genotype. We found for the first time that changes in HDL function and size were *APOE* genotype-specific, and that there is a link between HDL function, size, and cognitive function.

# Chapter 1: HDL Across the Lifespan: From Early Childhood, Through Pregnancy, and Into Old Age

Brian V. Hong[1], Jack Jingyuan Zheng[1], Angela M. Zivkovic[1]

[1]Department of Nutrition, University of California – Davis, Davis, CA, 95616

## ABSTRACT

The function of high-density lipoprotein (HDL) particles has emerged as a promising therapeutic target and the measurement of HDL function is a promising diagnostic across several disease states. The vast majority of research on HDL functional biology has focused on adult participants with underlying chronic diseases, whereas limited research has investigated the role of HDL in childhood, pregnancy, and old age. Yet, it is apparent that functional HDL is essential at all life stages for maintaining health. In this review, we discuss current data regarding the role of HDL during childhood, pregnancy and in the elderly, how disturbances in HDL may lead to adverse health outcomes, and knowledge gaps in the role of HDL across these life stages.

**Keywords**: Children; Cholesterol efflux capacity, Elderly; High-density lipoproteins; Pregnancy

## INTRODUCTION

High-density lipoprotein (HDL) particles are often recognized for their role in removing excess cholesterol from the body, known as reverse cholesterol transport [1]. However, in addition to or as part of this role in cholesterol clearance, HDL particles are critical during development [2] and have been implicated in several inflammation-related diseases including diabetes [3,4], chronic kidney disease [5,6], cardiovascular disease (CVD) [7,8], and Alzheimer's disease [9,10]. The cholesterol content in HDL (HDL-C) has been well documented as an inverse marker of cardiovascular mortality across several prospective studies [11–13], and when measured earlier in life, is predictive of health status later in life. For example, low HDL-C measured during early adulthood (35-50 years) [14] and midlife (51-60 years) [14,15] is associated with increased Alzheimer's disease risk after age 60, which suggests that monitoring, as well as interventions to improve HDL, should begin early in order to prevent disease decades later.

However, high HDL-C does not guarantee protection against overall health risk [16], and a U-shaped curve in the association between HDL-C and mortality risk has been observed, with HDL-C concentrations of both < 30 mg/dL and > 90 mg/dL associated with increased mortality risk [17]. The reasons for this complex relationship are not yet clear, however, it is clear that the simple measurement of HDL-C concentration does not provide information about HDL functionality, such as its ability to promote cholesterol efflux and its anti-inflammatory, antioxidative, and vasodilatory activities among others [18]. The measurement of "dysfunctional" HDLs has emerged as an alternative therapeutic target over the measurement of HDL-C concentration [19]. For example, HDL's ability to promote cholesterol efflux from macrophages is a more reliable predictor of CVD risk than HDL-C concentration [20–22],

suggesting that the function of HDL is a promising approach for evaluating the complex role of HDL in health and disease.

The role of HDL in human health is largely studied during adulthood in participants with underlying chronic diseases, but limited studies have examined the role of HDL in the health of children, during pregnancy, and in the elderly. This review highlights the current evidence on the role of HDL across the lifespan, particularly in children, during pregnancy, and in the elderly, and describes the limitations of current HDL research among these populations.

## 2. Children and adolescents (0-20 years)

### 2.1. HDL and preterm infants

Preterm birth is associated with cardiovascular events later in life [23], and is one of the main causes of neonatal death in the world [24]. Yet, early HDL-based therapies in preterm infants have not been fully explored. A long-term prospective study in preterm infants (n = 216) followed for 13-16 years into adolescence found improvement in both the low-density lipoprotein (LDL):HDL and apolipoprotein B (ApoB): Apolipoprotein A-1 (ApoA1) ratios in those infants who were breastfed compared to those who were fed preterm formula [25]. These findings suggest that breastfeeding early in life may influence lipoprotein profiles and cardiovascular health later in adulthood. Similarly, the benefit of breast milk on HDL extends to healthy infants. For example, infants born full term (n = 37) given exclusively breast milk for the first 4 months of life showed higher HDL-C:LDL cholesterol ratios compared to healthy infants on regular formula [26]. These finding reveal that HDL is responsive to diet early in life and that these early life exposures may have lasting impacts on lipoprotein metabolism into adolescence and adulthood.

HDL particle concentration and subclass distribution are additional parameters that have been found to be distinct in preterm infants. A small pilot study observed lower HDL particle concentration in preterm (29 – 34 weeks gestational age, n = 9) and very preterm (≤ 28 weeks gestational age, n = 6) infants at enrollment (0-5 days of age) compared to at 2 weeks, with small HDL and small LDL particles being the predominant subclasses among the two groups [27]. Elevated small dense LDL particles have been linked to the development of CVD in children [28] and adults [29]. Dietary intervention in preterm infants (≤ 32 weeks gestational age) can ameliorate lipoprotein profiles, with early total parenteral nutrition from birth to 5 days of age increasing both ApoA1 concentration and shifting HDL subclasses, as measured by gradient gel electrophoresis, from large $HDL_{2b}$ towards smaller and more dense $HDL_{3c}$ particles of similar value comparable to healthy term infants [30]. At 1 month of age, late-preterm infants (n = 25, 34-37 weeks gestational age) compared to term infants (n = 56) displayed lower cholesterol concentration within several HDL subclasses, including very large, large, medium, and very small HDL [31], which suggest a reduction in HDL maturation. Lecithin-cholesterol acyltransferase (LCAT) activity, an essential remodeling factor involved in HDL maturation [32] linked to CVD [33], was found to be low in cord blood [34] and remained decreased in preterm infants during the first week of life compared to infants born full-term [35]. These studies suggest that a lack of LCAT activity is a factor that reduces HDL maturation in preterm infants. In addition, HDL's ability to efflux cholesterol, measured as cholesterol efflux capacity (CEC), is the initial step in the reverse cholesterol transport pathway and relies on LCAT for efficient cholesterol transport [20]. It is currently unknown whether and how preterm pathophysiology alters HDL CEC and other functions in infants. These limited data on the effects of preterm birth on HDL outcomes both during infancy and later in life provide tantalizing evidence that early

life exposures may be critical in shaping life-long effects on HDL-mediated outcomes. Studies are needed to better understand how preterm birth alters pathways involved in HDL metabolism, and how these early life events shape future metabolic disease risk.

### 2.2. HDL in children and adolescents at risk for cardiovascular events

CEC is one of many key functions of HDL that has consistently been shown to be directly linked with CVD [20,21,36]. Dyslipidemia mediated by disturbances in genes involved in cholesterol trafficking resulting in diminished HDL CEC in pediatric patients (3.6 – 5.1y) makes these children susceptible to accelerated atherosclerosis compared to healthy children of similar age and sex [37]. In early childhood, HDL CEC was reported to be negatively associated with BMI at age 5, but not at age 9 [38], and impaired in children with familial hypercholesterolemia [39]. Similarly, in adults HDL CEC was also found to be negatively associated with BMI and waist circumference [40], and in fact, this observation of a negative effect of obesity on HDL concentrations is so well documented in adults that low HDL-C is one of the 5 criteria for the diagnosis of metabolic syndrome [41]. In addition to the effects on HDL-C concentrations, obesity also has negative effects on HDL functional parameters. Obese children (11.8 ± 1.9 y) had impaired HDL function, including reduced HDL CEC and antioxidant capacities as well as LCAT activity [32]. Both dyslipidemic and normolipidemic overweight adolescents with a mean age of 13 years had reduced HDL CEC compared with normolipidemic normal-weight children of similar age and sex [42]. Together, these data suggest that disturbances in HDL metabolism and function can already be present in childhood and adolescence, and that some of the same factors that diminish HDL function and increase CVD risk in adults (e.g., obesity) are already present early in life.

### 2.3. HDL in children and adolescents with diabetes and chronic kidney disease

Despite the fact that children with type 1 diabetes have normal HDL-C concentrations, loss of HDL function has been observed in this population [43–45]. Children (8 -18 y) with type 1 diabetes had an impaired ability of HDL to exchange ApoA1 [43], a key process in the RCT pathway that has been demonstrated to be associated with atherosclerosis [46] and positively correlated with ATP binding cassette transporter A1 (ABCA1)-mediated CEC, independent of HDL-C and ApoA1 levels [47]. In another study, children with type 1 diabetes and poor glycemic control had a lower proportion of large $HDL_{2b}$ by native gel electrophoresis compared to age and sex-matched non-diabetic children, despite a lack of difference in serum and HDL CEC [44]. By contrast, adults with type 1 diabetes were shown to have increased CEC and larger HDL particles measured by nuclear magnetic resonance (NMR) spectroscopy compared to non-diabetes adults, with no differences in HDL-C [48]. It is not clear whether these differences in HDL function and particle size in type 1 diabetic adults vs. children are due to underlying differences in biology or due to differences in the methods used to measure these HDL parameters [48]. Hyperglycemia is known to result in the glycation of ApoA1 and other HDL-associated proteins, which has been demonstrated to impair HDL function and affect HDL metabolism [49]. Thus, differences in glycemic control in both children and adults with type 1 diabetes may contribute to differences in HDL concentration, particle distribution and function.

Children with chronic kidney disease (9.8 ± 5.5 y) and end-stage renal disease (9.6 ± 5.0 y) also have dysfunctional HDL [50]. HDL of children with renal disease have an impaired ability to reduce monocyte adhesion to endothelial cells [50], however CEC was not affected in two different pediatric cohorts with chronic kidney disease (<16 y) [50,51]. Interestingly, this is similar to what has been observed for adult populations with kidney disease. Adult patients with end-stage renal disease also have impaired HDL functional parameters such as anti-oxidant

capacity [52] but CEC may increase due to a preponderance of pre-beta HDL which effectively perform cholesterol efflux but which do not mature into the large, spherical HDL that carry additional functional proteins and thus perform additional functions [53]. It has been observed in adult patients with end-stage renal disease that this loss of HDL maturation is due in part to the loss of LCAT activity [54,55]. It is unknown whether similar mechanisms lead to loss of HDL maturation in pediatric kidney disease patients. Further studies in children are needed, however, the limited existing evidence already suggests that HDL disturbances in childhood kidney disease are likely to be similar to those observed in adults, confirming the importance of early detection and intervention.

### 2.4 Interventions that improve HDL concentrations and/or function in childhood and adolescence

HDL functional biology during childhood and adolescence is poorly characterized. Nonetheless, interventions to improve child health measured through HDL markers have been investigated. Lipid-enriched supplements during pregnancy and postpartum in a malnourished population in Ghana can improve children's HDL CEC at 18 months of age [56], with marked benefits on linear growth [57]. Interestingly, in these children HDL CEC was associated with site-specific glycosylation of HDL-associated proteins [56]. Some of these associations were also observed in adults. For example, a positive correlation between alpha-1 antitrypsin (A1AT) glycosylation on amino acid position 70 (A1AT_70_5402 and A1AT_70_5412) and HDL CEC was observed in the children from Ghana as well as in healthy American adults [58]. These observations suggest that compositional parameters that are linked to HDL functionality in adulthood are already present in childhood, highlighting that similar markers can be used to assess the effects of interventions in children and adults.

In adolescents, interventions to mitigate atherogenic lipid profiles have been explored across several metrics of HDL function. A one year follow up after vertical sleeve gastrectomy in obese adolescent males (17.4 ± 1.6 y) enhanced both HDL CEC and anti-oxidative capacity, independent of both BMI and HDL-C concentrations [59]. Similarly, obese adults with type 2 diabetes undergoing sleeve gastrectomy showed improved ApoA1 exchange rate and ABCA1-independent CEC 5 years post procedure, though ABCA1-dependent CEC did not change [60]. In adolescents (14.9± 3.6 y), a 10-month lifestyle intervention program composed of dietary restriction, exercise, and psychological support improved both HDL CEC and antiatherogenic stimulation of endothelial nitric-oxide synthases [61]. Likewise in obese adult men, a one-year lifestyle modification consisting of aerobic activity and nutrition counseling improved both HDL-C and HDL CEC [62]. Together, these studies suggest that interventions to improve HDL in adults reflect similar changes to HDL in adolescents. Thus, therapeutic approaches and interventions that ameliorate HDL outcomes in adults may also be beneficial for adolescents. Given that chronic diseases like atherosclerotic CVD develop over the entire life course, interventions such as diet and exercise, which can be safely implemented over many decades and which have been shown to be effective in modifying HDL outcomes, are a desirable disease prevention strategy to implement early in life.

## 3. Pregnancy (18 years and over)

### 3.1. Role of HDL during pregnancy

HDL-C steadily increases during pregnancy and peaks between the 2$^{nd}$ and 3$^{rd}$ trimester [63,64], along with other lipid variables, including triglycerides, LDL cholesterol (LDL-C), and total cholesterol, which increase considerably throughout gestation [63]. Studies have shown that HDL-C can be positively associated with pregnancy outcomes, however, there is conflicting

evidence on the extent to which HDL influences birth outcomes. On the one hand, HDL-C measured at 36 weeks gestation (26.5 ± 5.2 y, n = 320) was positively associated with the duration of pregnancy [65], and pregnant women (28.5 ± 4.6 y, n = 335) with a pre-pregnancy body mass index (BMI) ≥ 25 kg/m$^2$ showed improved birth size when HDL-C increased by 10 mg/dL from preconception to 28 weeks gestation [66]. In contrast, others have found a negative association between maternal serum HDL-C and birth size measured during the second and third trimesters [67,68]. In a small cohort of healthy pregnant women (30.7 ± 3.96 y, n = 25), total HDL particle concentration measured by NMR during mid-gestation (22-28 weeks) was negatively associated with birth weight [69], which is in agreement with a case-control cohort (n = 5337) that found an association between higher total HDL particle concentration during mid-gestation (24 – 26 weeks) with small for gestation age (SGA) infants at term, but no difference in HDL-C was observed [70]. An important consideration in these conflicting findings is that how HDL is measured heavily influences the results. HDL-C is a crude measure of the total amount of cholesterol transported as part of all HDL particles, which includes large and small HDL particles carrying different proportions of that cholesterol. However, there are significant differences between large and small HDL particles, despite the fact that the total size range is only about 5 nm. HDL particles that are 7 nm in diameter transport 10X fewer cholesterol molecules than HDL particles that are 12 nm in diameter [71]. There are also important differences in HDL particle composition by size. For example, apolipoprotein E (ApoE) and apolipoprotein J (or clusterin, CLU), which are both critically involved in Alzheimer's disease, are enriched in large HDL, whereas A1AT, a protein known for its critical role in attenuating proteolytic damage during inflammation is found mainly on small HDL [72]. In addition to or as part of their ability to remove excess cholesterol from cells HDL confer anti-oxidant, anti-

inflammatory, immuno-modulatory, anti-coagulant, anti-infectious, and many other functions, with as many as 16 functional subclasses dictated by protein composition [73]. Conditions where the pathology is a loss of HDL particles, such as that which has been observed in obesity and metabolic syndrome, can be measured by simple metrics such as HDL-C concentration, total amount of ApoA1, and total particle number. However, these simple measures may not capture the complexity of HDL, such as the relative proprotions of different functional subclasses, which may be the critical differentiating factor in other conditions, such as in pregnancy.

### 3.1. HDL subclasses during pregnancy

Different subclasses of HDL transport different clusters of HDL proteins [72,73], and the presence of specific protein clusters can dictate HDL function [74]. A few studies have reported an increased proportion of larger HDL particles during pregnancy [75,76]. In a small cohort of healthy women at 9 weeks gestation, the authors observed a race-specific association with higher levels of large HDL measured by NMR among African American women with preterm birth (n = 12) versus African American women who gave birth at term (n = 14), but not in white women with preterm (n = 6) versus term (n = 17) births [77]; however, this observation needs further investigation in a larger cohort. In healthy Serbian women, large HDL2a particles measured by NMR before delivery (37.2 - 37.3 weeks, n = 41) were shown to be negatively associated with birth length and head circumference [64]. In pregnant women from the Pregnancy, Infection, and Nutrition study (PIN) cohort (mean age 28 y, n = 715), composed of women with heterogenous pre-pregnancy weight (underweight to obese), a higher concentration of medium HDL by NMR during pregnancy at mid-gestation (24-28 weeks) but not at < 20 weeks gestation was associated with an increased risk for preterm birth (<37 weeks), [78]. In a nested case-control study of mothers without severe chronic illnesses or preeclampsia (n = 323 cases, n = 671 controls)

birthing children at term (≥ 37 weeks), NMR analysis during mid gestation (24-26 weeks) showed a higher proportion of small HDL and medium HDL as well as ApoA1 in mothers birthing small for gestational age infants compared to mothers with infants of normal birth weight [70]. However, the authors postulate that this observation was a consequence of placental dysfunction which may have increased maternal HDL levels, but future studies should define this relationship in detail. These studies suggest that the influence of HDL on birth outcomes differs substantially depending on the time of gestation at which HDL was measured. Furthermore, the changes to HDL during pregnancy suggest that modifications to HDL may also influence its function that may be linked to birth outcomes. The mechanisms of HDL metabolism leading to different HDL subclass distributions during pregnancy and its relationship with birth outcomes needs further investigation. A common limitation in these studies is the cross-sectional measurement of HDL that conceals the changes to HDL over the course of pregnancy as it relates to birth outcomes. Most cohorts were relatively healthy women, and, to our knowledge, it is not known yet whether alteration in specific HDL subclasses in chronic diseases (e.g. diabetes) during pregnancy associate with birth outcomes. Other factors such as dietary intake before and during pregnancy were not reported, which may also influence HDL profiles.

### 3.2. HDL and adverse outcomes

Paraoxonase 1 (PON1) is an essential HDL-associated protein that has been linked to cardiovascular disease and is protective against LDL oxidation through its antioxidant activity [79]. PON1 activity was found to be impaired in both obese adults and children [80], and its activity has been shown to be reduced in chronic renal failure [81] and type 2 diabetes [82]. A longitudinal study in normal pregnant women (n = 50) found that PON1 activity decreases over the course of pregnancy which is accompanied by the natural increase in oxidative stress during

pregnancy [83]. However, uncontrolled oxidative stress can be detrimental, where higher levels of oxidative stress during pregnancy play a role in the pathophysiology of preeclampsia [84], and increase the susceptibility of the fetus to develop cardiovascular disease in adulthood [85]. Pregnant women with preeclampsia (29 ± 7 y, n = 19) during late pregnancy (35.2 ± 3.1 weeks) had reduced PON1 activity compared with healthy pregnant women of higher gestational age (38.4 ± 1.2 weeks) without preeclampsia (32 ± 3.8 y, n = 6), but no significant differences in serum lipid levels including HDL-C [86]. The decrease in HDL PON1 activity without changes to HDL-C suggest that the function of HDL may be a more reliable predictor of pregnancy complications, which is similar to the link between HDL function and cardiovascular disease, which is also independent of HDL-C [20]. Likewise, in a cohort of white European women, women with preeclampsia (31 ± 6.5 y, n = 17) and their fetuses had lower ABCA1-mediated CEC compared with healthy normotensive pregnant women (28.2 ± 7.2 y, n = 17), but there were no differences in the concentration of maternal HDL-C between the two groups [87]. In a separate cohort, women with preeclampsia during pregnancy (31.4 ± 4.8, n = 42) had reduced ABCA1-mediated CEC at 6 months postpartum compared with postpartum women (32.4 ± 4.7 y, n = 44) who were normotensive during pregnancy, but, again, there were no differences in HDL-C concentration among the two groups [88]. The disturbance in HDL function during preeclampsia supports the importance of functional HDL on pregnancy outcomes. However, the limitations of these studies are the low number of participants, lack of follow up on mothers and their children, and uncertainty of HDL function returning to normal value after delivery. Other analyses such as the association between maternal HDL function across pregnancy and birth outcomes (e.g. birth size) can be further explored. Furthermore, it is uncertain whether the changes in HDL function during pregnancy are causal for pregnancy complications. Many

questions remain unanswered, yet current data point to important differences in HDL metabolism and function in healthy pregnancy vs. pregnancy with poor outcomes.

## 4. Elderly (65 years and over)

### 4.1. HDL Cholesterol in Aging

Emerging evidence highlights the importance of HDL particles in aging biology. HDL-C concentrations, HDL particle size and particle number have all been found to be associated with age, but also with physical activity, metabolic diseases, and cognitive decline. In elderly individuals, HDL-C is a strong predictor of longevity across several prospective studies [89–91]. Compared to healthy younger adults (41.0 ± 10.6 years, n = 200), octogenarians from Sicily (84.18 ± 3.6 years, n = 100) had significantly higher HDL-C concentrations [92]. Higher HDL-C concentration was also positively associated with better physical functions (4-m walking speed, the short physical performance battery score, the basic and instrumental activities of daily living scales scores) in the octogenarian subjects (85.9 ± 4.9 years) of the ilSIRENTE study (n = 364) [89]. In a genome wide association study examining relationships between the age at death of parents of middle-aged UK Biobank participants of European decent (n = 75, 244) showed that longevity was associated with HDL-C concentration only in the parent group with extreme longevity (father ≥ 95 years, mother ≥ 98 years, n = 1339) [93]. In a similar study, researchers recruited 312 offspring from longevity historical families and 298 controls from non-longevity historical families in the Bama Aging Study cohort, aiming to understand the potential relationships between HDL-C, apolipoprotein E (*APOE*) genotype, and longevity [90]. HDL-C was significantly higher in the longevity group, while *APOE* genotype was not associated with either HDL-C or age [90].

In a study of 139 Ashkenazi Jewish centenarians (age 95 – 107 y), plasma HDL-C concentrations were significantly and directly correlated to Mini-Mental State Examination (MMSE) scores [94]. When compared within the centenarians, female, but not male, subjects with lower MMSE score (<25) had significantly lower plasma HDL-C compared to those stratified as having higher MMSE score (25 – 30) [94]. Compared to female nonagenarians (92 ± 4.0 y, n = 280), male nonagenarians (91 ± 1.0 y, n = 107) had significantly lower HDL-C concentrations, which were associated with MMSE scores only in the male subjects after adjustment for covariates [95]. In the Baltimore Longitudinal Study of Aging, high LDL:HDL ratio was associated with an increased risk of developing Alzheimer's Disease (AD) 5 to 7 years before diagnosis [96]. However, low HDL-C (< 50 mg/dL) alone did not show significantly higher relative risk for developing AD compared to normal HDL-C concentration [96]. Together these data suggest a relationship between high HDL-C concentrations and protection from cognitive decline in elderly individuals, and particularly the oldest old.

However, HDL-C concentrations have also been found to be higher in populations with cognitive decline. In a study investigating the relationship between cognitive decline, fat-soluble vitamins, and *APOE* genotype in aging adults and elderly (65.31 ± 6.30 y), HDL-C was higher in the mild cognitive impairment (MCI) group (n = 583) compared to the normal group (n = 1171), though this comparison was not adjusted for any genotype effects [97]. Higher HDL-C was found in the cognitively normal group with *APOE*2 genotype, and in the MCI group with *APOE*2 and *APOE*4 genotype, compared to those with the *APOE*3 genotypes [97]. HDL functional capacity was recently found to be differential by *APOE* genotype in elderly individuals, with *APOE4* carriers having lower CEC and LCAT activity regardless of dementia diagnosis [98]. *APOE* genotype is also well known to be associated with HDL-C concentrations, LDL-C and

total cholesterol concentrations [99], as well as CVD risk and mortality [100]. Thus, in studying the relationships between HDL and aging outcomes, it is important to account for APOE genotype, especially in the elderly, where individuals who have not already died from heart disease will be disproportionately overrepresented.

HDL-C status in elderly was also shown to be differentiated by eating patterns. In a study group of community-dwelling elders in Northern China with normal HDL-C (67.3 ± 5.9 y, n = 2646) and low HDL-C (67.3 ± 5.9 y, n = 741), participants with the highest scores for a balanced diet had a decreased risk for developing low HDL-C, characterized as having <1.04 mmol/L (40 mg/dL) in the group with a BMI of 27.1 kg/m$^2$ or above (overweight to over obese) [101]. Conversely, there was a significant negative association between the highest intake of the Western diet and low HDL-C in the group with a BMI of 21.6 to 24.8 kg/m$^2$ (normal to slight overweight). Interestingly, higher score for the thrifty dietary pattern was also associated with increased risk of low HDL-C, especially in the group with a BMI of 21.6 kg/m$^2$ and below (normal to underweight). These observational data suggest that diet quality is linked with HDL-C status and that the effects of diet on HDL are mediated by BMI status, where both low and high BMI can affect HDL-C concentrations. Together, the data on HDL-C in aging indicate that HDL-C concentrations are associated with differential outcomes in aging, that *APOE* genotype is an important mediator in the relationship between HDL-C and aging related outcomes, and that dietary patterns influence HDL-C in elderly individuals.

### 4.2. HDL functions in Aging: Cholesterol efflux and Aβ carrying capacity

To fully understand the role of HDL, measurement of HDL functionality is crucial in addition to measurement of HDL size, number, and concentrations. This includes HDL CEC and other non-conventional functions such as Aβ carrying capacity. In a small study, compared to young subjects (20 – 30 y, n = 8), elderly individuals (65 – 70 y, n = 9) presented lower ABCA-1 mediated CEC, specifically for the $HDL_3$ subclass [102]. This reduction in efflux capacity may have been due to higher levels of damage to the HDL particles since the HDL of elderly subjects had higher amounts of ApoA1 modification (i.e. oxidation) [102]. In a larger study investigating the effects of extra virgin olive oil (EVOO) consumption on HDL CEC, elderly subjects (70.72 ± 5.6 y, n = 57) had lower HDL CEC than young subjects (31.81 ± 6.79 y, n = 27), as well as higher small HDL and lower large HDL at baseline [103]. The study also showed that 12-week consumption of EVOO at 25 ml/day improved HDL CEC of the elderly subjects to the same level comparable to young subjects at baseline, though HDL CEC for the young subjects also improved and was significantly higher than that of the elderly subjects at the end of the study [103].

In an early study on the role of cerebrospinal fluid HDL on the development of AD, the $HDL_1$ subclass was found to have increased soluble amyloid β (sAβ) and increased apolipoprotein in the AD group compared to age-matched aging normal human subjects, and that sAβ molecules were associated with ApoE and apolipoprotein J in the $HDL_2$ and $HDL_3$ subclasses [104]. HDL levels were shown to positively associate with serum neurofilament light levels, a biomarker for neuro-axonal damage, in study subjects age over 60 years, but not in subjects below the age of 60 [105].

### 4.3. Activity of HDL-associated protein/components (PON-1, PAF-AH) in aging

HDL presents additional functions besides cargo transportation. These include antioxidation, anti-inflammation, anti-proteolysis, and anti-platelet formation [18]. Components associated with HDL are suggested to be the mediators of these additional functions, and changes to these components have been shown over the course of aging.

HDL composition was altered in elderly subjects' samples, showing increased abundance of acute phase proteins, for example serum amyloid A and complement C3, and decreased abundance of ApoE, compared to young healthy subjects [106]. In elderly subjects' samples, the phospholipid layer fluidity and the phosphatidyl choline/sphingomyelin (PC/SPM) ratio of HDLs, which was shown previously to affect non-ABCA1-mediated cholesterol efflux, were both lower compared to young subjects [102]. Another study observed increased SPM concentrations in HDL from elderly subjects [106], which could explain the decrease in the PC/SPM ratio observed previously.

Compared to HDL from young subjects (24.70 ± 3.09 y), elderly (74.86 ± 13.18 y) HDL samples had reduced antioxidation capacity, as shown by shorter lag-phase in the production of conjugated dienes when incubated with copper ion [107]. The reduced antioxidative capacity was highly associated with a reduced enzymatic activity of PON1, an HDL-associated protein responsible for preventing lipid oxidation. [107]. This observation was also supported by other groups, which confirmed defective antioxidant properties, lower PON1 activity, and higher uptake rate by macrophages in elderly HDL samples compared to HDL from healthy young subjects [106,108].

HDL from elderly subjects (n 71 ± 4 years, n = 26) compared to that from young males (22 ± 2 years, n = 18), showed increased glycation with ApoA1 multimerization and decreased HDL phospholipid content, which induced more severe cellular senescence, foam cell formation

when incubated with human dermal fibroblasts and macrophage, and induced cholesterol influx into macrophages [109]. Thus, in addition to the loss of beneficial functions, such as antioxidant capacity, HDL from elderlies have also been shown to have gain of deleterious function, such as induction of senescence and net delivery of cholesterol to cells, rather than efflux.

Compared to healthy younger adults (41.0 ± 10.6 y, n = 200), octogenarians from Sicily (84.18 ± 3.6 y, n = 100) had significantly higher plasma and HDL-associated platelet-activating factor acetyl hydrolase (PAH-AH) activity level, which is an enzyme that inactivates platelet activation, preventing LDL oxidation, and thus protective against the development of atherosclerosis [92].

## CONCLUSION

HDL has the potential to be a predictive measurement of health status across all life stages, from infancy, through pregnancy, and in aging. Similar to adults, children with underlying conditions, such as dyslipidemia, diabetes, and chronic kidney disease, exhibit some degree of HDL dysfunctionality, but many gaps in knowledge remain and much further work is needed to understand the role of HDL in children on health status later in life. HDL-C and HDL CEC are established predictors of CVD risk, but HDL-C does not reflect HDL function. During pregnancy, the time at which HDL is measured is essential. Changes to HDL subclasses occur throughout gestation, but it is uncertain how these alterations relate to HDL function at different stages of pregnancy, and how these alterations affect pregnancy outcomes and life-long trajectories in the infant. In certain conditions such as metabolic syndrome where the underlying mechanism of loss of function is the loss of total numbers of HDL particles, the measurement of

HDL-C, total HDL particle count, and total ApoA1 in plasma may be adequate. However, in other physiological states such as pregnancy, simple measurements of total amounts of HDL in circulation do not appear to be useful because it is instead the loss of certain subsets of HDL particles or certain functional aspects of HDL that have negative health effects. In elderly individuals, HDL function and composition change as part of the aging process, however this is highly differential between healthy elderly individuals achieving very long lifespans, such as octogenarians, nonagenarians, and centenarians, compared with elderly individuals with chronic diseases such as CVD and AD. The study of healthy vs. unhealthy elderly individuals will be an important area of research for the discovery of protective mechanisms that improve health outcomes, as well as loss-of-beneficial-function and gain-of-deleterious-function aspects of HDL. Most work on HDL in elderly, as well as in children and in pregnancy, is cross-sectional, hindering our ability to derive casual relationships. Thus, in addition to further studies on the functional biology of HDL at these under-studied life stages, future work is needed to fully understand how different conditions and physiological states affect the components of HDL, including function, subclass distribution, and composition. HDL particles have multi-faceted links with health status across life stages, with early influences as early as in utero and in infancy having lasting life-long effects. As the HDL field continues to advance and as more sophisticated methods for isolating and characterizing HDL emerge, new discoveries will lead to novel HDL-based diagnostics and interventions/therapeutics. Interventions to modify and improve the functionality of HDL particles can improve health outcomes and health trajectories at all life stages.

## REFERENCES


1. Chiesa, S.T.; Charakida, M. High-Density Lipoprotein Function and Dysfunction in Health and Disease. *Cardiovasc Drugs Ther* **2019**, *33*, 207–219, doi:10.1007/s10557-018-06846-w.
2. Herrera, E.; Ortega-Senovilla, H. Maternal Lipid Metabolism during Normal Pregnancy and Its Implications to Fetal Development. *null* **2010**, *5*, 899–911, doi:10.2217/clp.10.64.
3. Haase, C.L.; Tybjærg-Hansen, A.; Nordestgaard, B.G.; Frikke-Schmidt, R. HDL Cholesterol and Risk of Type 2 Diabetes: A Mendelian Randomization Study. *Diabetes* **2015**, *64*, 3328–3333, doi:10.2337/db14-1603.
4. Lee, S.-H.; Kim, H.-S.; Park, Y.-M.; Kwon, H.-S.; Yoon, K.-H.; Han, K.; Kim, M.K. HDL-Cholesterol, Its Variability, and the Risk of Diabetes: A Nationwide Population-Based Study. *The Journal of Clinical Endocrinology & Metabolism* **2019**, *104*, 5633–5641, doi:10.1210/jc.2019-01080.
5. Kawachi, K.; Kataoka, H.; Manabe, S.; Mochizuki, T.; Nitta, K. Low HDL Cholesterol as a Predictor of Chronic Kidney Disease Progression: A Cross-Classification Approach and Matched Cohort Analysis. *Heart Vessels* **2019**, *34*, 1440–1455, doi:10.1007/s00380-019-01375-4.
6. Zoppini, G.; Targher, G.; Chonchol, M.; Perrone, F.; Lippi, G.; Muggeo, M. Higher HDL Cholesterol Levels Are Associated with a Lower Incidence of Chronic Kidney Disease in Patients with Type 2 Diabetes. *Nutr Metab Cardiovasc Dis* **2009**, *19*, 580–586, doi:10.1016/j.numecd.2008.11.003.
7. Cooney, M.T.; Dudina, A.; De Bacquer, D.; Wilhelmsen, L.; Sans, S.; Menotti, A.; De Backer, G.; Jousilahti, P.; Keil, U.; Thomsen, T.; et al. HDL Cholesterol Protects against Cardiovascular Disease in Both Genders, at All Ages and at All Levels of Risk. *Atherosclerosis* **2009**, *206*, 611–616, doi:10.1016/j.atherosclerosis.2009.02.041.
8. Assmann, G.; Gotto, A.M. HDL Cholesterol and Protective Factors in Atherosclerosis. *Circulation* **2004**, *109*, III8-14.
9. Button, E.B.; Robert, J.; Caffrey, T.M.; Fan, J.; Zhao, W.; Wellington, C.L. HDL from an Alzheimer's Disease Perspective. *Curr Opin Lipidol* **2019**, *30*, 224–234, doi:10.1097/MOL.0000000000000604.
10. Marsillach, J.; Adorni, M.P.; Zimetti, F.; Papotti, B.; Zuliani, G.; Cervellati, C. HDL Proteome and Alzheimer's Disease: Evidence of a Link. *Antioxidants* **2020**, *9*, doi:10.3390/antiox9121224.
11. Barter, P.; Gotto, A.M.; LaRosa, J.C.; Maroni, J.; Szarek, M.; Grundy, S.M.; Kastelein, J.J.P.; Bittner, V.; Fruchart, J.-C.; Investigators, T. to N.T. HDL Cholesterol, Very Low Levels of LDL Cholesterol, and Cardiovascular Events. *N Engl J Med* **2007**, *357*, 1301–1310.
12. Wilson, P.W.; Abbott, R.D.; Castelli, W.P. High Density Lipoprotein Cholesterol and Mortality. The Framingham Heart Study. *Arteriosclerosis: An Official Journal of the American Heart Association, Inc.* **1988**, *8*, 737–741, doi:10.1161/01.ATV.8.6.737.
13. Penson, P.; Long, D.L.; Howard, G.; Howard, V.J.; Jones, S.R.; Martin, S.S.; Mikhailidis, D.P.; Muntner, P.; Rizzo, M.; Rader, D.J.; et al. Associations between Cardiovascular Disease, Cancer, and Very Low High-Density Lipoprotein Cholesterol in the REasons for Geographical and Racial Differences in Stroke (REGARDS) Study. *Cardiovascular Research* **2019**, *115*, 204–212, doi:10.1093/cvr/cvy198.

14. Zhang, X.; Tong, T.; Chang, A.; Ang, T.F.A.; Tao, Q.; Auerbach, S.; Devine, S.; Qiu, W.Q.; Mez, J.; Massaro, J.; et al. Midlife Lipid and Glucose Levels Are Associated with Alzheimer's Disease. *Alzheimers Dement* **2022**, doi:10.1002/alz.12641.
15. Svensson, T.; Sawada, N.; Mimura, M.; Nozaki, S.; Shikimoto, R.; Tsugane, S. The Association between Midlife Serum High-Density Lipoprotein and Mild Cognitive Impairment and Dementia after 19 Years of Follow-Up. *Translational Psychiatry* **2019**, *9*, 26, doi:10.1038/s41398-018-0336-y.
16. März, W.; Kleber, M.E.; Scharnagl, H.; Speer, T.; Zewinger, S.; Ritsch, A.; Parhofer, K.G.; von Eckardstein, A.; Landmesser, U.; Laufs, U. HDL Cholesterol: Reappraisal of Its Clinical Relevance. *Clin Res Cardiol* **2017**, *106*, 663–675, doi:10.1007/s00392-017-1106-1.
17. Huang, Y.-Q.; Liu, X.-C.; Lo, K.; Liu, L.; Yu, Y.-L.; Chen, C.-L.; Huang, J.-Y.; Feng, Y.-Q.; Zhang, B. The U Shaped Relationship Between High-Density Lipoprotein Cholesterol and All-Cause or Cause-Specific Mortality in Adult Population. *Clin Interv Aging* **2020**, *15*, 1883–1896, doi:10.2147/CIA.S271528.
18. Soran, H.; Hama, S.; Yadav, R.; Durrington, P.N. HDL Functionality. *Curr Opin Lipidol* **2012**, *23*, 353–366.
19. Woudberg, N.J.; Pedretti, S.; Lecour, S.; Schulz, R.; Vuilleumier, N.; James, R.W.; Frias, M.A. Pharmacological Intervention to Modulate HDL: What Do We Target? *Frontiers in Pharmacology* **2018**, *8*.
20. Rohatgi, A.; Khera, A.; Berry, J.D.; Givens, E.G.; Ayers, C.R.; Wedin, K.E.; Neeland, I.J.; Yuhanna, I.S.; Rader, D.R.; de Lemos, J.A.; et al. HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events. *N Engl J Med* **2014**, *371*, 2383–2393, doi:10.1056/NEJMoa1409065.
21. Khera, A.V.; Cuchel, M.; de la Llera-Moya, M.; Rodrigues, A.; Burke, M.F.; Jafri, K.; French, B.C.; Phillips, J.A.; Mucksavage, M.L.; Wilensky, R.L.; et al. Cholesterol Efflux Capacity, High-Density Lipoprotein Function, and Atherosclerosis. *N Engl J Med* **2011**, *364*, 127–135, doi:10.1056/NEJMoa1001689.
22. Khera, A.V.; Demler, O.V.; Adelman, S.J.; Collins, H.L.; Glynn, R.J.; Ridker, P.M.; Rader, D.J.; Mora, S. Cholesterol Efflux Capacity, High-Density Lipoprotein Particle Number, and Incident Cardiovascular Events: An Analysis From the JUPITER Trial (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin). *Circulation* **2017**, *135*, 2494–2504, doi:10.1161/CIRCULATIONAHA.116.025678.
23. Vrselja, A.; Pillow, J.J.; Black, M.J. Effect of Preterm Birth on Cardiac and Cardiomyocyte Growth and the Consequences of Antenatal and Postnatal Glucocorticoid Treatment. *J Clin Med* **2021**, *10*, doi:10.3390/jcm10173896.
24. Blencowe, H.; Cousens, S.; Oestergaard, M.Z.; Chou, D.; Moller, A.-B.; Narwal, R.; Adler, A.; Vera Garcia, C.; Rohde, S.; Say, L.; et al. National, Regional, and Worldwide Estimates of Preterm Birth Rates in the Year 2010 with Time Trends since 1990 for Selected Countries: A Systematic Analysis and Implications. *The Lancet* **2012**, *379*, 2162–2172, doi:10.1016/S0140-6736(12)60820-4.
25. Singhal, A.; Cole, T.J.; Fewtrell, M.; Lucas, A. Breastmilk Feeding and Lipoprotein Profile in Adolescents Born Preterm: Follow-up of a Prospective Randomised Study. *The Lancet* **2004**, *363*, 1571–1578, doi:10.1016/S0140-6736(04)16198-9.

26. Bayley, T.M.; Alasmi, M.; Thorkelson, T.; Krug-Wispe, S.; Jones, P.J.H.; Bulani, J.L.; Tsang, R.C. Influence of Formula versus Breast Milk on Cholesterol Synthesis Rates in Four-Month-Old Infants. *Pediatric Research* **1998**, *44*, 60–67, doi:10.1203/00006450-199807000-00010.
27. Scoble, J.A.; Smilowitz, J.T.; Argov-Argaman, N.; German, J.B.; Underwood, M.A. Plasma Lipoprotein Particle Subclasses in Preterm Infants. *Am J Perinatol* **2018**, *35*, 369–379.
28. Burns, S.F.; Lee, S.J.; Arslanian, S.A. Surrogate Lipid Markers for Small Dense Low-Density Lipoprotein Particles in Overweight Youth. *J Pediatr* **2012**, *161*, 991–996, doi:10.1016/j.jpeds.2012.06.013.
29. Ivanova, E.A.; Myasoedova, V.A.; Melnichenko, A.A.; Grechko, A.V.; Orekhov, A.N. Small Dense Low-Density Lipoprotein as Biomarker for Atherosclerotic Diseases. *Oxid Med Cell Longev* **2017**, *2017*, 1273042, doi:10.1155/2017/1273042.
30. Genzel-Boroviczény, O.; Göbel, Y.; Koletzko, B. Effect of Early Enteral Feeding on Apolipoprotein AI Levels and High-Density Lipoprotein Heterogeneity in Preterm Infants. *Ann Nutr Metab* **2002**, *46*, 121–127, doi:10.1159/000063080.
31. Nagano, N.; Okada, T.; Yonezawa, R.; Yoshikawa, K.; Fujita, H.; Usukura, Y.; Makimoto, M.; Hosono, S.; Fujita, Y.; Takahashi, S.; et al. Early Postnatal Changes of Lipoprotein Subclass Profile in Late Preterm Infants. *Clinica Chimica Acta* **2012**, *413*, 109–112, doi:10.1016/j.cca.2011.09.010.
32. Martin, M.; Gaete, L.; Tetzlaff, W.; Ferraro, F.; Lozano Chiappe, E.; Botta, E.E.; Osta, V.; Saez, M.S.; Lorenzon Gonzalez, M.V.; Palenque, P.; et al. Vascular Inflammation and Impaired Reverse Cholesterol Transport and Lipid Metabolism in Obese Children and Adolescents. *Nutr Metab Cardiovasc Dis* **2022**, *32*, 258–268, doi:10.1016/j.numecd.2021.09.025.
33. Rousset, X.; Vaisman, B.; Amar, M.; Sethi, A.A.; Remaley, A.T. Lecithin: Cholesterol Acyltransferase--from Biochemistry to Role in Cardiovascular Disease. *Curr Opin Endocrinol Diabetes Obes* **2009**, *16*, 163–171, doi:10.1097/med.0b013e328329233b.
34. Nagasaka, H.; Chiba, H.; Kikuta, H.; Akita, H.; Takahashi, Y.; Yanai, H.; Hui, S.-P.; Fuda, H.; Fujiwara, H.; Kobayashi, K. Unique Character and Metabolism of High Density Lipoprotein (HDL) in Fetus. *Atherosclerosis* **2002**, *161*, 215–223, doi:10.1016/S0021-9150(01)00663-3.
35. Jain, S.K.; Diaz, J.J. Plasma Lecithin-Cholesterol Acyltransferase Activity and Cholesterol and Phospholipid Levels in Premature Newborn Infants. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* **1991**, *1086*, 225–229, doi:10.1016/0005-2760(91)90011-6.
36. Kosmas, C.E.; Martinez, I.; Sourlas, A.; Bouza, K.V.; Campos, F.N.; Torres, V.; Montan, P.D.; Guzman, E. High-Density Lipoprotein (HDL) Functionality and Its Relevance to Atherosclerotic Cardiovascular Disease. *Drugs Context* **2018**, *7*, 212525–212525, doi:10.7573/dic.212525.
37. Zimetti, F.; Favari, E.; Cagliero, P.; Adorni, M.P.; Ronda, N.; Bonardi, R.; Gomaraschi, M.; Calabresi, L.; Bernini, F.; Guardamagna, O. Cholesterol Trafficking-Related Serum Lipoprotein Functions in Children with Cholesteryl Ester Storage Disease. *Atherosclerosis* **2015**, *242*, 443–449, doi:https://doi.org/10.1016/j.atherosclerosis.2015.08.007.
38. Khalil, H.; Murrin, C.; O'Reilly, M.; Viljoen, K.; Segurado, R.; O'Brien, J.; Somerville, R.; McGillicuddy, F.; Kelleher, C.C. Total HDL Cholesterol Efflux Capacity in Healthy

Children - Associations with Adiposity and Dietary Intakes of Mother and Child. *Nutr Metab Cardiovasc Dis* **2017**, *27*, 70–77.
39. Christensen, J.J.; Narverud, I.; Ruuth, M.; Heier, M.; Jauhiainen, M.; Ulven, S.M.; Bogsrud, M.P.; Kovanen, P.T.; Halvorsen, B.; Oda, M.N.; et al. Children with Familial Hypercholesterolemia Display Changes in LDL and HDL Function: A Cross-Sectional Study. *Journal of Internal Medicine* **2021**, *290*, 1083–1097, doi:10.1111/joim.13383.
40. Härdfeldt, J.; Cariello, M.; Simonelli, S.; Ossoli, A.; Scialpi, N.; Piglionica, M.; Pasculli, E.; Noia, A.; Berardi, E.; Suppressa, P.; et al. Abdominal Obesity Negatively Influences Key Metrics of Reverse Cholesterol Transport. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **2022**, *1867*, 159087, doi:10.1016/j.bbalip.2021.159087.
41. Rochlani, Y.; Pothineni, N.V.; Kovelamudi, S.; Mehta, J.L. Metabolic Syndrome: Pathophysiology, Management, and Modulation by Natural Compounds. *Ther Adv Cardiovasc Dis* **2017**, *11*, 215–225, doi:10.1177/1753944717711379.
42. Medina-Urrutia, A.; Juarez-Rojas, J.G.; Cardoso-Saldaña, G.; Jorge-Galarza, E.; Posadas-Sánchez, R.; Martínez-Alvarado, R.; Caracas-Portilla, N.; Mendoza Pérez, E.; Posadas-Romero, C. Abnormal High-Density Lipoproteins in Overweight Adolescents With Atherogenic Dyslipidemia. *Pediatrics* **2011**, *127*, e1521–e1527, doi:10.1542/peds.2010-1395.
43. Heier, M.; Borja, M.S.; Brunborg, C.; Seljeflot, I.; Margeirsdottir, H.D.; Hanssen, K.F.; Dahl-Jørgensen, K.; Oda, M.N. Reduced HDL Function in Children and Young Adults with Type 1 Diabetes. *Cardiovasc Diabetol* **2017**, *16*, 85, doi:10.1186/s12933-017-0570-2.
44. Medina-Bravo, P.; Medina-Urrutia, A.; Juárez-Rojas, J.G.; Cardoso-Saldaña, G.; Jorge-Galarza, E.; Posadas-Sánchez, R.; Coyote-Estrada, N.; Nishimura-Meguro, E.; Posadas-Romero, C. Glycemic Control and High-Density Lipoprotein Characteristics in Adolescents with Type 1 Diabetes. *Pediatric Diabetes* **2013**, *14*, 399–406, doi:10.1111/j.1399-5448.2012.00924.x.
45. Gourgari, E.; Nadeau, K.J.; Pyle, L.; Playford, M.P.; Ma, J.; Mehta, N.N.; Remaley, A.T.; Gordon, S.M. Effect of Metformin on the High-Density Lipoprotein Proteome in Youth with Type 1 Diabetes. *Endocrinol Diabetes Metab* **2021**, *4*, e00261–e00261, doi:10.1002/edm2.261.
46. Borja, M.S.; Zhao, L.; Hammerson, B.; Tang, C.; Yang, R.; Carson, N.; Fernando, G.; Liu, X.; Budamagunta, M.S.; Genest, J.; et al. HDL-ApoA-I Exchange: Rapid Detection and Association with Atherosclerosis. *PLoS One* **2013**, *8*, e71541, doi:10.1371/journal.pone.0071541.
47. Borja, M.S.; Ng, K.F.; Irwin, A.; Hong, J.; Wu, X.; Isquith, D.; Zhao, X.-Q.; Prazen, B.; Gildengorin, V.; Oda, M.N.; et al. HDL-Apolipoprotein A-I Exchange Is Independently Associated with Cholesterol Efflux Capacity. *J Lipid Res* **2015**, *56*, 2002–2009, doi:10.1194/jlr.M059865.
48. Ahmed, M.O.; Byrne, R.E.; Pazderska, A.; Segurado, R.; Guo, W.; Gunness, A.; Frizelle, I.; Sherlock, M.; Ahmed, K.S.; McGowan, A.; et al. HDL Particle Size Is Increased and HDL-Cholesterol Efflux Is Enhanced in Type 1 Diabetes: A Cross-Sectional Study. *Diabetologia* **2021**, *64*, 656–667, doi:10.1007/s00125-020-05320-3.
49. Kashyap, S.R.; Osme, A.; Ilchenko, S.; Golizeh, M.; Lee, K.; Wang, S.; Bena, J.; Previs, S.F.; Smith, J.D.; Kasumov, T. Glycation Reduces the Stability of ApoAI and Increases

HDL Dysfunction in Diet-Controlled Type 2 Diabetes. *The Journal of Clinical Endocrinology & Metabolism* **2018**, *103*, 388–396, doi:10.1210/jc.2017-01551.
50. Kaseda, R.; Jabs, K.; Hunley, T.E.; Jones, D.; Bian, A.; Allen, R.M.; Vickers, K.C.; Yancey, P.G.; Linton, M.F.; Fazio, S.; et al. Dysfunctional High-Density Lipoproteins in Children with Chronic Kidney Disease. *Metabolism* **2015**, *64*, 263–273, doi:10.1016/j.metabol.2014.10.020.
51. Meier, S.M.; Wultsch, A.; Hollaus, M.; Ammann, M.; Pemberger, E.; Liebscher, F.; Lambers, B.; Fruhwürth, S.; Stojakovic, T.; Scharnagl, H.; et al. Effect of Chronic Kidney Disease on Macrophage Cholesterol Efflux. *Life Sciences* **2015**, *136*, 1–6, doi:https://doi.org/10.1016/j.lfs.2015.06.005.
52. Moradi, H.; Vaziri, N.D.; Kashyap, M.L.; Said, H.M.; Kalantar-Zadeh, K. Role of HDL Dysfunction in End-Stage Renal Disease: A Double-Edged Sword. *J Ren Nutr* **2013**, *23*, 203–206, doi:10.1053/j.jrn.2013.01.022.
53. Yamatani, K.; Hirayama, S.; Seino, U.; Hirayama, A.; Hori, A.; Suzuki, K.; Idei, M.; Kitahara, M.; Miida, T. Preβ1-High-Density Lipoprotein Metabolism Is Delayed in Patients with Chronic Kidney Disease Not on Hemodialysis. *Journal of Clinical Lipidology* **2020**, *14*, 730–739, doi:10.1016/j.jacl.2020.07.011.
54. Bories, P.C.; Subbaiah, P.V.; Bagdade, J.D. Lecithin: Cholesterol Acyltransferase Activity in Dialyzed and Undialyzed Chronic Uremic Patients. *Nephron* **1982**, *32*, 22–27, doi:10.1159/000182726.
55. Miida, T.; Miyazaki, O.; Hanyu, O.; Nakamura, Y.; Hirayama, S.; Narita, I.; Gejyo, F.; Ei, I.; Tasaki, K.; Kohda, Y.; et al. LCAT-Dependent Conversion of Preβ1-HDL into α-Migrating HDL Is Severely Delayed in Hemodialysis Patients. *Journal of the American Society of Nephrology* **2003**, *14*, 732–738, doi:10.1097/01.ASN.0000046962.43220.8A.
56. Hong, B.V.; Zhu, C.; Wong, M.; Sacchi, R.; Rhodes, C.H.; Kang, J.W.; Arnold, C.D.; Adu-Afarwuah, S.; Lartey, A.; Oaks, B.M.; et al. Lipid-Based Nutrient Supplementation Increases High-Density Lipoprotein (HDL) Cholesterol Efflux Capacity and Is Associated with Changes in the HDL Glycoproteome in Children. *ACS Omega* **2021**, *6*, 32022–32031, doi:10.1021/acsomega.1c04811.
57. Adu-Afarwuah, S.; Lartey, A.; Okronipa, H.; Ashorn, P.; Peerson, J.M.; Arimond, M.; Ashorn, U.; Zeilani, M.; Vosti, S.; Dewey, K.G. Small-Quantity, Lipid-Based Nutrient Supplements Provided to Women during Pregnancy and 6 Mo Postpartum and to Their Infants from 6 Mo of Age Increase the Mean Attained Length of 18-Mo-Old Children in Semi-Urban Ghana: A Randomized Controlled Trial. *Am J Clin Nutr* **2016**, *104*, 797–808.
58. Zhu, C.; Wong, M.; Li, Q.; Sawrey-Kubicek, L.; Beals, E.; Rhodes, C.H.; Sacchi, R.; Lebrilla, C.B.; Zivkovic, A.M. Site-Specific Glycoprofiles of HDL-Associated ApoE Are Correlated with HDL Functional Capacity and Unaffected by Short-Term Diet. *J Proteome Res* **2019**, *18*, 3977–3984.
59. Davidson, W.S.; Inge, T.H.; Sexmith, H.; Heink, A.; Elder, D.; Hui, D.Y.; Melchior, J.T.; Kelesidis, T.; Shah, A.S. Weight Loss Surgery in Adolescents Corrects High-Density Lipoprotein Subspecies and Their Function. *Int J Obes (Lond)* **2017**, *41*, 83–89, doi:10.1038/ijo.2016.190.
60. Lorkowski, S.W.; Brubaker, G.; Rotroff, D.M.; Kashyap, S.R.; Bhatt, D.L.; Nissen, S.E.; Schauer, P.R.; Aminian, A.; Smith, J.D. Bariatric Surgery Improves HDL Function Examined by ApoA1 Exchange Rate and Cholesterol Efflux Capacity in Patients with Obesity and Type 2 Diabetes. *Biomolecules* **2020**, *10*, doi:10.3390/biom10040551.

61. Wesnigk, J.; Bruyndonckx, L.; Hoymans, V.Y.; De Guchtenaere, A.; Fischer, T.; Schuler, G.; Vrints, C.J.; Adams, V. Impact of Lifestyle Intervention on HDL-Induced ENOS Activation and Cholesterol Efflux Capacity in Obese Adolescent. *Cardiol Res Pract* **2016**, *2016*, 2820432–2820432, doi:10.1155/2016/2820432.
62. Boyer, M.; Mitchell, P.L.; Poirier, P.; Alméras, N.; Tremblay, A.; Bergeron, J.; Després, J.-P.; Arsenault, B.J. Impact of a One-Year Lifestyle Modification Program on Cholesterol Efflux Capacities in Men with Abdominal Obesity and Dyslipidemia. *American Journal of Physiology-Endocrinology and Metabolism* 2018, *315*, E460–E468.
63. Wiznitzer, A.; Mayer, A.; Novack, V.; Sheiner, E.; Gilutz, H.; Malhotra, A.; Novack, L. Association of Lipid Levels during Gestation with Preeclampsia and Gestational Diabetes Mellitus: A Population-Based Study. *Am J Obstet Gynecol* **2009**, *201*, 482.e1-482.e4828, doi:10.1016/j.ajog.2009.05.032.
64. Zeljkovic, A.; Vekic, J.; Spasic, S.; Jelic-Ivanovic, Z.; Spasojevic-Kalimanovska, V.; Gojkovic, T.; Ardalic, D.; Mandic-Markovic, V.; Cerovic, N.; Mikovic, Z. Changes in LDL and HDL Subclasses in Normal Pregnancy and Associations with Birth Weight, Birth Length and Head Circumference. *Matern Child Health J* **2013**, *17*, 556–565, doi:10.1007/s10995-012-1031-x.
65. Oaks, B.M.; Stewart, C.P.; Laugero, K.D.; Adu-Afarwuah, S.; Lartey, A.; Vosti, S.A.; Ashorn, P.; Dewey, K.G. Maternal Plasma Cholesterol and Duration of Pregnancy: A Prospective Cohort Study in Ghana. *Matern Child Nutr* **2017**, *13*, 10.1111/mcn.12418.
66. Bever, A.M.; Mumford, S.L.; Schisterman, E.F.; Sjaarda, L.; Perkins, N.J.; Gerlanc, N.; DeVilbiss, E.A.; Silver, R.M.; Kim, K.; Nobles, C.J.; et al. Maternal Preconception Lipid Profile and Gestational Lipid Changes in Relation to Birthweight Outcomes. *Sci Rep* **2020**, *10*, 1374–1374, doi:10.1038/s41598-019-57373-z.
67. Wang, H.; Dang, Q.; Zhu, H.; Liang, N.; Le, Z.; Huang, D.; Xiao, R.; Yu, H. Associations between Maternal Serum HDL-c Concentrations during Pregnancy and Neonatal Birth Weight: A Population-Based Cohort Study. *Lipids in Health and Disease* **2020**, *19*, 93, doi:10.1186/s12944-020-01264-0.
68. Boghossian, N.S.; Mendola, P.; Liu, A.; Robledo, C.; Yeung, E.H. Maternal Serum Markers of Lipid Metabolism in Relation to Neonatal Anthropometry. *Journal of Perinatology* **2017**, *37*, 629–635, doi:10.1038/jp.2017.22.
69. Rideout, T.C.; Wen, X.; Choudhary, D.; Catanzaro, M.; Browne, R.W.; Barnabei, V.M.; Kong, K.L. Associations of Maternal Lipoprotein Particle Distribution in Mid-Pregnancy with Birth Outcomes: A Pilot Study. *Lipids Health Dis* **2022**, *21*, 53, doi:10.1186/s12944-022-01664-4.
70. Kramer, M.S.; Kahn, S.R.; Dahhou, M.; Otvos, J.; Genest, J.; Platt, R.W.; Evans, R.W. Maternal Lipids and Small for Gestational Age Birth at Term. *The Journal of Pediatrics* **2013**, *163*, 983–988, doi:10.1016/j.jpeds.2013.05.014.
71. Segrest, J.P.; Cheung, M.C.; Jones, M.K. Volumetric Determination of Apolipoprotein Stoichiometry of Circulating HDL Subspecies. *J Lipid Res* **2013**, *54*, 2733–2744, doi:10.1194/jlr.M039172.
72. Zheng, J.J.; Agus, J.K.; Hong, B.V.; Tang, X.; Rhodes, C.H.; Houts, H.E.; Zhu, C.; Kang, J.W.; Wong, M.; Xie, Y.; et al. Isolation of HDL by Sequential Flotation Ultracentrifugation Followed by Size Exclusion Chromatography Reveals Size-Based Enrichment of HDL-Associated Proteins. *Scientific Reports* **2021**, *11*, 16086, doi:10.1038/s41598-021-95451-3.

73. Furtado, J.D.; Yamamoto, R.; Melchior, J.T.; Andraski, A.B.; Gamez-Guerrero, M.; Mulcahy, P.; He, Z.; Cai, T.; Davidson, W.S.; Sacks, F.M. Distinct Proteomic Signatures in 16 HDL (High-Density Lipoprotein) Subspecies. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2018**, *38*, 2827–2842, doi:10.1161/ATVBAHA.118.311607.
74. Melchior, J.T.; Street, S.E.; Andraski, A.B.; Furtado, J.D.; Sacks, F.M.; Shute, R.L.; Greve, E.I.; Swertfeger, D.K.; Li, H.; Shah, A.S.; et al. Apolipoprotein A-II Alters the Proteome of Human Lipoproteins and Enhances Cholesterol Efflux from ABCA1. *Journal of Lipid Research* **2017**, *58*, 1374–1385, doi:10.1194/jlr.M075382.
75. Alvarez, J.J.; Montelongo, A.; Iglesias, A.; Lasunción, M.A.; Herrera, E. Longitudinal Study on Lipoprotein Profile, High Density Lipoprotein Subclass, and Postheparin Lipases during Gestation in Women. *J Lipid Res* **1996**, *37*, 299–308.
76. Melchior, J.T.; Swertfeger, D.K.; Morris, J.; Street, S.E.; Warshak, C.R.; Welge, J.A.; Remaley, A.T.; Catov, J.M.; Davidson, W.S.; Woollett, L.A. Pregnancy Is Accompanied by Larger High Density Lipoprotein Particles and Compositionally Distinct Subspecies. *Journal of Lipid Research* **2021**, *62*, doi:10.1016/j.jlr.2021.100107.
77. Catov, J.M.; Mackey, R.H.; Scifres, C.M.; Bertolet, M.; Simhan, H.N. Lipoprotein Heterogeneity Early in Pregnancy and Preterm Birth. *Am J Perinatol* **2017**, *34*, 1326–1332.
78. Grace, M.R.; Vladutiu, C.J.; Nethery, R.C.; Siega-Riz, A.M.; Manuck, T.A.; Herring, A.H.; Savitz, D.; Thorp, J.T. Lipoprotein Particle Concentration Measured by Nuclear Magnetic Resonance Spectroscopy Is Associated with Gestational Age at Delivery: A Prospective Cohort Study. *BJOG* **2018**, *125*, 895–903, doi:10.1111/1471-0528.14927.
79. González, F.E.M.; Ponce-RuÍz, N.; Rojas-GarcÍa, A.E.; Bernal-Hernández, Y.Y.; Mackness, M.; Ponce-Gallegos, J.; Cardoso-Saldaña, G.; Jorge-Galarza, E.; Torres-Tamayo, M.; Medina-Díaz, I.M. PON1 Concentration and High-Density Lipoprotein Characteristics as Cardiovascular Biomarkers. *Arch Med Sci Atheroscler Dis* **2019**, *4*, e47–e54, doi:10.5114/amsad.2019.84447.
80. Seres, I.; Bajnok, L.; Harangi, M.; Sztanek, F.; Koncsos, P.; Paragh, G. Alteration of PON1 Activity in Adult and Childhood Obesity and Its Relation to Adipokine Levels. *Adv Exp Med Biol* **2010**, *660*, 129–142, doi:10.1007/978-1-60761-350-3_12.
81. Dantoine, T.F.; Debord, J.; Charmes, J.P.; Merle, L.; Marquet, P.; Lachatre, G.; Leroux-Robert, C. Decrease of Serum Paraoxonase Activity in Chronic Renal Failure. *J Am Soc Nephrol* **1998**, *9*, 2082–2088, doi:10.1681/ASN.V9112082.
82. El-said, N.H.; Nasr-Allah, M.M.; Sadik, N.A.; Sharaf, S.A. Paraoxonase-1 Activity in Type 2 Diabetes Mellitus with and without Nephropathy. *The Egyptian Journal of Internal Medicine* **2015**, *27*, 63–68, doi:10.4103/1110-7782.159451.
83. Stefanović, A.; Ardalic, D.; Kotur-Stevuljević, J.; Vujović, A.; Spasic, S.; Spasojević-Kalimanovska, V.; Jelic-Ivanovic, Z.; Mandic-Markovic, V.; Mikovic, Z.; Cerovic, N. Longitudinal Changes in PON1 Activities, PON1 Phenotype Distribution and Oxidative Status throughout Normal Pregnancy. *Reproductive Toxicology* **2012**, *33*, 20–26, doi:10.1016/j.reprotox.2011.10.009.
84. Aouache, R.; Biquard, L.; Vaiman, D.; Miralles, F. Oxidative Stress in Preeclampsia and Placental Diseases. *Int J Mol Sci* **2018**, *19*, doi:10.3390/ijms19051496.
85. Rodríguez-Rodríguez, P.; Ramiro-Cortijo, D.; Reyes-Hernández, C.G.; López de Pablo, A.L.; González, M.C.; Arribas, S.M. Implication of Oxidative Stress in Fetal

Programming of Cardiovascular Disease. *Front Physiol* **2018**, *9*, 602, doi:10.3389/fphys.2018.00602.
86. Einbinder, Y.; Biron-Shental, T.; Agassi-Zaitler, M.; Tzadikevitch-Geffen, K.; Vaya, J.; Khatib, S.; Ohana, M.; Benchetrit, S.; Zitman-Gal, T. High-Density Lipoproteins (HDL) Composition and Function in Preeclampsia. *Archives of Gynecology and Obstetrics* **2018**, *298*, 405–413, doi:10.1007/s00404-018-4824-3.
87. Mistry, H.D.; Kurlak, L.O.; Mansour, Y.T.; Zurkinden, L.; Mohaupt, M.G.; Escher, G. Increased Maternal and Fetal Cholesterol Efflux Capacity and Placental CYP27A1 Expression in Preeclampsia. *J Lipid Res* **2017**, *58*, 1186–1195, doi:10.1194/jlr.M071985.
88. Kockx, M.; Roberts, L.; Wang, J.; Tran, C.; Brown, M.A.; Kritharides, L. Effects of Pre-Eclampsia on HDL-Mediated Cholesterol Efflux Capacity after Pregnancy. *Atherosclerosis Plus* **2022**, *48*, 12–19, doi:10.1016/j.athplu.2022.01.003.
89. Landi, F.; Russo, A.; Cesari, M.; Pahor, M.; Bernabei, R.; Onder, G. HDL-Cholesterol and Physical Performance: Results from the Ageing and Longevity Study in the Sirente Geographic Area (IlSIRENTE Study). *Age and ageing* **2007**, *36*, 514–520.
90. Wang, J.; Shi, L.; Zou, Y.; Tang, J.; Cai, J.; Wei, Y.; Qin, J.; Zhang, Z. Positive Association of Familial Longevity with the Moderate-High HDL-C Concentration in Bama Aging Study. *Aging (Albany NY)* **2018**, *10*, 3528.
91. Rahilly-Tierney, C.R.; Spiro, A. 3rd; Vokonas, P.; Gaziano, J.M. Relation between High-Density Lipoprotein Cholesterol and Survival to Age 85 Years in Men (from the VA Normative Aging Study). *Am J Cardiol* **2011**, *107*, 1173–1177, doi:10.1016/j.amjcard.2010.12.015.
92. Campo, S.; Sardo, M.A.; Trimarchi, G.; Bonaiuto, A.; Saitta, C.; Bitto, A.; Castaldo, M.; Cinquegrani, M.; Bonaiuto, M.; Cristadoro, S. Platelet Activating Factor-Acetylhydrolase (PAF-AH) Activity and HDL Levels, but Not PAF-AH Gene Polymorphisms, Are Associated with Successful Aging in Sicilian Octogenarians. *Aging clinical and experimental research* **2008**, *20*, 171–177.
93. Pilling, L.C.; Atkins, J.L.; Bowman, K.; Jones, S.E.; Tyrrell, J.; Beaumont, R.N.; Ruth, K.S.; Tuke, M.A.; Yaghootkar, H.; Wood, A.R. Human Longevity Is Influenced by Many Genetic Variants: Evidence from 75,000 UK Biobank Participants. *Aging (Albany NY)* **2016**, *8*, 547.
94. Atzmon, G.; Gabriely, I.; Greiner, W.; Davidson, D.; Schechter, C.; Barzilai, N. Plasma HDL Levels Highly Correlate with Cognitive Function in Exceptional Longevity. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **2002**, *57*, M712–M715.
95. Pancani, S.; Sofi, F.; Cecchi, F.; Macchi, C. HDL Cholesterol Is Independently Associated with Cognitive Function in Males but Not in Females within a Cohort of Nonagenarians: The Mugello Study. *The journal of nutrition, health & aging* **2019**, *23*, 552–557.
96. Corrada, M.; Kawas, C. Plasma Total Cholesterol, LDL, HDL Levels and the Risk of Alzheimer Disease: The Baltimore Longitudinal Study of Aging. *Neurology* **2002**, *58*, A106–A107.
97. Huang, X.; Zhang, H.; Zhen, J.; Dong, S.; Guo, Y.; Van Halm-Lutterodt, N.; Yuan, L. Diminished Circulating Retinol and Elevated α-TOH/Retinol Ratio Predict an Increased Risk of Cognitive Decline in Aging Chinese Adults, Especially in Subjects with ApoE2 or ApoE4 Genotype. *Aging (Albany NY)* **2018**, *10*, 4066.

98. Hong, B.V.; Zheng, J.; Agus, J.K.; Tang, X.; Lebrilla, C.B.; Jin, L.-W.; Maezawa, I.; Erickson, K.; Harvey, D.J.; DeCarli, C.S.; et al. High-Density Lipoprotein Changes in Alzheimer's Disease Are APOE Genotype-Specific. *Biomedicines* **2022**, *10*, doi:10.3390/biomedicines10071495.
99. Alvim, R.O.; Freitas, S.R.; Ferreira, N.E.; Santos, P.C.; Cunha, R.S.; Mill, J.G.; Krieger, J.E.; Pereira, A.C. APOE Polymorphism Is Associated with Lipid Profile, but Not with Arterial Stiffness in the General Population. *Lipids in Health and Disease* **2010**, *9*, 128, doi:10.1186/1476-511X-9-128.
100. Mahley, R.W. Apolipoprotein E: From Cardiovascular Disease to Neurodegenerative Disorders. *J Mol Med (Berl)* **2016**, *94*, 739–746, doi:10.1007/s00109-016-1427-y.
101. Song, P.; Man, Q.; Li, Y.; Jia, S.; Yu, D.; Zhang, J.; Ding, G. Association between Dietary Patterns and Low HDL-C among Community-Dwelling Elders in North China. *Nutrients* **2021**, *13*, 3308.
102. Berrougui, H.; Isabelle, M.; Cloutier, M.; Grenier, G.; Khalil, A. Age-Related Impairment of HDL-Mediated Cholesterol Efflux. *Journal of lipid research* **2007**, *48*, 328–336.
103. Otrante, A.; Trigui, A.; Walha, R.; Berrougui, H.; Fulop, T.; Khalil, A. Extra Virgin Olive Oil Prevents the Age-Related Shifts of the Distribution of HDL Subclasses and Improves Their Functionality. *Nutrients* **2021**, *13*, 2235.
104. Koudinov, A.R.; Berezov, T.T.; Koudinova, N.V. The Levels of Soluble Amyloid Beta in Different High Density Lipoprotein Subfractions Distinguish Alzheimer's and Normal Aging Cerebrospinal Fluid: Implication for Brain Cholesterol Pathology? *Neuroscience letters* **2001**, *314*, 115–118.
105. Koini, M.; Pirpamer, L.; Hofer, E.; Buchmann, A.; Pinter, D.; Ropele, S.; Enzinger, C.; Benkert, P.; Leppert, D.; Kuhle, J. Factors Influencing Serum Neurofilament Light Chain Levels in Normal Aging. *Aging (Albany NY)* **2021**, *13*, 25729.
106. Holzer, M.; Trieb, M.; Konya, V.; Wadsack, C.; Heinemann, A.; Marsche, G. Aging Affects High-Density Lipoprotein Composition and Function. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids* **2013**, *1831*, 1442–1448.
107. Cherki, M.; Berrougui, H.; Isabelle, M.; Cloutier, M.; Koumbadinga, G.A.; Khalil, A. Effect of PON1 Polymorphism on HDL Antioxidant Potential Is Blunted with Aging. *Experimental gerontology* **2007**, *42*, 815–824.
108. Jaouad, L.; de Guise, C.; Berrougui, H.; Cloutier, M.; Isabelle, M.; Fulop, T.; Payette, H.; Khalil, A. Age-Related Decrease in High-Density Lipoproteins Antioxidant Activity Is Due to an Alteration in the PON1's Free Sulfhydyl Groups. *Atherosclerosis* **2006**, *185*, 191–200.
109. Park, K.-H.; Cho, K.-H. High-Density Lipoprotein (HDL) from Elderly and Reconstituted HDL Containing Glycated Apolipoproteins AI Share Proatherosclerotic and Prosenescent Properties with Increased Cholesterol Influx. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences* **2011**, *66*, 511–520.

## Chapter 2: Lipid-Based Nutrient Supplementation Increases HDL Cholesterol Efflux Capacity and is Associated with Changes in the HDL Glycoproteome in Children

Brian V. Hong[a], Chenghao Zhu[a], Maurice Wong[b], Romina Sacchi[a], Christopher H. Rhodes[a], Jea Woo Kang[a], Charles D. Arnold[a], Seth Adu-Afarwuah[c], Anna Lartey[c], Brietta M. Oaks[d], Carlito B. Lebrilla[b], Kathryn G. Dewey[a], Angela M. Zivkovic[a*]

[a]Department of Nutrition and [b]Chemistry, University of California, Davis, Davis, CA, USA; [c]Department of Nutrition and Food Science, University of Ghana, Legon, Ghana; [d]Department of Nutrition and Food Sciences, University of Rhode Island, Kingston, RI, USA

**ABSTRACT**

Prenatal plus postnatal small-quantity lipid-based nutrient supplements (SQ-LNS) improved child growth at 18 mo in the International Lipid-Based Nutrient Supplements (iLiNS) DYAD trial in Ghana. In this secondary outcome analysis, we determined whether SQ-LNS vs. prenatal iron and folic acid (IFA) supplementation improves the cholesterol efflux capacity (CEC) of high-density lipoprotein (HDL) particles and alters their lipidomic, proteomic or glycoproteomic composition in a subset of 80 children at 18 mo of age. HDL CEC was higher among children in the SQ-LNS vs. IFA group (20.9 ± 4.1% vs. 19.4 ± 3.3%; one-tailed p = 0.038). There were no differences in HDL lipidomic or proteomic composition between groups. Twelve glycopeptides out of the 163 analyzed were significantly altered by SQ-LNS, but none of the group differences remained significant after correction for multiple testing. Exploratory analysis showed that 6 out of the 33 HDL-associated proteins monitored differed in glycopeptide enrichment between intervention groups and 6 out of the 163 glycopeptides were correlated with CEC. We conclude that prenatal plus postnatal SQ-LNS may modify HDL protein glycoprofiles and improve the CEC of HDL particles in children, which may have implications for subsequent child health outcomes.

This trial was registered at clinicaltrials.gov as NCT00970866.

## INTRODUCTION

Inadequate micronutrient intake is common in low- to middle-income countries, and is associated with adverse consequences including slower linear growth of children,[1] impaired cognitive development and diseases in later stages of life.[2] In addition, n-3 and n-6 polyunsaturated fatty acids may be low in food sources and breast milk in certain countries.[3] The International Lipid-Based Nutrient Supplements (iLiNS) Project developed small-quantity lipid-based nutrient supplements (SQ-LNS) to enrich home-prepared foods, particularly for women and children.[4] SQ-LNS provide vitamins, minerals, and essential fatty acids (linoleic acid and α-linolenic acid) to either infants or mothers during pregnancy and lactation.[4] In Ghana, prenatal SQ-LNS led to increased weight, length, and head circumference among infants of primiparous mothers, compared to those whose mothers received an iron and folic acid (IFA) or multiple micronutrient (MMN) supplement,[5] and improved the length, weight, and stunting prevalence in the entire cohort at 18 mo of age.[6]

High-density lipoprotein (HDL) particles are lipid-protein complexes in circulation that play essential roles in maintaining lipid and cholesterol metabolic homeostasis.[7] In addition to the well-known atheroprotective effects of HDL,[7,8] including the role of HDL particles in the regulation of cellular cholesterol concentrations via cholesterol efflux,[9] recent evidence suggests that HDL particles may also be important for the mother-child dyad. Very little is known about the role of HDL during pregnancy and in early development. In the United States, low maternal HDL cholesterol (HDL-C) was associated with low birth weight z-score,[10] and in Ghana we previously reported that high HDL-C at 36 wk gestation was positively associated with the duration of gestation.[11] Even less is known about HDL particles, particularly in infants, however limited evidence in preterm infants indicates that a lower concentration of HDL particles is found

in infants with chronic lung disease compared to those without lung disease.[12] HDL particles display a variety of immuno-modulatory capabilities,[13] including boosting the ability of innate immune cells to fight infection,[14] and may thus be particularly important in settings with high infection burden.

The functionality of HDL particles is known to be dictated by their composition, including both the lipid and protein components,[15–17] and these components are modifiable by diet.[18,19] We have previously demonstrated that in addition to the proteins and lipids, the glycan components may also play an important role in determining the functional capacity of HDL particles.[20,21] Specific components such as α-linolenic acid (ALA), an essential n-3 polyunsaturated fatty acid, have been shown to improve the cholesterol efflux capacity (CEC) of HDL particles in vitro.[22] We have also demonstrated that the glycoprofiles of specific HDL-associated proteins are associated with HDL CEC and can be modified by diet.[21]

Because SQ-LNS deliver essential fatty acids along with proteins and other fats, they may improve the composition and function of HDL particles. In this pilot study and secondary outcome analysis of samples from the iLiNS-DYAD-Ghana trial, we hypothesized that SQ-LNS provided to the mother during pregnancy and the first 6 mo postpartum, followed by SQ-LNS provided to the infant from 6 to 18 mo of age, would increase HDL CEC by altering HDL lipidomic and glycoproteomic composition.

## MATERIALS AND METHODS

### Samples and Subjects

The complete study design and subject characteristics of the iLiNS DYAD-Ghana trial were described in detail previously.[5] The compositions of IFA and SQ-LNS are presented in **Supporting Information Table S6**. In total, 1320 women were enrolled at a mean gestational

age of 16.3 wk and were randomized to receive IFA, MMN, or SQ-LNS until delivery, followed by placebo (200 mg calcium), MMN, or SQ-LNS, respectively during 6 mo postpartum. The infants whose mothers were supplemented with SQ-LNS received SQ-LNS formulated for infants from 6 to 18 mo of age; children in the IFA group did not receive supplements. Women were visited biweekly during pregnancy and weekly after birth to deliver fresh supplies of supplements and monitor supplement consumption and morbidity. Blood was drawn from children at 18 mo at the laboratory, and plasma was separated after centrifugation at 1,252 g for 15 min at room temperature. Plasma samples were stored at -33°C in Ghana before they were airmailed on dry ice to Davis, CA, USA, after which time the samples were stored at -80°C.[42]

In this study, a subset of 40 children from the IFA group and 40 from the SQ-LNS group were selected, and their plasma samples at 18 mo were analyzed. The 80 children were selected according to the following maternal criteria: 1) randomized to either SQ-LNS or IFA (the MMN group was excluded); 2) primiparous; 3) not overweight (body mass index (BMI) < 25 kg/m$^2$ at enrollment); and 4) enrolled between October 2010 and December 2011 (to avoid inclusion of women enrolled earlier who may have had mixed exposure to IFA and MMN, as explained previously).[5] The maternal criteria were selected to maximize the probability of observing a response to SQ-LNS, as the effects of SQ-LNS on birth size were greater among infants born to primiparous mothers,[5] and longer-term effects on child growth were greater among children born to non-overweight women.[43] In total, 116 children met these criteria, 53 in the SQ-LNS group and 63 in the IFA group. These children were randomly sorted and the first 40 in each group were selected (**Supporting Information Figure S1**).

The study protocol was approved by the ethics committees of the University of California, Davis; the Ghana Health Service; and the University of Ghana Noguchi Memorial Institute for Medical Research, and was registered on clinicaltrials.gov as NCT00970866.

**HDL isolation**

HDL particles were isolated through a two-step HDL isolation method modified from Holzer et al.[44] which isolates HDL particles first by density using sequential flotation ultracentrifugation as previously described,[45] followed by fast protein liquid chromatography (FPLC). Briefly, 500 µL of plasma was underlaid under KBr solution at a density of 1.006 g/mL to remove triglyceride-rich, low density (< 1.006 g/mL) particles, including chylomicrons and very low-density lipoproteins (VLDL), and submitted to ultracentrifugation in an Optima MAX-TL Ultracentrifuge with (Beckmann-Coulter) fixed angle rotor at 110,000 RPM and 14°C for 30 min. After centrifugation, the supernatant was removed by aspiration, and the remaining fraction containing HDL, low-density lipoproteins (LDL), albumin, and plasma proteins was adjusted to a density of 1.210 g/mL with 1.340 g/mL KBr solution and underlaid under clean 1.210 g/mL density solution, then submitted to ultracentrifugation at 110,000 RPM and 14°C for 3 h and 30 min. The supernatant was removed by aspiration and dialyzed using an Amicon Ultra-4 50kDa centrifugal filter (Millipore) by centrifugation at 4,500 RPM for 8 min. A final volume of 250 µl was then transferred to an amber vial for FPLC analysis using a single Superdex 200 Increase 10/300 GL agarose-crosslinked column (GE Healthcare) on an AKTA P-920 FPLC (Amersham Biosciences) connected to a fraction collector. Four 1-mL fractions eluting in the HDL size range were pooled together and dialyzed to 100 µL, of which one aliquot was used for glycoproteomic analysis, another for lipidomics analysis, and one for analysis of CEC.

**HDL Protein and Glycoprotein Identification**

Isolated HDL samples were run on an Agilent 1290 Infinity II High-Performance Liquid Chromatography (HPLC) coupled to a Fusion Lumos MS/MS Orbitrap (Thermo Fisher Scientific). Peptides and glycopeptides were identified with Byonic software (Protein Metrics Inc) from the Orbitrap MS data (see **Supporting Information Material 1** for additional details).

A total of 33 HDL-associated proteins were monitored in this study including apolipoprotein A-I (APOA1), apolipoprotein(a) (LPA), apolipoprotein A-II (APOA2), apolipoprotein A-IV (APOA4), apolipoprotein A-V (APOA5), apolipoprotein C-II (APOC2), apolipoprotein C-IV (APOC4), apolipoprotein F (APOF), apolipoprotein L1 (APOL1), haptoglobin-related protein (HPR), phosphatidylcholine-sterol acyltransferase (LCAT), phospholipid transfer protein (PLTP), alpha-1-antitrypsin (A1AT), alpha-1B-glycoprotein (A1BG), alpha-1-antichymotrypsin (AACT), apolipoprotein B-100 (APOB100), apolipoprotein C-I (APOC1), apolipoprotein C-III (APOC3), apolipoprotein D (APOD), apolipoprotein E (APOE), beta-2-glycoprotein 1 (APOH), apolipoprotein M (APOM), complement C1s subcomponent (C1S), clusterin (CLUS or APOJ), complement C3 (C3), alpha-2-HS-glycoprotein (FETUA or AHSG), hemopexin (HPX), heparin cofactor 2 (HCF2), kininogen-1 (KNG1), serum paraoxonase/arylesterase 1 (PON1), serum amyloid A-4 (SAA4) , serum amyloid A-1 (SAA1) , serum amyloid A-2 (SAA2).

**Targeted glycoproteomics analysis**

(Glyco)peptides were quantified on an Agilent 1290 Infinity II LC system coupled to an Agilent 6495B Triple Quadrupole MS. A commercially available human serum (Sigma-Aldrich) was also digested to serve as sample preparation controls. Protein standards (APOA1, APOC1, APOD, APOE, CLUS; all from Sigma-Aldrich) were mixed and digested with the batch to serve as calibration standards (see **Supporting Information Material 2** for additional details).

A transition list for target analytes was created by combining previously reported transitions[30,46] with new transitions selected from the Orbitrap analysis. The transition list included 47 peptides and 163 glycopeptides from 33 proteins. The instrument was run on Dynamic Multiple Reaction Monitoring (DMRM) mode to minimize the number of transitions being monitored at each scan cycle. For peptides, at least 2 product ions were selected for monitoring. Quantitation was based on the area of the more abundant product ion while the other ions monitored were for qualitative identification. Abundance is the amount of a glycopeptide in ion counts normalized to the ion counts of the non-glycosylated peptide, which is used as a measure of the total amount of that protein. Product ions for glycopeptides were based on diagnostic glycan fragments.

**HDL lipidomic analysis**

The HDL lipidomic profile was measured at the West Coast Metabolomics Center, using a previously reported protocol.[47] Briefly, 225 μL of cold internal standard mixture was added into 25 μL purified HDL sample, followed by adding 750 μL cold methyl *tert*-butyl ether containing cholesteryl ester (CE) 22:1, and 188 μL of distilled water was added after shaking at 4°C for 6 min. Following centrifugation at 14,000 × g for 2 min, 350 μL supernatant was extracted, dried down, and resuspended with 65 μL methanol/toluene (9:1, v/v) solution, and 3 μL of the resuspended sample was then injected into a LCMS for analysis. Each sample was injected in parallel into an Agilent 6530b quadrupole time-of-flight (QTOF) and a 6550 QTOF for positive and negative mode respectively to capture as many complex lipid species as possible. Liquid chromatography separation was done on a Waters Ultra-Performance Liquid Chromatography CSH C18 column (1.7 μM, 2.1 mm 100 mm), using a gradient method. A quality control (QC) sample was run every 11$^{th}$ injection. The QC samples all came from the same human plasma

pool. The internal standard mixture contained ceramide (Cer) d18:1/17:0, d7-cholesterol, diacylglycerol (DG)12:0/12:0, lysophosphatidylcholine (LPC) 17:0, lysophosphatidylethanolamine(LPE) 17:1, monoradyglycerol (MG) 17:0, phosphatidylcholine (PC) 12:0/13:0, phosphatidylethanolamine (PE) 17:0/17:0, sphingomyelin (SM) d18:1/17:0, sphingosine (d17:1), and $d_5$-triacylglycerol (TG) 17:0/17:1/17:0 dissolved in methanol. Each lipid species was calibrated to the internal standard which belongs to the same lipid class. The concentration of lipid classes was calculated by aggregating all lipid species which belong to the same lipid class. Lipidomics summarized variables including equivalent of double-bound per 18 carbons ($EOD_{18}$), average chain length (ACL), and surface/core lipid ratios were calculated as reported previously.[18]

**Analysis of CEC**

HDL CEC was measured using a commercially available kit (Abcam, ab19685) using a protocol as reported previously.[48] J774A.1 (ATCC TIB-67) macrophage cells were first cultured for 4 h in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum (FBS) and fluorescently labeled with cholesterol labeling reagent for another 4 h. Cells were then washed and incubated for 2 h with isolated HDL fractions, together with acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors and 3',5'-cyclic adenosine monophosphate (cAMP). Cellular supernatant was removed, and cells were lysed using MPER cell lysis buffer (78501; Thermo Scientific). The fluorescent intensity in the supernatant and cells were measured separately using a Synergy H1 plate reader (BioTek). The cellular cholesterol capacity was calculated as follows:

$$\%\ \text{cholesterol efflux capacity} = \frac{\text{fluorescence intensity of the media}}{\text{fluorescence intensity of the cell lysate + media}} \times 100$$

**Statistical analysis**

The statistical analysis plan was posted before analysis (https://ilins.ucdavis.edu/). With our sample size of 40 per group, we can detect an effect size of 0.64 SD in the mean difference between groups, assuming an alpha of 0.05 and 80% power. Data were analyzed on an intention-to-treat basis whereby children were included regardless of adherence to the intervention. Data analysis was performed in R (3.6.1). The distributions of outcome variables and key baseline variables were inspected for normality using the Shapiro-Wilks test. A Shapiro-Wilks statistic larger than 0.95 was considered normally distributed. Outcome variables were transformed as necessary with a natural log transformation and if this still did not normalize the distribution, normalized ranks or categories were created. Maternal background characteristics were summarized as mean ± SD according to their intervention group assignment at enrollment (IFA or SQ-LNS). The household assets index, housing index, and household food insecurity access score were calculated as proxy indicators for women's socioeconomic status as reported previously.[5] The children's growth status was expressed as weight for age Z score (WAZ), length for age Z score (LAZ), weight for length Z score (WLZ), and head circumference Z score (HCZ), calculated according to the World Health Organization standard.[49]

Unadjusted and adjusted linear models were used to test the impact of the intervention group (SQ-LNS vs. IFA) on the HDL lipidome, proteome, and CEC. The adjusted model included the mother's baseline characteristics, including height, BMI, age, years of formal education, household food insecurity access score, asset index, housing index, malaria status, maternal alpha-1-acid glycoprotein (AGP) and C-reactive protein (CRP), and child's sex. A covariate was included in the adjusted model if it was correlated with the outcome variable using Pearson's correlation test ($p < 0.1$). We hypothesized that SQ-LNS provided to both mothers and their children would increase child CEC; we conducted a one-tailed test for significance because

it is very unlikely that SQ-LNS could cause a decrease in this outcome, given that SQ-LNS contain ALA, which has been shown to increase CEC in vitro.[22] Two-tailed tests were performed for the lipidomics primary outcomes, HDL $EOD_{18}$, ACL, and surface/core lipid ratio, and for the proteomics primary outcomes, HDL APOA1, SAA1, SAA2, and APOL1 level. We hypothesized that because the SQ-LNS supplement provided essential fatty acids, the average desaturation and chain length of the fatty acids within HDL particles, and the ratio of surface lipids (i.e. phospholipids) to core lipids (i.e. cholesterol esters) would be altered among children in the SQ-LNS group. We also hypothesized that the content of the main apolipoprotein associated with HDL, APOA1, as well as the content of proteins linked to immune activation and inflammation (i.e. SAA1, SAA2, and APOL1), would be altered in the SQ-LNS group.

An exploratory analysis was performed to examine whether intervention group was related to the secondary outcomes, HDL lipidome species or glycopeptides, using linear models with two-tailed tests. In the exploratory analysis, a Benjamini-Hochberg test was performed to correct for multiple testing. Enrichment analysis was performed using the phyper function in R's stats package to test whether the glycopeptides of each protein were enriched in either intervention group. Enrichment is characterized as the total amount of glycopeptides of a particular protein across all glycosylation sites as a measure of the degree of glycosylation of that protein. The glycan signals nomenclature follows the conventions of the Consortium for Functional Glycomics. The glycopeptides are labeled as Protein_Position_GlycanComposition_ChargeState. Glycan compositions are written as 4-digit numbers indicating the number of hexose (mannose or galactose), N-acetylhexosamine (N-acetylgalactosamine), fucose, N-acetylneuraminic acid or sialic acid (Neu5Ac), respectively. For example, A1AT_70_5402 represents the glycopeptide of A1AT consisting of 5 hexose, 4 N-acetylhexosamine, 0 fucose, and 2 Neu5Ac.

An additional exploratory analysis was performed to examine the association between HDL glycosylation and CEC to identify which compositional changes to the HDL particles were associated with improvement in cholesterol efflux. We have previously found that glycoprofiles of HDL-associated proteins were highly correlated with CEC.[21] For the association between CEC and HDL glycosylation, a Spearman's test was used to reduce the effect of outliers.
We also explored the associations between HDL variables (lipidome, proteome, and CEC) and growth outcomes, including growth status at 18 mo (WAZ, LAZ, WLZ and HCZ) and change in WAZ, LAZ, WLZ, and HCZ from 12 to 18 mo, using both unadjusted and adjusted models. The potential covariates in the adjusted models included maternal age, height, BMI, malaria status, hemoglobin, asset, and housing index, household food insecurity access score, and years of formal education, as well as child morbidity (number of episodes of respiratory infections, fever, loose stool, and poor appetite from 6 to 18 mo). Covariates were included in the adjusted model if they were correlated with the growth outcome using Pearson's correlation test ($p < 0.1$).

## RESULTS

The baseline characteristics of the mothers of the 80 selected children, and the child morbidity variables from 6 to 18 mo, are presented in **Table 1**, by intervention group. There were no significant differences in the baseline characteristics between the SQ-LNS and IFA groups. Child growth status at 18 mo and the change in z-scores from 12 to 18 mo are presented in **Table 2**, by intervention group. The children in the SQ-LNS group had an increase instead of a decrease in LAZ from 12 to 18 mo, and this difference in change of LAZ score was statistically significant ($p = 0.044$).

**Table 1:** Background characteristics at enrollment of mothers in the iron+folic acid (IFA) and small-quantity lipid-based nutrient supplement (SQ-LNS) groups, and child morbidity from 6 to 18 months in this subsample[*].

| **Background characteristics**[*] | IFA (n = 40) | SQ-LNS (n = 40) | p-value |
|---|---|---|---|
| Age, y | 22.1 ± 3.1 (40) | 23.3 ± 3.6 (40) | 0.109 |
| Estimated pre-pregnancy BMI[†], kg/m$^2$ | 21.7 ± 2.0 (40) | 21.4 ± 2.0 (36) | 0.564 |
| Years of formal education, y | 8.3 ± 2.7 (40) | 8.3 ± 3.1 (40) | 1.000 |
| Mother's height, cm | 158.1 ± 5.0 (40) | 160.4 ± 5.9 (36) | 0.065 |
| Household Food Insecurity Access score | 1.7 ± 3.5 (39) | 0.9 ± 2.6 (40) | 0.241 |
| Gestational age at enrollment, wk | 39.0 ± 2.2 (40) | 39.5 ± 1.7 (40) | 0.228 |
| Asset Index[‡] | 0.02 ± 0.86 (40) | 0.01 ± 0.89 (40) | 0.952 |
| Housing Index[‡] | -0.27 ± 1.07 (40) | 0.05 ± 0.87 (40) | 0.139 |
| Maternal malaria RDT[§] | 4/40 (10.0) | 6/40 (10.0) | 0.505 |
| Mother's blood hemoglobin conc., g/L | 109.1 ± 11.0 (40) | 107.6 ± 9.8 (40) | 0.521 |
| **Child morbidity variables from 6 to 18 months** | | | |
| Any illness episodes | 12.4 ± 5.2 (36) | 13.9 ± 7.5 (39) | 0.327 |
| Fever episodes | 2.0 ± 1.7 (36) | 2.3 ± 2.2 (39) | 0.529 |
| Loose stool episodes | 2.2 ± 2.4 (36) | 3.2 ± 3.2 (39) | 0.114 |
| Respiratory infection episodes | 7.8 ± 3.4 (36) | 7.7 ± 3.8 (39) | 0.945 |
| Poor appetite episodes | 3.1 ± 2.5 (36) | 3.6 ± 3.3 (39) | 0.465 |

[*]Values are presented as mean ± SD (n). Values are presented as n/N = number of participants whose response was "yes" in question/n of participants analyzed. RDT, rapid diagnostic test. IFA, iron and folic acid; SQ-LNS, small-quantity lipid-based nutrient supplement.

[†]Pre-pregnancy body mass index (BMI) was estimated from height and weight at enrollment using polynomial regression with gestational age, gestational age squared, and gestational age cubed as predictors. Mean estimated pre-pregnancy BMI in this subcohort was lower than in the larger study population[6] because of the selection criteria for this subcohort (non-overweight women).

[‡]Proxy indicators for household socioeconomic status. Higher index value means higher socioeconomic status.

[§] Clearview Malarial Combo, Vision Biotech

**Table 2:** Anthropometric characteristics of children in the subsample, by intervention group*.

| Growth outcomes | Z-score at 18 months | | | Change in z-score from 12 to 18 months | | |
|---|---|---|---|---|---|---|
| | IFA (n = 40) | SQ-LNS (n = 40) | p-value | IFA (n = 40) | SQ-LNS (n = 39) | p-value |
| WAZ | -0.94 ± 1.01 (40) | -0.69 ± 1.09 (40) | 0.304 | -0.14 ± 0.41 (37) | -0.05 ± 0.54 (35) | 0.430 |
| LAZ | -0.97 ± 0.91 (40) | -0.63 ± 1.11 (40) | 0.138 | -0.16 ± 0.36 (37) | 0.05 ± 0.47 (35) | **0.044** |
| HCZ | -1.16 ± 1.04 (40) | -1.08 ± 0.87 (39) | 0.717 | -0.30 ± 0.50 (37) | -0.24 ± 0.44 (34) | 0.647 |
| WLZ | -0.66 ± 1.06 (40) | -0.54 ± 1.05 (40) | 0.635 | -0.10 ± 0.57 (37) | -0.11 ± 0.59 (35) | 0.944 |

*Values are represented as mean ± SDs (n). IFA, iron and folic acid; SQ-LNS, small-quantity lipid-based nutrient supplement; WAZ, weight for age z-score; LAZ, length for age z-score; HCZ, head circumference for age z-score.

**HDL composition and function by intervention group**

The primary HDL outcome variables are shown in **Table 3**. HDL CEC was significantly higher among children in the SQ-LNS group compared to those in the IFA group (p = 0.038, one-tailed test). HDL lipidome characteristics including $EOD_{18}$, ACL, and surface/core lipid ratio were not significantly different between groups (**Table 3**). HDL APOA1, SAA1, SAA2, and APOL1 were also not significantly different by intervention group.

Three-hundred and thirteen lipid species from 12 lipid classes were quantified (**Supporting Information Table S1**). The effects of the intervention on lipid species are presented in the volcano plot of **Figure 1A**. Only two lipid species, phosphatidylcholine 35:4.1 (p = 0.0188) and phosphatidylcholine 33:1.1 (p = 0.033) were significantly different between intervention groups, and these differences did not remain statistically significant after correction for multiple testing (p = 0.998 and p = 0.998, respectively).

Thirty-three HDL associated proteins and 163 glycopeptides from 21 proteins were quantified; the remaining 12 out of the 33 HDL-associated proteins did not contain glycopeptides (**Supporting Information Tables S2 and S3**). There were no statistically significant differences between intervention groups in any of the 33 HDL-associated proteins. The effects of the intervention on the glycopeptides are presented in the volcano plot of **Figure 1B**. The abundances of 12 glycopeptides differed between intervention groups. Four sialylated A1AT glycopeptides (i.e., A1AT_70_5402, A1AT_271_5511, A1AT_271_5402, and A1AT_107_5402) were higher in children in the SQ-LNS group (p = 0.008, p = 0.019, p = 0.043, and p = 0.028, respectively, before correction for multiple testing). The sialylated glycopeptides AACT_271_6502, FETUA_176_6501, and C1S_174_5401 were also higher in children given SQ-LNS (p = 0.004 and 0.017, and 0.047, respectively). Two APOC3

glycopeptides (APOC3_94AMC_1202 and APOC3_94_1101, p = 0.030 and 0.027, respectively) and three CLUS glycopeptides (CLUS_291_5421/5402, CLUS_374_6520/6501 and CLUS_291_5400, p = 0.014, p = 0.043, and p = 0.047, respectively) were lower in children given SQ-LNS. However, none of the group differences in these glycopeptides remained significant after correction for multiple testing.

Enrichment analysis results for 21 proteins showed whether the glycopeptides of a particular protein were enriched within either group (**Supporting Information Table S4**). Glycopeptides of A1AT, FETUA, and AACT were significantly enriched in the SQ-LNS group ($p < 0.001$, $p = 0.004$, and $p = 0.006$, respectively), whereas glycopeptides of APOC3, CLUS, and PON1 were significantly enriched in the IFA group ($p = 0.001$, $p = 0.004$, and $p = 0.002$, respectively; **Figure 2**).

**Table 3:** Primary HDL outcomes at 18 months of age, by intervention group[*].

| Variable | IFA (N=40) | SQ-LNS (N=40) | Unadjusted Model | | Adjusted Model[¶] | |
|---|---|---|---|---|---|---|
| | | | Difference in means (95% CI) | p-value | Difference in means (95% CI) | p-value |
| Cholesterol Efflux (%)[#] | 19.4 ± 3.3 | 20.9 ± 4.1 | 1.5(-0.2,3.2) | **0.038** | 1.5(-0.2,3.2) | **0.038** |
| Overall $EOD_{18}$ [†**] | 1.4 ± 0.1 | 1.4 ± 0.1 | 0.0(-0.0,0.1) | 0.429 | 0.0(-0.0,0.1) | 0.429 |
| Overall ACL[†**] | 15.6 ± 0.4 | 15.6 ± 0.5 | -0.0(-0.2,0.2) | 0.960 | -0.0(-0.2,0.2) | 0.831 |
| Surface/core lipids ratio[‡**] | 2.0 ± 0.3 | 2.0 ± 0.4 | 0.0(-0.2,0.2) | 0.942 | 0.0(-0.2,0.2) | 0.942 |
| APOA1[§**] | $1.4\pm0.5\times10^6$ | $1.5\pm0.5\times10^6$ | $0.1(-0.1,0.3)\times10^6$ | 0.410 | $0.1(-0.1,0.3)\times10^6$ | 0.410 |
| SAA1[**] | $8.4\pm5.9\times10^3$ | $9.5\pm5.7\times10^3$ | $1.1(-1.4,3.7)\times10^3$ | 0.387 | $1.9(-0.7,4.5)\times10^3$ | 0.146 |
| SAA2[**] | $1.4\pm0.9\times10^4$ | $1.6\pm1.0\times10^4$ | $0.2(-0.2,0.6)\times10^4$ | 0.344 | $0.2(-0.2,0.6)\times10^4$ | 0.344 |
| APOL1[**] | $8.6\pm8.6\times10^3$ | $7.8\pm6.5\times10^3$ | $-0.7(-4.1,2.7)\times10^3$ | 0.671 | $0.3(-3.0,3.6)\times10^3$ | 0.864 |

[*]Values are represented as mean ± SDs (n). IFA, iron and folic acid; SQ-LNS, small-quantity lipid-based nutrient supplement.

[†]$EOD_{18}$: Equivalent of double-bond per 18 carbons; ACL: Average chain length.

[‡]Surface lipids include amphipathic phospholipids, lysophospholipids, sphingomyelin, ceramides, free cholesterol, diacylglycerol, and monoacylglycerol. Core lipids include hydrophobic cholesteryl esters and triacylglycerol.

[§]The mass spectrometry intensity was reported for APOA1 (apolipoprotein A-I), SAA1 (serum amyloid A-1), SAA2 (serum amyloid A-2), and APOL1 (apolipoprotein L-1).

[¶]The adjusted model included mother's baseline characteristics, including height, body mass index (BMI), age, years of formal education, household food insecurity access score, asset index, housing index, malaria status, maternal alpha-1-acid glycoprotein (AGP) and C-reactive protein (CRP), and child's sex.

[#]One-tailed test was performed.

[**]Two-tailed test was performed.

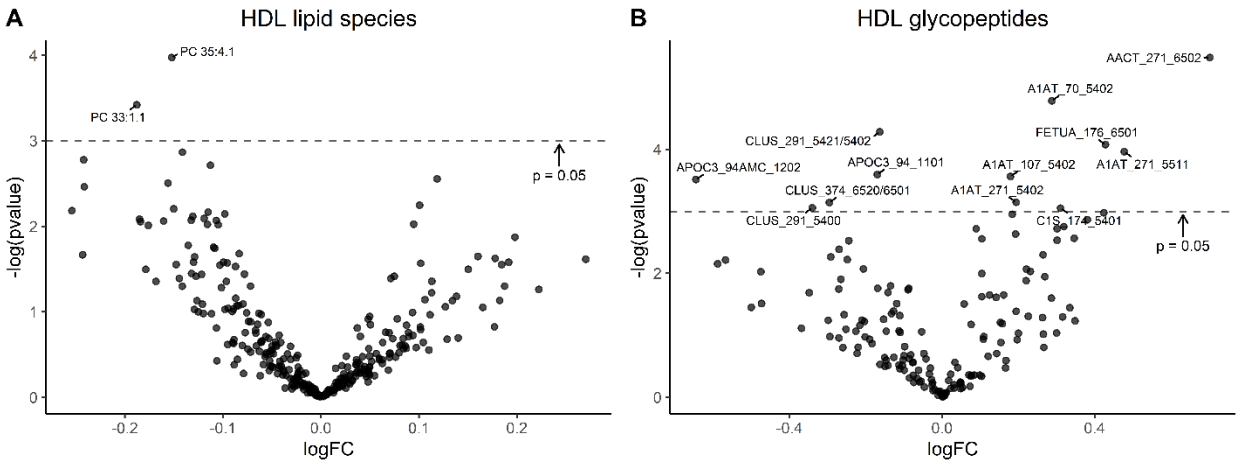


**Figure 1:** Volcano plots of the intervention effects on high-density lipoprotein (HDL) lipid species (**A**) and HDL glycopeptides (**B**). The log fold changes of all measured variable are displayed on the x-axis and the -log(p-value) on the y-axis. Variables with p-value < 0.05 were labeled. P values were not corrected for multiple testing.

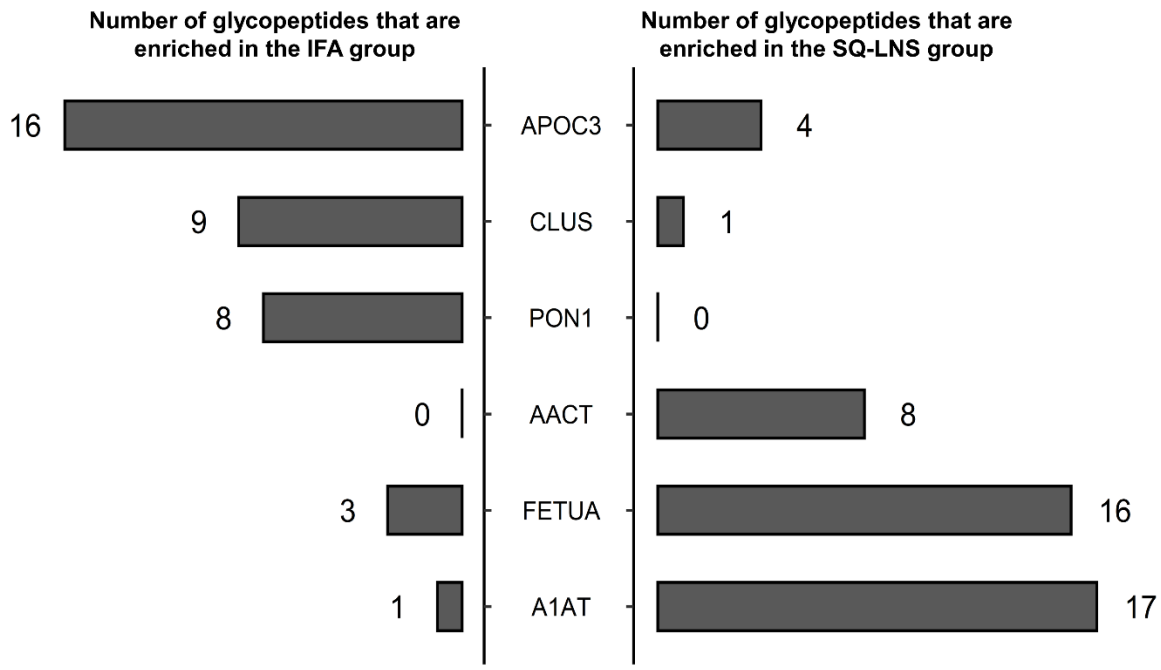


**Figure 2:** Enrichment analysis of glycopeptides in the IFA and SQ-LNS group. Out of the 33 HDL-associated proteins monitored, 21 contained glycopeptides. Six glycopeptides from a subset of the 21 proteins differed significantly in enrichment between intervention groups. Enrichment is characterized as the total amount of glycopeptides of a particular protein across all glycosylation sites as a measure of the degree of glycosylation of that protein. Number of glycopeptides of APOC3 (apolipoprotein C-III), CLUS (clusterin), PON1 (paraoxonase 1), AACT (alpha 1-antichymotrypsin), FETUA (fetuin A), and A1AT (alpha-1-antitrypsin) that are lower (left panel) or higher (right panel) in children in the SQ-LNS compared to the IFA intervention group. SQ-LNS, small-quantity lipid-based nutrient supplements; IFA, iron and folic acid; HDL, high-density lipoproteins.

### Associations between site-specific glycosylation and CEC

Among the 12 glycopeptides that were altered by SQ-LNS intervention (**Figure 3A**), A1AT_70_5402 was also positively associated with HDL CEC (Spearman's unadjusted p = 0.006, **Figure 3B**). Five additional glycopeptides (not altered by SQ-LNS) were significantly correlated with HDL CEC (**Figure 3C-G**): A1AT_70_5412 (unadjusted p = 0.049), FETUA_156_6513 (unadjusted p = 0.015), and two sialylated APOD glycopeptides, APOD_98_5402 and APOD_6503 (unadjusted p = 0.031 and p = 0.034, respectively), were positively associated with HDL CEC, whereas PON1_324_6503 was negatively associated (unadjusted p = 0.017) with HDL CEC. However, none of these associations remained significant after correction for multiple testing.

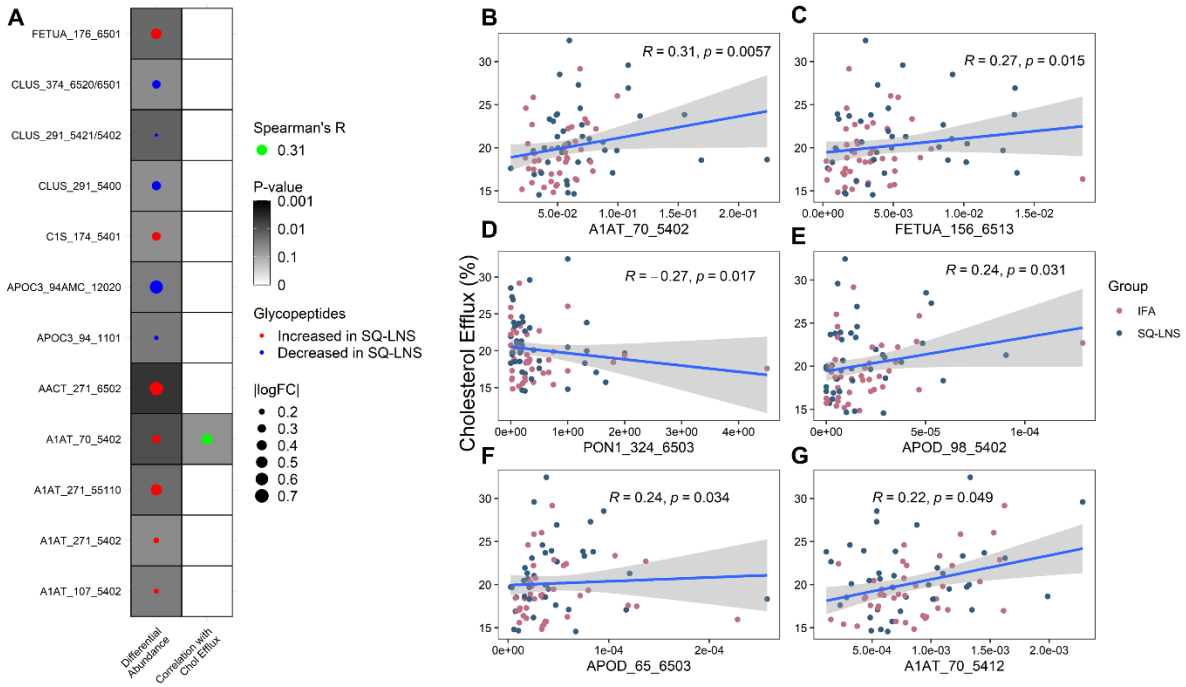


**Figure 3: A:** Dotmap of the SQ-LNS effects on HDL glycopeptides and glycopeptide correlation with CEC. Glycopeptides that were significantly different ($P \leq 0.05$) between intervention groups are shown. The darkness of the background indicates p-value. The dot size represents glycopeptide log fold changes in the abundance analysis. **B-G:** Scatterplot of all glycopeptides associated with HDL CEC, including glycopeptides A1AT_70_5402 (B), FETUA_156_6513 (C), PON1_324_6503 (D), APOD_98_5402 (E), APOD_65_6503 (F), and A1AT_70_5412 (G). CEC, cholesterol efflux capacity; HDL, high-density lipoprotein; SQ-LNS, small-quantity lipid-based nutrient supplements; A1AT, alpha-1-antitrypsin; FETUA, alpha-2-HS-glycoprotein; PON1, serum paraoxonase/arylesterase 1; APOD, apolipoprotein D.

**Associations between HDL composition and function and growth outcomes**

We determined the associations between the primary HDL variables (HDL CEC, overall $EOD_{18}$ and ACL, surface/core lipid ratio, HDL APOA1, SAA1, SAA2, and APOL1) and the growth outcomes (**Supporting Information Table S5**). Overall $EOD_{18}$ was positively associated with the change in LAZ from 12 to 18 mo in the adjusted model ($p = 0.041$). HDL APOA1 was positively associated with change in WAZ and WLZ from 12 to 18 mo ($p = 0.017$ and $p = 0.0105$, respectively). HDL SAA1 and SAA2 were both positively associated with change in WAZ ($p < 0.001$ for both), and change in WLZ ($p < 0.001$ for both) from 12 to 18 mo. APOL1 was positively associated with change in HCZ ($p = 0.035$), WAZ ($p < 0.001$) and WLZ ($p < 0.001$) from 12 to 18 mo.

**DISCUSSION**

In this secondary outcome analysis of a subgroup of participants in the iLiNS-DYAD-Ghana study, we explored whether SQ-LNS given to both mothers and their children was related to child HDL composition and function at 18 mo, and whether these HDL characteristics were associated with growth outcomes. The previously published results using the complete set of participants ($N = 1228$) demonstrated that children in the SQ-LNS group had significantly higher WAZ and LAZ at 18 mo.[6] In this study of a subset of 80 children, the results support our hypothesis that HDL from children in the SQ-LNS group had an increased capacity to efflux cholesterol from macrophage cells compared to HDL from children in the IFA group. We did not observe any significant differences in the level of HDL-associated proteins including APOA1, SAA, or APOL1, or their glycosylation compositions. However, there was a significant enrichment of glycopeptides in A1AT, FETUA, and AACT in the SQ-LNS group and an enrichment of glycopeptides in APOC3, CLUS, and PON1 in the IFA group. We also found that the HDL lipidome $EOD_{18}$ was positively associated with the change in LAZ from 12 to 18 mo,

whereas the HDL-associated proteins including APOA1, SAA1, SAA2, and APOL1 were associated with other aspects of growth from 12 to 18 mo.

To our knowledge, the HDL CEC of children in lower-income populations has not been previously studied. In Ireland, HDL CEC was negatively correlated with waist circumference and BMI in children at age 5 and 9 years, suggesting that over-nutrition may have a detrimental effect on HDL cholesterol efflux.[23] Among children under 6 years of age in India, decreased PON1 activity and anti-oxidant capacity were observed in 30 malnourished children compared to 30 healthy controls.[24] Our results suggest that improved maternal and child nutrition may improve HDL CEC among children at 18 mo of age. Cholesterol efflux is a critical function of HDL particles, regulating cellular cholesterol homeostasis and thus affecting a wide array of fundamental cellular activities.[25] For example, by regulating cellular plasma membrane cholesterol content, HDL particles potentiate the innate immune response by increasing the ability of macrophages to clear respiratory tract bacterial infections.[14] HDL particles also potentiate the adaptive immune response by regulating dendritic cell phenotype and activity.[26]

APOA1, as the defining HDL protein, is strongly linked with the ability of HDL particles to efflux cholesterol and is essential for the binding of HDL particles to the ABCA1 receptor.[27] In this study, child HDL APOA1 concentration was not significantly higher in the SQ-LNS group, suggesting either that a larger sample size is needed to determine whether SQ-LNS increases HDL APOA1, or that another HDL parameter was responsible for the increase in efflux capacity.

The lack of effect of SQ-LNS on HDL lipidomic composition was somewhat surprising. We expected that providing SQ-LNS, which is enriched in fatty acids from soybean oil and peanut, would result in an enrichment of these fatty acids, and a change in the overall saturation

and chain length of fatty acids within HDL lipids. We expect that this lack of effect on the HDL lipidome may be due to the background diet and/or breastmilk fatty acid composition, or that the amount of lipid in the supplement was less than what is needed to change the composition of HDL. $EOD_{18}$ and ACL are estimations of the overall unsaturation and chain length, respectively, of fatty acids in the HDL lipidome. A higher $EOD_{18}$ value means more unsaturated fatty acids in a sample, while a higher ACL means more long-chain fatty acids. The $EOD_{18}$ and ACL observed in this sample of children at 18 mo were 1.4 ± 0.1 double bonds and 15.6 ± 0.5 carbons, respectively. Breastmilk fatty acids tend to be enriched in medium-chain fatty acids (e.g. 12:0, 14:0, 14:1), which may be contributing to the observed ACL in these children at 18 mo of age, given that many of them were still breastfeeding.[28] In fact, women in the SQ-LNS group had higher median ALA levels and ALA:arachidonic acid ratios in breast milk at 6 mo postpartum compared to women in the MMN group ($p = 0.02$ and $p = 0.02$, respectively) and IFA group (non-significant for both), although these differences did not remain statistically significant after adjusted analysis.[29]

Proteins associated with HDL have different glycosylation patterns compared to the proteins in plasma, suggesting that glycosylation may be important for either directing proteins to HDL particles or conferring HDL-specific functions.[30] We have also previously shown that the glycosylation profiles of APOC3, but not A1AT, FETUA, and APOE, were changed in response to short-term diet change.[21] However, the effects of longer-term diet change on HDL glycosylation profiles have not been previously reported. Our exploratory findings in this subset of 80 children suggest that SQ-LNS may alter the glycosylation of several key HDL-associated proteins. The abundances of 12 specific glycopeptides on 6 different HDL proteins were different in response to long-term provision of maternal and infant SQ-LNS. Seven of the

glycopeptides that differed were derived from 3 acute-phase proteins (A1AT, AACT, and FETUA) and 1 immune protein (C1S), and these exhibited higher abundance in the SQ-LNS group. Moreover, A1AT, AACT, and FETUA, but not C1S, were found to be more highly glycosylated in the SQ-LNS group through enrichment analysis. Both A1AT and AACT are protease inhibitors released in response to a wide variety of inflammatory stimuli, to protect tissues from proteolytic degradation associated with the activity of immune cells, particularly neutrophils.[31,32] FETUA is a lipid binding protein that has multiple roles, including its role as a negative acute-phase protein during sepsis and endotoxemia, its role in promoting wound healing, and its role in neuroprotection.[33,34] C1S is a critical component in the activation of the classical complement pathway, which is involved in innate immunity.[35] Five of the 12 glycopeptides that differed by intervention group were derived from 2 other HDL proteins, APOC3 and CLUS. For these, lower glycopeptide abundance and enrichment were observed in the SQ-LNS group. APOC3 is involved in the regulation of lipoprotein metabolism, and its concentration in pre-school children born preterm has been found to be higher than in those born full term.[36] CLUS is a glycoprotein with a multitude of functions including lipid transport and as a chaperone protein.[37] , In the enrichment analysis, PON1 glycosylation was also lower in the SQ-LNS group. PON1 is an enzyme that influences HDL antioxidant capacity, and its activity was found to be diminished in obese children compared with normal weight children.[38,39] The implications of these glycoprofile changes associated with SQ-LNS in the context of growth and development in lower-income settings are currently unknown and further work on their mechanism is needed.

Glycosylation involvement with HDL cholesterol efflux is largely unknown. Here, we found that 6 glycosylation sites on four HDL proteins were associated with CEC. Among these,

two disialylated A1AT glycopeptides, A1AT_70_5402 and ATAT_70_5412, were positively associated with CEC. Interestingly, both of these glycopeptides have been previously reported to be associated with CEC in healthy adults.[21] Thus, the site-specific glycosylation of A1AT may play a role in modulating HDL functional capacity, but the underlying mechanism by which glycosylation alters HDL function remains to be explored.

The HDL lipid $EOD_{18}$ was significantly correlated with change in LAZ from 12 to 18 mo in this study, which suggests that higher levels of fatty acid unsaturation or higher dietary intake of unsaturated fatty acids may have beneficial effects on linear growth. Indeed, a cross-sectional study in Malawi found that stunted young children aged 12 to 59 mo had lower levels of serum ω-3 and ω-6 polyunsaturated fatty acids.[40] We found that HDL APOA1 was positively associated with WAZ and WLZ changes from 12 to 18 mo, which suggests that higher HDL APOA1 content during early development may be associated with increased ponderal growth. However, these results may not be generalizable to populations of different geographical regions. For example, the iLiNS-DYAD trial in Malawi showed no changes in linear growth in the SQ-LNS group compared with the IFA group at 18 mo of age.[41]

The strengths of this study include a detailed analysis of HDL composition and in vitro assessment of HDL functional capacity in a well-characterized cohort of young children. The sub-cohort of 80 children selected for this study were similar to the whole cohort (n = 440 for IFA and n = 441 for SQ-LNS) from the larger iLiNS-DYAD-Ghana study with respect to the mean and standard deviation of the WAZ, LAZ, HCZ, and WLZ scores.[6] The exploratory nature of the study and the small sample size are limitations of the study. While the aim of this pilot study is to investigate the function and composition of HDL particles in children, further research should explore whether the composition of their LDL particles is altered by SQ-LNS.

## CONCLUSION

In this sample of children, we demonstrated that provision of SQ-LNS had a beneficial effect on HDL CEC and potentially also on HDL protein glycosylation, but not lipidomic composition or protein abundance in HDL. Further research is needed to evaluate whether an improvement in CEC due to SQ-LNS is evident in other populations and to investigate the long-term impact of improved HDL functional capacity on the growth and development of young children.

## ACKNOWLEDGEMENTS

We thank all of the co-investigators, collaborators, study team members, participants and local communities involved in the iLiNS-DYAD Ghana trial. The project described was supported by Bill & Melinda Gates Foundation grant (OPP124589) to the University of California, Davis. The findings and conclusions contained within this work are those of the authors and do not necessarily reflect the positions or policies of the Bill & Melinda Gates Foundation.

## SUPPORTING INFORMATION

Flowchart of study profile (Supporting Information Figure S1). Detailed experimental procedures for HDL protein and glycoprotein identification and target glycoproteomic analysis (Supporting Information Material 1-2). Supplemental tables for lipid species relative abundance, HDL-associated proteins mean abundance, glycopeptide analysis, enrichment analysis, and supplement composition (Supporting Information Tables S1-6). Supporting information are available free of charge via Internet at http://pubs.acs.org.

## REFERENCES


(1) Bailey, R. L.; West, K. P.; Black, R. E. The Epidemiology of Global Micronutrient Deficiencies. *Ann Nutr Metab* **2015**, *66 Suppl 2*, 22–33.

(2) Adair, L. S.; Fall, C. H. D.; Osmond, C.; Stein, A. D.; Martorell, R.; Ramirez-Zea, M.; Sachdev, H. S.; Dahly, D. L.; Bas, I.; Norris, S. A.; Micklesfield, L.; Hallal, P.; Victora, C. G.; group, C. Associations of Linear Growth and Relative Weight Gain during Early Life with Adult Health and Human Capital in Countries of Low and Middle Income: Findings from Five Birth Cohort Studies. *Lancet* **2013**, *382*, 525–534.

(3) Michaelsen, K. F.; Dewey, K. G.; Perez-Exposito, A. B.; Nurhasan, M.; Lauritzen, L.; Roos, N. Food Sources and Intake of N-6 and n-3 Fatty Acids in Low-Income Countries with Emphasis on Infants, Young Children (6-24 Months), and Pregnant and Lactating Women. *Matern Child Nutr* **2011**, *7 Suppl 2*, 124–140.

(4) Arimond, M.; Zeilani, M.; Jungjohann, S.; Brown, K. H.; Ashorn, P.; Allen, L. H.; Dewey, K. G. Considerations in Developing Lipid-Based Nutrient Supplements for Prevention of Undernutrition: Experience from the International Lipid-Based Nutrient Supplements (ILiNS) Project. *Matern Child Nutr* **2015**, *11 Suppl 4*, 31–61.

(5) Adu-Afarwuah, S.; Lartey, A.; Okronipa, H.; Ashorn, P.; Zeilani, M.; Peerson, J. M.; Arimond, M.; Vosti, S.; Dewey, K. G. Lipid-Based Nutrient Supplement Increases the Birth Size of Infants of Primiparous Women in Ghana. *Am J Clin Nutr* **2015**, *101*, 835–846.

(6) Adu-Afarwuah, S.; Lartey, A.; Okronipa, H.; Ashorn, P.; Peerson, J. M.; Arimond, M.; Ashorn, U.; Zeilani, M.; Vosti, S.; Dewey, K. G. Small-Quantity, Lipid-Based Nutrient Supplements Provided to Women during Pregnancy and 6 Mo Postpartum and to Their Infants from 6 Mo of Age Increase the Mean Attained Length of 18-Mo-Old Children in Semi-Urban Ghana: A Randomized Controlled Trial. *Am J Clin Nutr* **2016**, *104*, 797–808.

(7) Assmann, G.; Gotto, A. M. HDL Cholesterol and Protective Factors in Atherosclerosis. *Circulation* **2004**, *109*, III8-14.

(8) Barter, P.; Gotto, A. M.; LaRosa, J. C.; Maroni, J.; Szarek, M.; Grundy, S. M.; Kastelein, J. J. P.; Bittner, V.; Fruchart, J.-C.; Investigators, T. to N. T. HDL Cholesterol, Very Low Levels of LDL Cholesterol, and Cardiovascular Events. *N Engl J Med* **2007**, *357*, 1301–1310.

(9) Luo, J.; Yang, H.; Song, B.-L. Mechanisms and Regulation of Cholesterol Homeostasis. *Nat Rev Mol Cell Biol* **2020**, *21*, 225–245.

(10) Mudd, L. M.; Holzman, C. B.; Evans, R. W. Maternal Mid-Pregnancy Lipids and Birthweight. *Acta Obstet Gynecol Scand* **2015**, *94*, 852–860.

(11) Oaks, B. M.; Stewart, C. P.; Laugero, K. D.; Adu-Afarwuah, S.; Lartey, A.; Vosti, S. A.; Ashorn, P.; Dewey, K. G. Maternal Plasma Cholesterol and Duration of Pregnancy: A Prospective Cohort Study in Ghana. *Matern Child Nutr* **2017**, *13*, 10.1111/mcn.12418.

(12) Scoble, J. A.; Smilowitz, J. T.; Argov-Argaman, N.; German, J. B.; Underwood, M. A. Plasma Lipoprotein Particle Subclasses in Preterm Infants. *Am J Perinatol* **2018**, *35*, 369–379.

(13) Bonacina, F.; Pirillo, A.; Catapano, A. L.; Norata, G. D. Cholesterol Membrane Content Has a Ubiquitous Evolutionary Function in Immune Cell Activation: The Role of HDL. *Curr Opin Lipidol* **2019**, *30*, 462–469.

(14) van der Vorst, E. P. C.; Theodorou, K.; Wu, Y.; Hoeksema, M. A.; Goossens, P.; Bursill, C. A.; Aliyev, T.; Huitema, L. F. A.; Tas, S. W.; Wolfs, I. M. J.; Kuijpers, M. J. E.; Gijbels, M. J.; Schalkwijk, C. G.; Koonen, D. P. Y.; Abdollahi-Roodsaz, S.; McDaniels, K.; Wang, C.-C.; Leitges, M.; Lawrence, T.; Plat, J.; Van Eck, M.; Rye, K.-A.; Touqui, L.; de Winther, M. P. J.; Biessen, E. A. L.; Donners, M. M. P. C. High-Density Lipoproteins Exert Pro-Inflammatory Effects on Macrophages via Passive Cholesterol Depletion and PKC-NF-KB/STAT1-IRF1 Signaling. *Cell Metab* **2017**, *25*, 197–207.

(15) Rached, F.; Lhomme, M.; Camont, L.; Gomes, F.; Dauteuille, C.; Robillard, P.; Santos, R. D.; Lesnik, P.; Serrano, C. V. J.; Chapman, M. J.; Kontush, A. Defective Functionality of Small, Dense HDL3 Subpopulations in ST Segment Elevation Myocardial Infarction: Relevance of Enrichment in Lysophosphatidylcholine, Phosphatidic Acid and Serum Amyloid A. *Biochim. Biophys. Acta* **2015**, *1851* (9), 1254–1261. https://doi.org/10.1016/j.bbalip.2015.05.007.

(16) Agarwala, A. P.; Rodrigues, A.; Risman, M.; McCoy, M.; Trindade, K.; Qu, L.; Cuchel, M.; Billheimer, J.; Rader, D. J. High-Density Lipoprotein (HDL) Phospholipid Content and Cholesterol Efflux Capacity Are Reduced in Patients With Very High HDL Cholesterol and Coronary Disease. *Arterioscler. Thromb. Vasc. Biol.* **2015**, *35* (6), 1515–1519. https://doi.org/10.1161/ATVBAHA.115.305504.

(17) Vaisar, T.; Tang, C.; Babenko, I.; Hutchins, P.; Wimberger, J.; Suffredini, A. F.; Heinecke, J. W. Inflammatory Remodeling of the HDL Proteome Impairs Cholesterol Efflux Capacity. *J. Lipid Res.* **2015**, *56* (8), 1519–1530. https://doi.org/10.1194/jlr.M059089.

(18) Zhu, C.; Sawrey-Kubicek, L.; Beals, E.; Hughes, R. L.; Rhodes, C. H.; Sacchi, R.; Zivkovic, A. M. The HDL Lipidome Is Widely Remodeled by Fast Food versus Mediterranean Diet in 4 Days. *Metabolomics* **2019**, *15*, 114.

(19) Richard, C.; Couture, P.; Desroches, S.; Nehmé, B.; Bourassa, S.; Droit, A.; Lamarche, B. Effect of an Isoenergetic Traditional Mediterranean Diet on the High-Density Lipoprotein Proteome in Men with the Metabolic Syndrome. *J Nutr. Nutr.* **2014**, *7*, 48–60.

(20) Krishnan, S.; Shimoda, M.; Sacchi, R.; Kailemia, M. J.; Luxardi, G.; Kaysen, G. A.; Parikh, A. N.; Ngassam, V. N.; Johansen, K.; Chertow, G. M.; Grimes, B.; Smilowitz, J. T.; Maverakis, E.; Lebrilla, C. B.; Zivkovic, A. M. HDL Glycoprotein Composition and Site-Specific Glycosylation Differentiates Between Clinical Groups and Affects IL-6 Secretion in Lipopolysaccharide-Stimulated Monocytes. *Sci Rep* **2017**, *7*, 43728.

(21) Zhu, C.; Wong, M.; Li, Q.; Sawrey-Kubicek, L.; Beals, E.; Rhodes, C. H.; Sacchi, R.; Lebrilla, C. B.; Zivkovic, A. M. Site-Specific Glycoprofiles of HDL-Associated ApoE Are Correlated with HDL Functional Capacity and Unaffected by Short-Term Diet. *J Proteome Res* **2019**, *18*, 3977–3984.

(22) Zhang, J.; Kris-Etherton, P. M.; Thompson, J. T.; Hannon, D. B.; Gillies, P. J.; Heuvel, J. P. V. Alpha-Linolenic Acid Increases Cholesterol Efflux in Macrophage-Derived Foam Cells by Decreasing Stearoyl CoA Desaturase 1 Expression: Evidence for a Farnesoid-X-Receptor Mechanism of Action. *J Nutr Biochem* **2012**, *23*, 400–409.

(23) Khalil, H.; Murrin, C.; O'Reilly, M.; Viljoen, K.; Segurado, R.; O'Brien, J.; Somerville, R.; McGillicuddy, F.; Kelleher, C. C. Total HDL Cholesterol Efflux Capacity in Healthy Children - Associations with Adiposity and Dietary Intakes of Mother and Child. *Nutr Metab Cardiovasc Dis* **2017**, *27*, 70–77.

(24) Mogarekar, M. R.; Dhabe, M. G.; Palmate, M. M. PON1 Arylesterase Activity, HDL Functionality and Their Correlation in Malnourished Children. *J Pediatr Endocrinol Metab* **2019**, *32*, 321–326.
(25) Soran, H.; Hama, S.; Yadav, R.; Durrington, P. N. HDL Functionality. *Curr Opin Lipidol* **2012**, *23*, 353–366.
(26) Westerterp, M.; Gautier, E. L.; Ganda, A.; Molusky, M. M.; Wang, W.; Fotakis, P.; Wang, N.; Randolph, G. J.; D'Agati, V. D.; Yvan-Charvet, L.; Tall, A. R. Cholesterol Accumulation in Dendritic Cells Links the Inflammasome to Acquired Immunity. *Cell Metab* **2017**, *25*, 1294-1304.e6.
(27) Tall, A. R.; Yvan-Charvet, L.; Terasaka, N.; Pagler, T.; Wang, N. HDL, ABC Transporters, and Cholesterol Efflux: Implications for the Treatment of Atherosclerosis. *Cell Metab* **2008**, *7*, 365–375.
(28) Andreas, N. J.; Kampmann, B.; Mehring Le-Doare, K. Human Breast Milk: A Review on Its Composition and Bioactivity. *Early Hum Dev* **2015**, *91*, 629–635.
(29) Oaks, B. M.; Young, R. R.; Adu-Afarwuah, S.; Ashorn, U.; Jackson, K. H.; Lartey, A.; Maleta, K.; Okronipa, H.; Sadalaki, J.; Baldiviez, L. M.; Shahab-Ferdows, S.; Ashorn, P.; Dewey, K. G. Effects of a Lipid-Based Nutrient Supplement during Pregnancy and Lactation on Maternal Plasma Fatty Acid Status and Lipid Profile: Results of Two Randomized Controlled Trials. *Prostaglandins Leukot. Essent. Fatty Acids* **2017**, *117*, 28–35. https://doi.org/10.1016/j.plefa.2017.01.007.
(30) Kailemia, M. J.; Wei, W.; Nguyen, K.; Beals, E.; Sawrey-Kubicek, L.; Rhodes, C.; Zhu, C.; Sacchi, R.; Zivkovic, A. M.; Lebrilla, C. B. Targeted Measurements of O- and N-Glycopeptides Show That Proteins in High Density Lipoprotein Particles Are Enriched with Specific Glycosylation Compared to Plasma. *J Proteome Res* **2018**, *17*, 834–845.
(31) de Serres, F.; Blanco, I. Role of Alpha-1 Antitrypsin in Human Health and Disease. *J Intern Med* **2014**, *276*, 311–335.
(32) Baker, C.; Belbin, O.; Kalsheker, N.; Morgan, K. SERPINA3 (Aka Alpha-1-Antichymotrypsin). *Front Biosci* **2007**, *12*, 2821–2835.
(33) Trepanowski, J. F.; Mey, J.; Varady, K. A. Fetuin-A: A Novel Link between Obesity and Related Complications. *Int. J. Obes.* **2015**, *39*, 734–741.
(34) Mukhopadhyay, S.; Mondal, S. A.; Kumar, M.; Dutta, D. Proinflammatory and Antiinflammatory Attributes of Fetuin-a: A Novel Hepatokine Modulating Cardiovascular and Glycemic Outcomes in Metabolic Syndrome. *Endocr Pr.* **2014**, *20*, 1345–1351.
(35) Müller-Eberhard, H. J. Molecular Organization and Function of the Complement System. *Annu Rev Biochem* **1988**, *57*, 321–347.
(36) Posod, A.; Pechlaner, R.; Yin, X.; Burnap, S. A.; Kiechl, S. J.; Willeit, J.; Witztum, J. L.; Mayr, M.; Kiechl, S.; Kiechl-Kohlendorfer, U. Apolipoprotein Profiles in Very Preterm and Term-Born Preschool Children. *J. Am. Heart Assoc.* **2019**, *8* (8), e011199–e011199. https://doi.org/10.1161/JAHA.118.011199.
(37) Jones, S. E.; Jomary, C. Clusterin. *Int. J. Biochem. Cell Biol.* **2002**, *34* (5), 427–431. https://doi.org/10.1016/S1357-2725(01)00155-8.
(38) Ferré, N.; Feliu, A.; García-Heredia, A.; Marsillach, J.; París, N.; Zaragoza-Jordana, M.; Mackness, B.; Mackness, M.; Escribano, J.; Closa-Monasterolo, R.; Joven, J.; Camps, J. Impaired Paraoxonase-1 Status in Obese Children. Relationships with Insulin Resistance and Metabolic Syndrome. *Clin. Biochem.* **2013**, *46* (18), 1830–1836. https://doi.org/10.1016/j.clinbiochem.2013.08.020.

(39) Krzystek-Korpacka, M.; Patryn, E.; Hotowy, K.; Czapińska, E.; Majda, J.; Kustrzeba-Wójcicka, I.; Noczyńska, A.; Gamian, A. Paraoxonase (PON)-1 Activity in Overweight and Obese Children and Adolescents: Association with Obesity-Related Inflammation and Oxidative Stress. *Adv. Clin. Exp. Med. Off. Organ Wroclaw Med. Univ.* **2013**, *22* (2), 229–236.

(40) Semba, R. D.; Trehan, I.; Li, X.; Salem, N.; Moaddel, R.; Ordiz, M. I.; Maleta, K. M.; Kraemer, K.; Manary, M. J. Low Serum ω-3 and ω-6 Polyunsaturated Fatty Acids and Other Metabolites Are Associated with Poor Linear Growth in Young Children from Rural Malawi. *Am J Clin Nutr* **2017**, *106*, 1490–1499.

(41) Ashorn, P.; Alho, L.; Ashorn, U.; Cheung, Y. B.; Dewey, K. G.; Gondwe, A.; Harjunmaa, U.; Lartey, A.; Phiri, N.; Phiri, T. E.; Vosti, S. A.; Zeilani, M.; Maleta, K. Supplementation of Maternal Diets during Pregnancy and for 6 Months Postpartum and Infant Diets Thereafter with Small-Quantity Lipid-Based Nutrient Supplements Does Not Promote Child Growth by 18 Months of Age in Rural Malawi: A Randomized Controlled Trial. *J Nutr* **2015**, *145*, 1345–1353.

(42) Adu-Afarwuah, S.; Young, R. T.; Lartey, A.; Okronipa, H.; Ashorn, P.; Ashorn, U.; Oaks, B. M.; Arimond, M.; Dewey, K. G. Maternal and Infant Supplementation with Small-Quantity Lipid-Based Nutrient Supplements Increases Infants' Iron Status at 18 Months of Age in a Semiurban Setting in Ghana: A Secondary Outcome Analysis of the ILiNS-DYAD Randomized Controlled Trial. *J Nutr* **2019**, *149*, 149–158.

(43) Kumordzie, S. M.; Adu-Afarwuah, S.; Arimond, M.; Young, R. R.; Adom, T.; Boatin, R.; Ocansey, M. E.; Okronipa, H.; Prado, E. L.; Oaks, B. M.; Dewey, K. G. Maternal and Infant Lipid-Based Nutritional Supplementation Increases Height of Ghanaian Children at 4-6 Years Only If the Mother Was Not Overweight Before Conception. *J. Nutr.* **2019**, *149* (5), 847–855. https://doi.org/10.1093/jn/nxz005.

(44) Holzer, M.; Kern, S.; Birner-Grünberger, R.; Curcic, S.; Heinemann, A.; Marsche, G. Refined Purification Strategy for Reliable Proteomic Profiling of HDL(2/3): Impact on Proteomic Complexity. *Sci. Rep.* **2016**, *6*, 38533–38533. https://doi.org/10.1038/srep38533.

(45) Zheng, J. J.; Agus, J. K.; Hong, B. V.; Tang, X.; Rhodes, C. H.; Houts, H. E.; Zhu, C.; Kang, J. W.; Wong, M.; Xie, Y.; Lebrilla, C. B.; Mallick, E.; Witwer, K. W.; Zivkovic, A. M. Isolation of HDL by Sequential Flotation Ultracentrifugation Followed by Size Exclusion Chromatography Reveals Size-Based Enrichment of HDL-Associated Proteins. *Sci. Rep.* **2021**, *11* (1), 16086. https://doi.org/10.1038/s41598-021-95451-3.

(46) Li, Q.; Kailemia, M. J.; Merleev, A. A.; Xu, G.; Serie, D.; Danan, L. M.; Haj, F. G.; Maverakis, E.; Lebrilla, C. B. Site-Specific Glycosylation Quantitation of 50 Serum Glycoproteins Enhanced by Predictive Glycopeptidomics for Improved Disease Biomarker Discovery. *Anal Chem* **2019**, *91*, 5433–5445.

(47) Cajka, T.; Davis, R.; Austin, K. J.; Newman, J. W.; German, J. B.; Fiehn, O.; Smilowitz, J. T. Using a Lipidomics Approach for Nutritional Phenotyping in Response to a Test Meal Containing Gamma-Linolenic Acid. *Metabolomics* **2016**, *12* (8), 127.

(48) Sawrey-Kubicek, L.; Zhu, C.; Bardagjy, A. S.; Rhodes, C. H.; Sacchi, R.; Randolph, J. M.; Steinberg, F. M.; Zivkovic, A. M. Whole Egg Consumption Compared with Yolk-Free Egg Increases the Cholesterol Efflux Capacity of High-Density Lipoproteins in Overweight, Postmenopausal Women. *Am J Clin Nutr* **2019**, *110*, 617–627.

(49) Group, W. M. G. R. S. WHO Child Growth Standards Based on Length/Height, Weight and Age. *Acta Paediatr Suppl* **2006**, *450*, 76–85.

# Chapter 3: Activities of Enzymes Involved in HDL Metabolism are Influenced by Season Among Pregnant Mothers in Ghana

Brian V. Hong[a], Jack Jingyuan Zheng[a], Eduardo Romo[a], Joanne K. Agus[a], Xinyu Tang[a], Charles D. Arnold[a], Seth Adu-Afarwuah[b], Anna Lartey[b], Harriet Okronipa[c], Kathryn G. Dewey[a], Angela M. Zivkovic[a*]

[a]Department of Nutrition, University of California, Davis, Davis, CA, USA; [b]Department of Nutrition and Food Science, University of Ghana, Legon, Ghana; [c]Department of Nutritional Sciences, Oklahoma State University, Stillwater, OK, USA.

[*]To whom correspondence should be addressed.

3245 Meyer Hall, One Shield Ave, Davis, CA, 95616

(530) 752-3973

Email: amzivkovic@ucdavis.edu

**Keywords:** High-Density Lipoprotein, HDL, Cholesterol Efflux Capacity, LCAT, PLTP, CETP, iLiNS, small-quantity lipid-based nutrient supplements, SQ-LNS

## ABBREVIATIONS

CEC, cholesterol efflux capacity; CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; IFA, iron and folic acid; LCAT, lecithin-cholesterol acyltransferase; PLTP, phospholipid transfer protein; SQ-LNS, small-quantity lipid-based nutrient supplements.

## ABSTRACT

**BACKGROUND**: Small-quantity lipid-based nutrient supplements (SQ-LNS) during pregnancy and postnatally improved child cholesterol efflux capacity (CEC) and length at 18 mo in the International Lipid-Based Nutrient Supplements (iLiNS) DYAD trial in Ghana. In this secondary outcome analysis, we determined whether SQ-LNS alters high-density lipoprotein (HDL) CEC and the activities of three additional enzymes involved in HDL metabolism.

**METHODS**: Plasma samples were obtained from a subsample of mothers at 36 weeks gestation enrolled in the iLiNS-DYAD trial in Ghana ($N$ = 197). We investigated HDL CEC and the activities of three enzymes in mothers supplemented with either SQ-LNS or iron and folic acid (IFA). We further explored whether these functional metrics varied by season.

**RESULTS**: SQ-LNS did not have a significant effect on HDL CEC or plasma lecithin-cholesterol acyltransferase (LCAT) activity, cholesteryl transfer protein (CETP) activity, or phospholipid transfer protein (PLTP) activity at 36 weeks gestation (P > 0.05, for all). LCAT activity was negatively correlated with plasma inflammatory markers alpha-1-acid glycoprotein (R = -0.19, 95% CI [-0.33, -0.05], $P$ = 0.007) and C-reactive protein (R = -0.28, 95% CI [-0.41, -0.14], $P$ < 0.001). Regardless of supplemental group, median ($25^{th}$ percentile, $75^{th}$ percentile) CETP (28.98 (23.18, 38.71) vs 21.69 (7.06, 34.12] pmol/μl/h, $P$ < 0.001) and LCAT activity (1.46 (1.36, 1.54) vs 1.33 (1.28, 1.41) 390/470 nm, $P$ < 0.001) were increased during the dry season compared to the wet season, whereas PLTP activity was increased in the wet season compared to the dry season (125.55 (69.92, 178.74) vs 90.34 (66.03, 115.46) pmol/μl/h, $P$ = 0.001).

**CONCLUSION**: This study shows that the activities of enzymes involved in HDL metabolism and the capacity of HDL to efflux cholesterol are affected by factors that vary by season in pregnant women in Ghana, and that these seasonal factors have stronger effects than the potential effect of SQ-LNS.

This trial was registered at clinicaltrials.gov as NCT00970866.

## INTRODUCTION

Inadequate nutrient intake during pregnancy has long-lasting health effects in children that increase the risk for impaired cognitive development (1) and growth (2). Limited access to safe and nutritious foods is one of many factors that can detrimentally affect health and socioeconomic status and, consequently, women from low- and middle-income countries are often at risk of adverse pregnancy outcomes and of having children at risk for low birth weight and other negative health effects (3).

The International Lipid-Based Nutrient-Supplement (iLiNS) Project developed small-quantity lipid-based nutrient supplements (SQ-LNS) to address the issue of inadequate maternal and child nutrition, where the primary objective is to prevent malnutrition, promote growth, and decrease morbidity outcomes in children (4). SQ-LNS provides multiple micronutrients and essential fatty acids, including linoleic and α-linolenic acid (ALA) to enrich the diet and complement home-prepared meals. In Ghana, the efficacy of SQ-LNS is quite promising. The children of mothers who were supplemented with SQ-LNS had improved birth size (4), mean attained length at 18 mo (5), and iron status (6) compared to the children of mothers who were provided the standard of care iron and folic acid (IFA) supplements during pregnancy (4–6). Among women in this trial, the beneficial effect of SQ-LNS on children's growth was further augmented in primiparous mothers (4).

High-density lipoprotein (HDL) particles are most well studied for their role in reverse cholesterol transport, a key process to remove excess cholesterol from the body (7). However, HDL particles are involved in many additional functions of great relevance to maternal and child health, including protection from infectious pathogens (8,9). HDL particles are heterogeneous, and their size, lipid, and protein components are known to influence their functional capacity

(10,11). HDL particles perform multiple pleiotropic functions including anti-inflammatory and antioxidant functions among others, both of which are well established and important in response to oxidative stress, placental vascular function, and normal pregnancy outcomes (12,13). HDL particles are highly dynamic and can be rapidly altered by diet. For example, our group has shown that just 4 days of dietary intervention alters the HDL lipidome (14). In Ghana, high HDL cholesterol (HDL-C) at 36 weeks gestation is positively associated with longer gestational age at delivery (15). Importantly, dietary interventions can influence the function of HDL particles without changing total HDL-C. For example, we and others have found that consumption of eggs improves HDL CEC without altering total HDL-C (16), highlighting the importance of assessing parameters beyond the simple measurement of HDL-C to understand the potential impact of diet on HDL. Although HDL have been extensively studied in the context of heart disease, little is known about HDL biology during pregnancy.

We have previously shown that long-term SQ-LNS supplementation from enrollment to 18 mo of age improved child HDL CEC (17). Thus, the aim of this secondary outcome analysis is to determine whether SQ-LNS supplementation also affected multiple measures of HDL functional capacity and metabolism among women at 36 weeks gestation from the same mother-child dyad cohort. We further explored whether these HDL functional parameters varied by season. We hypothesized that mothers supplemented with SQ-LNS have altered HDL CEC as well as altered activities of enzymes involved in HDL metabolism compared to mothers supplemented with IFA.

## MATERIALS AND METHODS

### Participants

The study design and participant characteristics from the main trial have been described in detail elsewhere (4). Women (*N* = 1320, mean gestation age = 16.3 weeks) were enrolled in the iLiNS trial in Ghana to assess the effects of SQ-LNS on maternal and child growth outcomes. Briefly, women were enrolled year-round during the wet (May – October) and the dry (November – April) seasons and were randomized to receive either SQ-LNS, IFA, or multiple micronutrient supplements. Details of the three supplements have been described previously (4). Project staff conducted biweekly visits to ensure fresh delivery of supplements and to monitor supplement consumption and maternal and child morbidity. Blood was drawn at 36 weeks gestation and plasma samples were separated at 1,252 x g for 15 minutes at room temperature and then stored at -33$^{o}$C in Ghana before being airmailed on dry ice to Davis, CA, USA. Samples were stored at -80$^{o}$C upon arrival.

In this subset of pregnant mothers, plasma samples from 197 women at 36 weeks gestation were randomly selected based on 1) enrollment between October 2010 and December 2011 to avoid the inclusion of women enrolled earlier who received a mixed exposure of supplements as previously described elsewhere (4) and 2) plasma samples that were not subjected to more than 2 freeze-thaw cycles.

The study protocol was approved by the ethics committees of the University of California, Davis; the Ghana Health Service; and the University of Ghana Noguchi Memorial Institute for Medical Research and was registered on clinicaltrials.gov as NCT00970866.

**Apolipoprotein-B depletion**

Plasma apolipoprotein-B (ApoB) was precipitated by 20% polyethylene glycol (PEG, molecular weight 6000, Sigma-Aldrich, St. Louis, MO, USA, catalog 25322-68-3) in water as previously described by Davidson et al. (18). PEG mixture (40 uL) was added to 100 uL plasma

for 20 minutes at room temperature, followed by centrifugation at 10,000 rpm for 30 minutes. The HDL-containing supernatant was aliquoted and stored at -80°C until used.

**HDL Cholesterol Efflux**

HDL CEC in ApoB-depleted plasma was measured using a commercially available kit (Abcam, ab19685) as previously described (17,19) with modifications. J774A.1 (ATCC, Manassas, VA, USA, catalog TIB-67) were seeded at 50,000 cells in 96-well plates for 18 hours in Roswell Park Memorial Institute media containing 10% fetal bovine serum and 100 ug/ml penicillin and streptomycin. Cells were washed and labeled with fluorescent cholesterol along with acyl-CoA cholesterol acyltransferase inhibitor and cyclic adenosine monophosphate for 4 hours. Cells were washed with FluroBrite™ DMEM (Thermo Fisher Scientific, Waltham, MA, USA, catalog A1896701) followed by incubation with ApoB-depleted plasma (2%) in FluroBrite™ DMEM for another 4 hours. Cellular supernatant was removed, and the remaining cells were lysed with M-PER cell lysis buffer (Thermo Scientific, Waltham, MA, USA, catalog 78501) for 30 minutes. The fluorescence in the supernatant and lysed fraction was measured at 485/523 nm (emission/excitation) on a Synergy H1 plate reader (Biotek, Winooski, VT, USA). The percentage of cholesterol efflux (i.e. CEC) was calculated as followed:

$$\%\ \text{cholesterol efflux capacity} = \frac{\text{fluorescence intensity of the media}}{\text{fluorescence intensity of the cell lysate + media}} \times 100$$

**LCAT, PLTP, CETP Activity Assay**

Commercially available kits were used to measure plasma LCAT, PLTP, and CETP activities (all from Roar Biomedical Inc., New York, NY, USA) in duplicates following the manufacturer's instructions with modifications.

LCAT activity (catalog mak107) measurements were collected on a Synergy H1 plate reader (BioTek, Winooski, VT) read at 340 nm excitation and two emission wavelengths at 390 nm and 470 nm, which represent the hydrolyzed and intact substrate, respectively. The increase in LCAT activity is indicated as increased $\lambda_{em}390$ / $\lambda_{em}470$ nm ratios.

CETP activity (catalog MAK106) was measured in 20 uL of plasma (1:10 dilution in 10 mM Tris, 1 mM EDTA, 150 mM NaCl, pH 7.4) combined with synthetic cholesterol ester donor particles and lipoprotein acceptor particles provided by the kit. Plates were incubated for 3 hours at 37°C then measured at 465 nm excitation and 535 emission wavelengths on a Synergy H1 plate reader (Biotek, Winooski, VT, USA). The increase in fluorescence intensity measures CETP's ability to catalyze the transfer of the fluorescent cholesterol ester from the donor to the acceptor particle.

PLTP activity (catalog MAK 108) was measured in 50 uL of diluted plasma (1:10 dilution in 10 mM Tris, 1mM EDTA, 150 mM NaCl, pH 7.4) by combining both donor and acceptor particles. Plates were incubated for 30 minutes at 37°C. The transferred fluorescence intensity was measured at 465 nm excitation and 535 emission wavelengths on a Synergy H1 plate reader (Biotek, Winooski, VT, USA).

**Statistical Analysis**

The statistical analysis plan was posted before analysis (https://ilins.ucdavis.edu/). Sample size calculation is based on an effect size of 0.4 from previous CEC data among children at 18 mo of age for mothers and their children enrolled in the iLiNS-DYAD trial in Ghana (17). With a sample size of 100 per group, we can detect an effect size of 0.40 SDs mean difference between groups, assuming an alpha of 0.05 and 80% power. All data analyses were performed in R version 4.1.1 (R Project for Statistical Computing, Vienna, Austria). Data from women were

analyzed based on an intention-to-treat basis where women were included regardless of adherence to intervention. Variables were inspected for normality using the Shapiro-Wilks test. Non-normally distributed variables were natural log-transformed first. Normalized ranks were created if the natural log-transformed data did not normalize the distribution.

Unadjusted and adjusted linear models were used to assess the effect of treatment on HDL CEC and plasma LCAT, PLTP, and CETP activities. For the unadjusted model, a two-sample t-test was used to compare intervention groups on normally distributed data. A Wilcoxon rank-sum test was performed on data that did not follow a normal distribution. We also performed unadjusted and adjusted linear models to compare the association between HDL CEC and enzyme activities with birth outcomes, including infant weight, infant head circumference, infant length, and duration of gestation. The adjusted model included the following participant baseline characteristics if the outcome variables were Pearson correlated ($P < 0.1$) with the following baseline covariates: body mass index (BMI), age, years of formal education, height, gestational age at enrollment, season at enrollment (dry or wet), household food insecurity access score, asset index, parity, primiparous status, malaria status, maternal alpha-1-acid glycoprotein (AGP), and maternal C-reactive protein (CRP), hemoglobin levels. The associations between treatment and each HDL measure were considered significant at $P < 0.05$. A chi-squared test or Fisher exact test was used to compare categorical variables.

An exploratory analysis was performed to examine the associations between HDL CEC and plasma LCAT, PLTP, and CETP activities. For the association analysis, a Spearman's test was used, and the R values were reported. We also explored whether these metrics were associated with inflammatory markers (AGP and CRP) and hemoglobin levels at 36 weeks gestation. Lastly, we examined whether HDL CEC and plasma LCAT, PLTP, and CETP

activities were altered by season at 36 weeks gestation. Since ApoA-I, the main HDL protein, has a turnover rate of approximately 4 days (20), the function of HDL may be influenced by factors mediated by seasonal variation at the time of blood draw.

## RESULTS

### Participants characteristics

The characteristics of the mothers ($N$ = 197) at baseline and 36 weeks gestation are presented in **Table 1**. There were no significant differences in baseline or 36 weeks gestation values between the SQ-LNS and IFA groups.

**Table 1.** Median (25th percentile, 75th percentile) characteristics of study cohort of pregnant women in Ghana.

| Characteristics | IFA (*N* = 104) | SQ-LNS (*N* = 93) | *P* value |
|---|---|---|---|
| ≤20 weeks gestation | | | |
| Body mass index (BMI), kg/m$^2$ | 24.6 (22.0, 28.3) (n = 101) | 24.8 (21.4, 27.9) (n = 92) | 0.637 |
| Maternal age, years | 26.5 (24.0, 30.0) (n = 104) | 26.0 (22.0, 30.0) (n = 93) | 0.168 |
| Education, completed years | 9.0 (6.0, 9.0) (n = 104) | 9.0 (6.0, 9.0) (n = 93) | 0.938 |
| Mother's height, cm | 158.7 (154.8, 162.2) (n = 101) | 158.3 (155.7, 162.2) (n = 92) | 0.729 |
| Gestational age at enrollment, weeks | 16.9 (14.1, 18.7) (n = 104) | 15.9 (13.9, 18.7) (n = 93) | 0.389 |
| Season at blood draw, dry[a] | 49.0% (n = 104) | 50.1% (n = 93) | 0.834 |
| Primiparous (%)[a] | 27.9% (n = 104) | 34.4% (n = 93) | 0.323 |
| Parity (total births) | 1.0 (0.0, 2.0) (n = 104) | 1.0 (0.0, 2.0) (n = 93) | 0.113 |
| Household food insecurity access score | 0.0 (0.0. 3.2) (n = 104) | 0.0 (0.0. 2.0) (n = 92) | 0.513 |
| Asset index[b] | 0.1 (-0.1, 0.9) (n = 104) | 0.0 (-0.1, 0.6) (n = 93) | 0.262 |
| Overweight or obese (% BMI ≥ 25 kg/m$^2$)[a] | 46.5% (n = 101) | 47.8% (n = 92) | 0.858 |
| Women with anemia (Hb < 100 g/l)[a] | 8.7% (n = 104) | 10.8% (n = 93) | 0.618 |
| Positive for malaria[ac] | 6.7% (n = 104) | 6.5% (n = 93) | 0.937 |
| AGP (g/L) | 0.6 (0.5, 0.8) (n = 99) | 0.6 (0.5, 0.8) (n = 89) | 0.857 |
| CRP (mg/L) | 4.0 (1.8, 8.7) (n = 99) | 4.6 (1.6, 7.9) (n = 89) | 0.564 |
| 36 weeks gestation | | | |
| Body mass index (BMI), kg/m2 | 27.7 (25.1, 31.2) (n = 95) | 27.5 (25.2, 30.9) (n = 89) | 0.997 |
| Season at blood draw, dry[a] | 48.1% (n = 104) | 53.8% (n = 93) | 0.425 |
| Women with anemia (Hb < 100 g/l)[a] | 2.9% (n = 104) | 6.5% (n = 93) | 0.231 |
| Positive for malaria[ac] | 6.7% (n = 104) | 6.5% (n = 93) | 0.937 |
| AGP (g/L) | 0.4 (0.3, 0.5) (n = 104) | 0.4 (0.3, 0.5) (n = 93) | 0.535 |
| CRP (mg/L) | 2.1 (1.0, 4.5) (n = 104) | 2.5 (1.1, 5.3) (n = 93) | 0.456 |

[a]Chi-square test, for indicated categorical variables
[b]Proxy indicators for household socioeconomic status.
[c]Rapid diagnostic test by Clearview Malarial Combo, Vision Biotech.

AGP, alpha-1-acid glycoprotein; CRP, C-reactive protein; IFA, iron and folic acid; SQ-LNS, small-quantity lipid-based nutrient supplement.

**Effects of SQ-LNS supplementation on HDL functional measures and associations with birth outcomes.**

The primary HDL outcome variables are shown in **Table 2**. HDL CEC and plasma LCAT, PLTP, and CETP activities were not significantly different by intervention group in the unadjusted or the adjusted model ($P > 0.05$ for all).

**Table 2.** Primary HDL CEC and enzyme activities by supplement group at 36 weeks gestation.

| | Result by study group | | Unadjusted Model | | Adjusted Model[c] | |
|---|---|---|---|---|---|---|
| **Outcome** | **IFA (*N* = 104)** | **SQ-LNS (*N* = 93)** | **Difference in means (95% CI)** | ***P* value** | **Difference in means (95% CI)** | ***P* value** |
| **Cholesterol efflux capacity (%)[a]** | 38.09 ± 4.78 | 37.71 ± 4.45 | -0.38 (-1.68, 0.93) | 0.570 | -0.15 (-1.45, 1.15) | 0.821 |
| **LCAT activity (390/470nm ratio)[b]** | 1.37 [1.31, 1.47] | 1.41 [1.31, 1.50] | 0.03 (-0.01, 0.07) | 0.234 | 0.02 (-0.02, 0.06) | 0.325 |
| **PLTP activity (pmol/µl/h)[b]** | 97.54 [66.31, 134.06] | 101.47 [68.00, 147.59] | 2.99 (-12.9, 18.9) | 0.866 | 2.69 (-13.3, 18.7) | 0.740 |
| **CETP activity (pmol/µl/h)[b]** | 26.62 [18.03, 36.58] | 26.67 [15.82, 36.17] | 0.67 (-3.26, 4.61) | 0.643 | -0.08 (-3.98, 3.81) | 0.966 |

[a]Values are represented as mean ± SDs, two-tailed parametric test.
[b]Values are represented as median [25$^{th}$ percentile, 75$^{th}$ percentile], two-tailed nonparametric test.
[c]The adjusted model included the mother's baseline characteristics if the outcome variables were Pearson correlated ($P < 0.1$), which includes body mass index (BMI), age, years of education, height, gestational age, season, parity, primiparous, household food insecurity access score, asset index, malaria status, alpha-1-acid glycoprotein (AGP) and C-reactive protein (CRP), and hemoglobin levels. CETP, cholesteryl ester transfer protein; IFA, iron and folic acid; LCAT, lecithin-cholesterol acyltransferase; PLTP, phospholipid transfer protein; SQ-LNS, small-quantity lipid-based nutrient supplement.

We examined whether there were any associations between any of the HDL functional measures and birth outcomes or duration of gestation. None of the HDL variables were significantly correlated with infant birth weight, infant head circumference, infant length, or duration of gestation in the unadjusted or the adjusted models ($P > 0.05$, **Table 3**).

**Table 3.** Pregnancy correlation outcomes with HDL CEC and enzyme activities at 36 weeks gestation.

| | Unadjusted | | Adjusted[a] | |
|---|---|---|---|---|
| **Outcome** | **β (SE)** | ***P* value** | **β (SE)** | ***P* value** |
| **Cholesterol efflux capacity (%)** | | | | |
| Infant birth weight | 0.001 (0.006) | 0.854 | 0.001 (0.006) | 0.843 |
| Infant head circumference at birth | 0.03 (0.02) | 0.124 | 0.03 (0.02) | 0.125 |
| Infant length at birth | -0.02 (0.02) | 0.367 | -0.03 (0.03) | 0.223 |
| Duration of gestation | 0.02 (0.02) | 0.408 | 0.02 (0.02) | 0.397 |
| **LCAT activity (390/470nm ratio)** | | | | |
| Infant birth weight | -0.16 (0.17) | 0.366 | -0.09 (0.018) | 0.620 |
| Infant head circumference at birth | -0.29 (0.57) | 0.616 | 0.40 (0.61) | 0.510 |
| Infant length at birth | 0.49 (0.75) | 0.517 | 0.45 (0.78) | 0.568 |
| Duration of gestation | 0.34 (0.59) | 0.565 | 0.67 (0.64) | 0.301 |
| **PLTP activity (pmol/µl/h)** | | | | |
| Infant birth weight | -0.0002 (0.0005) | 0.683 | -0.0003 (0.0004) | 0.487 |
| Infant head circumference at birth | 0.0002 (0.002) | 0.899 | -0.0009 (0.001) | 0.540 |
| Infant length at birth | -0.002 (0.002) | 0.329 | -0.002 (0.002) | 0.236 |
| Duration of gestation | 0.0009 (0.002) | 0.595 | 0.0006 (0.001) | 0.702 |
| **CETP activity (pmol/µl/h)** | | | | |
| Infant birth weight | -0.002 (0.002) | 0.128 | -0.0001 (0.002) | 0.955 |
| Infant head circumference at birth | -0.008 (0.006) | 0.214 | 0.003 (0.006) | 0.631 |
| Infant length at birth | -0.003 (0.008) | 0.648 | 0.008 (0.009) | 0.352 |
| Duration of gestation | -0.01 (0.006) | 0.078 | -0.007 (0.007) | 0.327 |

[a]The adjusted model included the mother's baseline characteristics if the outcome variables were Pearson correlated ($P < 0.1$), which includes body mass index (BMI), age, years of education, height, gestational age, season, parity,

primiparous, household food insecurity access score, asset index, malaria status, alpha-1-acid glycoprotein (AGP) and C-reactive protein (CRP), and hemoglobin levels.

**Correlations between HDL CEC and enzyme activities**

We examined whether HDL CEC and plasma LCAT, PLTP and CETP activities were associated regardless of intervention (**Figure 1**). HDL CEC was significantly negatively correlated with CETP activity (R = -0.26, 95% CI (-0.39, -0.12), $P < 0.001$) and positively correlated with PLTP activity (R = 0.30, 95% CI (0.17, 0.43), $P < 0.001$). CETP activity was significantly positively correlated with LCAT activity (R = 0.46, 95% CI (0.33, 0.56), $P < 0.001$). Both CETP and LCAT activities were significantly negatively correlated with PLTP activity (R = -0.27, 95% CI (-0.40, -0.13), $P < 0.001$; R = -0.30, 95% CI (-0.43, -0.16), $P < 0.001$, respectively).

**Associations between HDL functional measures and inflammatory markers and hemoglobin levels**

We explored whether HDL CEC and plasma LCAT, PLTP and CETP activities were correlated with inflammatory markers AGP and CRP, as well as hemoglobin concentrations at 36 weeks gestation (**Figure 1**). AGP and CRP were significantly positively correlated (R = 0.49, 95% CI (0.37, 0.59), $P < 0.001$). LCAT activity was significantly negatively correlated with both AGP (R = -0.19, 95% CI (-0.33, -0.05), $P = 0.007$) and CRP (R = -0.28, 95% CI (-0.41, -0.14), $P < 0.001$), whereas HDL CEC, PLTP activity, and CETP activity were not significantly correlated with either AGP or CRP ($P > 0.05$). HDL CEC was positively correlated with hemoglobin (R = 0.15, 95% CI (0.01, 0.29), $P = 0.036$), but no significant correlations between plasma LCAT, PLTP, and CETP activities with hemoglobin were observed.

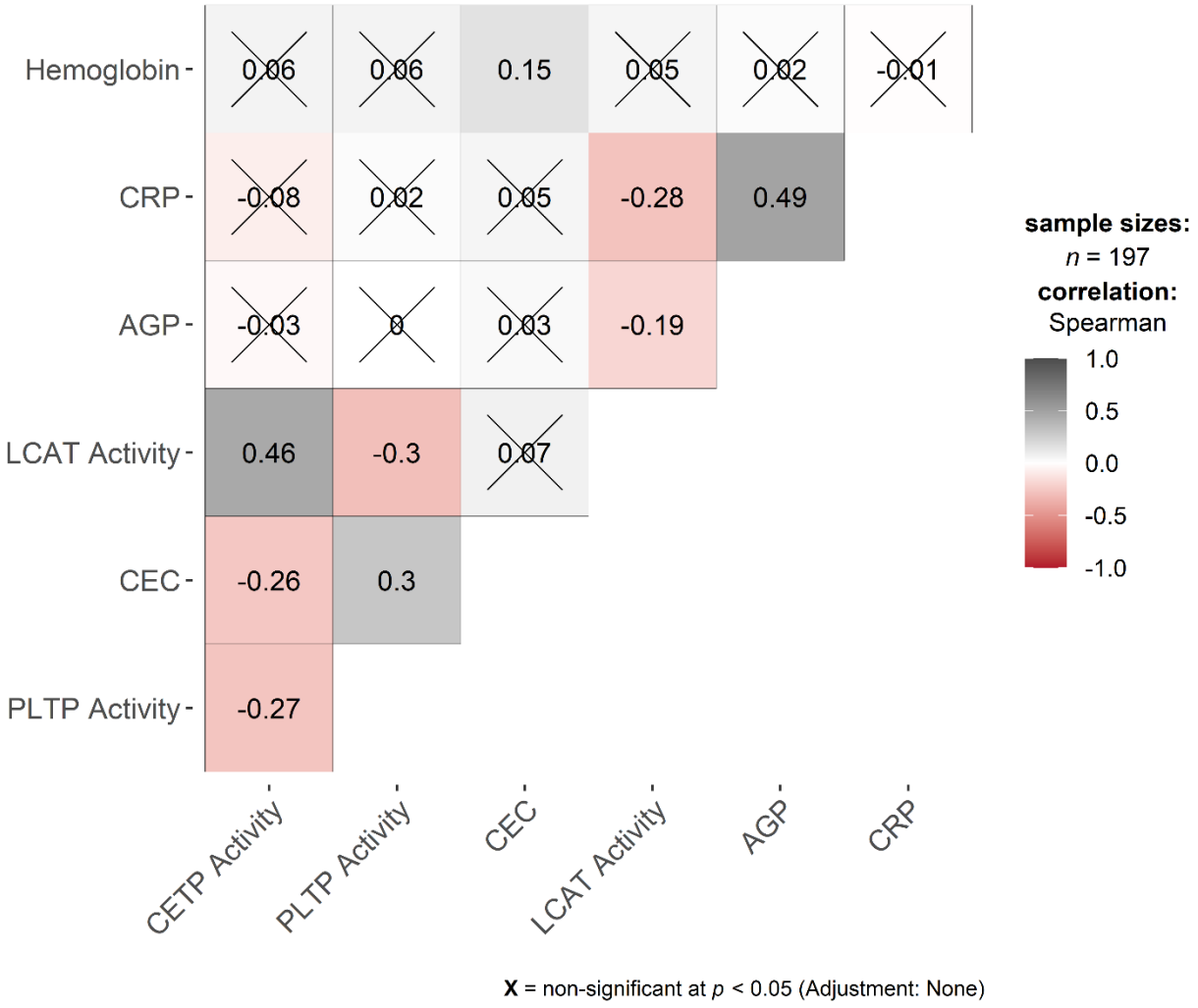


**Figure 1.** Spearman correlation analysis between HDL CEC, plasma enzyme activities, inflammation markers, and hemoglobin levels. AGP, alpha-1-acid glycoprotein; CEC, cholesterol efflux capacity; CETP, cholesteryl ester transfer protein; CRP, C-reactive protein; LCAT, lecithin-cholesterol acyltransferase; PLTP, phospholipid transfer protein.

**HDL enzyme activities differ by season**

We determined whether HDL CEC and plasma LCAT, PLTP, and CETP activities were altered by season at 36 weeks gestation. Regardless of treatment group, median ($25^{th}$ percentile, $75^{th}$ percentile) LCAT activity (1.46 (1.36, 1.54) vs 1.33 (1.28, 1.41) 390/470 nm, $P < 0.001$) and CETP activity (28.98 (23.18, 38.71) vs 21.69 (7.06, 34.12) pmol/µl/h, $P < 0.001$) were significantly higher in the dry season compared to the wet season (**Figure 2AB**). By contrast, PLTP activity was significantly higher during the wet season compared to the dry season (125.55 (69.92, 178.74) vs 90.34 (66.03, 115.46) pmol/µl/h, $P = 0.001$ **Figure 2C)**. HDL CEC was not significantly different by season ($P = 0.560$, **Figure 2D**). When treatment groups were stratified by season, there were no significant differences in HDL CEC or plasma LCAT, PLTP, and CETP activities between the SQ-LNS and IFA groups in either the wet or dry season ($P > 0.05$).

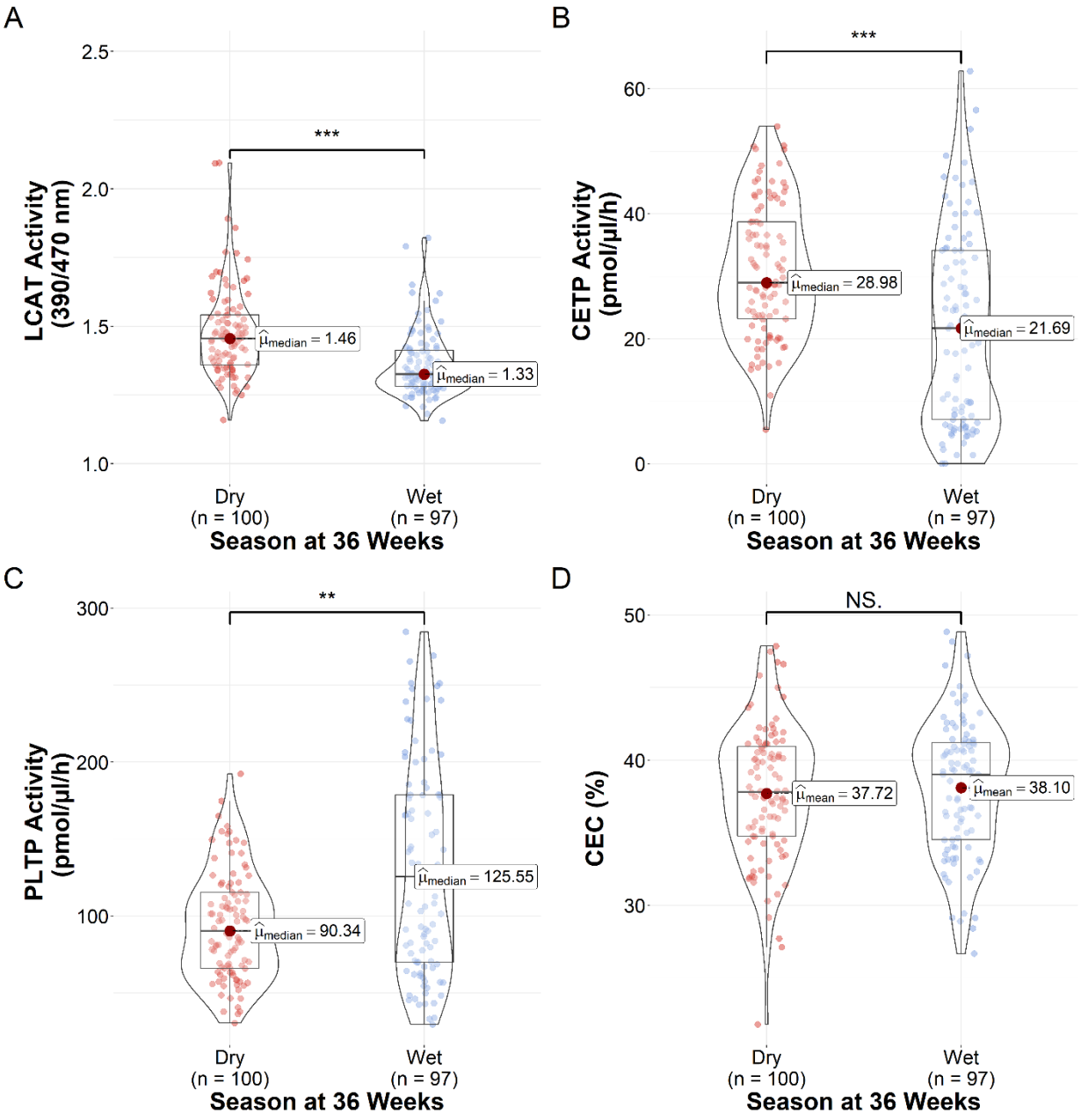


**Figure 2.** Plasma enzyme activities and high-density lipoproteins (HDL) cholesterol efflux capacity (CEC) by season (Dry: November - April and Wet: May - October) at 36 weeks gestation. Plasma A) lecithin-cholesterol acyltransferase (LCAT) activity, B) cholesteryl ester transfer protein (CETP) activity, C) phospholipid transfer protein (PLTP) activity, and D) HDL CEC. **P < 0.01, ***P < 0.001, NS = not significant.

## DISCUSSION

In this secondary outcome analysis, we explored for the first time whether HDL CEC and plasma enzymes involved in HDL metabolism were altered by SQ-LNS supplementation at 36 weeks gestation of mothers enrolled in the iLiNS-DYAD trial in Ghana. Our results demonstrate that maternal supplementation with SQ-LNS from enrollment (≤ 20 weeks) to 36 weeks gestation did not significantly alter HDL CEC or plasma LCAT, PLTP, and CETP activities, and none of these metrics were significantly associated with pregnancy and birth outcomes.

Both CEC and LCAT activity are measures of the ability of HDL to perform reverse cholesterol transport, involved in the removal of excess cholesterol from the body. LCAT is involved in the process of HDL maturation, in which cholesterol molecules are esterified and sequestered in the core of the particle, thus enabling a higher total cholesterol carrying capacity (21). Importantly, LCAT is also involved in anti-infectious function, as it is required for the ability of HDL particles to bind lipopolysaccharide (22). Likewise, higher PLTP activity was found to be associated with lower levels of LPS in plasma in cardiac surgery patients, which suggests PLTP is also involved in lipopolysaccharide elimination (23). By contrast, CETP is inhibited in an infectious state (24), which has been suggested to prevent the deleterious removal of HDL and to improve bacterial clearance (25). Together, LCAT, PLTP, and CETP activities are associated with infection by decreasing PLTP activity and increasing both LCAT and CETP activities which in turn can alter HDL metabolism (24,26). CETP can transfer cholesterol esters formed by LCAT to triglyceride-rich lipoproteins, including very-low-density lipoproteins and low-density lipoproteins, in exchange for triglycerides (27). Both the activities of CETP and LCAT have been shown to increase during pregnancy, and both are associated with increased concentrations of maternal plasma lipids (28,29). PLTP mediates the transfer of phospholipids

from triglyceride-rich lipoproteins to HDL particles and its activity is critical in part for the transfer of cholesterol from mother to fetus (30). It is possible that the lack of observed differences in HDL CEC and enzyme activities between the SQ-LNS and IFA groups is due to the natural changes to lipoproteins during pregnancy which may have outweighed the possible effects of SQ-LNS on these functional parameters.

We reported previously that HDL CEC of children at 18 mo of age assigned to the SQ-LNS group was increased compared to children in the IFA group (17). ALA, an essential n-3 polyunsaturated fatty acid and one of the nutrients provided in the SQ-LNS, has been shown to improve HDL CEC in vitro (31). In this cohort, at 36 weeks gestation the mothers had higher ALA:docosahexaenoic acid (DHA) ratios in the SQ-LNS group compared with the IFA group, but the median DHA concentration in plasma, as well as overall plasma fatty acids and lipid concentrations, were not significantly different (32). The lack of difference in HDL CEC between the SQ-LNS and IFA groups at 36 weeks gestation could be due to the lack of difference in fatty acid profiles in the two groups, or due to other unknown factors.

The lack of association between HDL functional measures and birth outcomes was somewhat surprising. Our group demonstrated that total HDL-C is positively associated with the duration of gestation (15). Even so, HDL CEC is recognized as a better indicator of health risk than HDL-C alone across many cohorts (33–35). Maternal plasma from preeclampsia patients has increased CEC, but lower ATP Binding Cassette Subfamily A Member 1 (ABCA1)-mediated CEC, compared to normotensive pregnant women, which the authors postulate as a rescue mechanism in preeclampsia to mitigate lipid peroxidation (36). Low plasma CETP activity at 36-38 weeks gestation is associated with giving birth to small-for-gestational-age

infants but not associated with large-for-gestational-age infants (37). More studies are needed to better understand the mechanisms by which HDL influences pregnancy and birth outcomes.

We found that plasma CETP and LCAT activities were positively correlated but were both negatively correlated with PLTP activity, whereas CETP activity was additionally negatively correlated with HDL CEC. LCAT activity was found to be negatively correlated with concentrations of the inflammatory markers AGP and CRP at 36 weeks gestation, which is in agreement with other observations of reduced LCAT activity during inflammation (24,26,38). HDL CEC and plasma PLTP activity were positively correlated and this association was also observed in participants with and without metabolic syndrome (39). Others have linked higher PLTP activity with an improved ability of HDL to efflux cholesterol (40,41) through its ability to stabilize and interact with ABCA1 (41). Moreover, PLTP participates in the reverse cholesterol transport pathway across the fetal placental barrier (42). By contrast, high PLTP activity has been linked to inflammation, particularly in sepsis patients (24,26). These studies suggest that the association between the enzymes involved in lipoprotein metabolism differ across health statuses and further work is needed to clarify the relationship between HDL function and metabolism in the context of pregnancy Longitudinal sampling throughout the course of pregnancy would be particularly informative to understand how HDL metabolism changes during normal pregnancy and in pregnancies where infection episodes or other events and environmental factors are present.

We observed a positive association between hemoglobin levels and HDL CEC. Iron deficiency induced in vitro contributes to reduced cholesterol efflux (43). Likewise, iron metabolism in macrophages affects the expression of transporters involved in cholesterol efflux, including ABCA1 (44). By contrast, higher hemoglobin concentrations were associated with a

higher proportion of smaller HDL particles, which increased the risk for cardiovascular disease (45). Thus, more work is needed to elucidate the relationship between HDL particles and hemoglobin during pregnancy.

We observed for the first time that both plasma CETP and LCAT activities were higher among participants in the dry season at 36 weeks gestation, while PLTP activity was higher during the wet season in this cohort of women in Ghana. We did not observe differences in AGP and CRP levels or differences in the positive rate of malaria by season (data not shown), which suggests that alterations in these enzymes were not influenced by inflammation. In Ghana, fish constitutes 50-80% of animal protein consumed (46), and the fat content of fish is at its highest during the dry season (November-May) compared to the wet season (June-September), whereas the fat content is reported to be the lowest and the water content is highest during the wet season (47). Furthermore, it has been reported that the availability of fish is increased from December – February during the dry season, but most available from July-September in the wet season (48). Thus, if fish consumption in this region is variable from the dry to the wet season, one plausible explanation for higher plasma LCAT and CETP activities during the dry season is related to an increase in the fat content of the diet, particularly the content of fat from fish intake. In the wet season, there is higher consumption of vitamin A-rich fruits, including mangoes, and vitamin A-rich dark leafy green vegetables among children in northern Ghana (49), which suggests that seasonality can affect the availability of certain foods, influence nutrient status, and possibly HDL metabolism. However, detailed dietary intake data was beyond the scope of this study and the relationship between seasonal dietary patterns and HDL particles in Ghana needs to be explored in future studies.

One strength of this study is that we included a subset of participants with similar characteristics among supplemental groups at baseline and 36 weeks. Thus, we were able to minimize the influence of background characteristics among mothers and assess the sole effects of SQ-LNS on HDL function and plasma enzymes involved in HDL metabolism. A limitation of the study was the use of cross-sectional samples which restricts our ability to detect changes in HDL function over time.

## CONCLUSION

Our study revealed changes in enzymes involved in lipoprotein metabolism by season in a subsample of pregnant women enrolled in the iLiNS-DYAD trial in Ghana and that these changes may be impacted by food availability, seasonal differences in the nutrient content of commonly consumed foods, or other unknown factors that vary by season and that influence HDL function and metabolism. By contrast, we did not observe differences in HDL CEC or plasma LCAT, PLTP, or CETP activities at 36 weeks gestation between mothers supplemented with SQ-LNS vs. the IFA control, which suggests that seasonal environmental factors had a stronger effect on HDL function and metabolism than the SQ-LNS supplement.

## ACKNOWLEDGEMENTS

We thank all of the co-investigators, collaborators, study team members, participants, and local communities involved in the iLiNS-DYAD-Ghana trial. The project described was supported by Bill & Melinda Gates Foundation grant (OPP124589) to the University of California, Davis. The findings and conclusions contained within this work are solely the responsibility of the authors and do not necessarily represent the official views of the Bill & Melinda Gates Foundation.

## REFERENCES


1. Prado EL, Dewey KG. Nutrition and brain development in early life. Nutr Rev 2014;72:267–84.

2. Christian P, Lee SE, Donahue Angel M, Adair LS, Arifeen SE, Ashorn P, Barros FC, Fall CH, Fawzi WW, Hao W, et al. Risk of childhood undernutrition related to small-for-gestational age and preterm birth in low- and middle-income countries. Int J Epidemiol 2013;42:1340–55.

3. Durao S, Visser M, Ramokolo V, Oliveira J, Schmidt B-M, Balakrishna Y, Brand A, Kristjansson E, Schoonees A. Community-level interventions for improving access to food in low- and middle-income countries. Cochrane Database Syst Rev [Internet] John Wiley & Sons, Ltd; 2020; Available from: https://doi.org//10.1002/14651858.CD011504.pub3

4. Adu-Afarwuah S, Lartey A, Okronipa H, Ashorn P, Zeilani M, Peerson JM, Arimond M, Vosti S, Dewey KG. Lipid-based nutrient supplement increases the birth size of infants of primiparous women in Ghana. Am J Clin Nutr 2015;101:835–46.

5. Adu-Afarwuah S, Lartey A, Okronipa H, Ashorn P, Peerson JM, Arimond M, Ashorn U, Zeilani M, Vosti S, Dewey KG. Small-quantity, lipid-based nutrient supplements provided to women during pregnancy and 6 mo postpartum and to their infants from 6 mo of age increase the mean attained length of 18-mo-old children in semi-urban Ghana: a randomized controlled trial. Am J Clin Nutr 2016;104:797–808.

6. Adu-Afarwuah S, Young RT, Lartey A, Okronipa H, Ashorn P, Ashorn U, Oaks BM, Arimond M, Dewey KG. Maternal and Infant Supplementation with Small-Quantity Lipid-Based Nutrient Supplements Increases Infants' Iron Status at 18 Months of Age in a Semiurban Setting in Ghana: A Secondary Outcome Analysis of the iLiNS-DYAD Randomized Controlled Trial. J Nutr 2019;149:149–58.

7. Chiesa ST, Charakida M. High-Density Lipoprotein Function and Dysfunction in Health and Disease. Cardiovasc Drugs Ther Springer US; 2019;33:207–19.

8. Bonacina F, Pirillo A, Catapano AL, Norata GD. Cholesterol membrane content has a ubiquitous evolutionary function in immune cell activation: the role of HDL. Curr Opin Lipidol 2019;30:462–9.

9. van der Vorst EPC, Theodorou K, Wu Y, Hoeksema MA, Goossens P, Bursill CA, Aliyev T, Huitema LFA, Tas SW, Wolfs IMJ, et al. High-Density Lipoproteins Exert Pro-inflammatory Effects on Macrophages via Passive Cholesterol Depletion and PKC-NF-κB/STAT1-IRF1 Signaling. Cell Metab 2017;25:197–207.

10. Kontush A, Lhomme M, Chapman MJ. Unraveling the complexities of the HDL lipidome. J Lipid Res 2013/03/30 ed. The American Society for Biochemistry and Molecular Biology; 2013;54:2950–63.

11. Zhang Y, Gordon SM, Xi H, Choi S, Paz MA, Sun R, Yang W, Saredy J, Khan M, Remaley AT, et al. HDL subclass proteomic analysis and functional implication of protein dynamic change during HDL maturation. Redox Biol 2019/05/17 ed. Elsevier; 2019;24:101222–101222.

12. Del Gaudio I, Hendrix S, Christoffersen C, Wadsack C. Neonatal HDL Counteracts Placental Vascular Inflammation via S1P-S1PR1 Axis. Int J Mol Sci MDPI; 2020;21:789.

13. Sulaiman WNW, Caslake MJ, Delles C, Karlsson H, Mulder MT, Graham D, Freeman DJ. Does high-density lipoprotein protect vascular function in healthy pregnancy? Clin Sci 2016;130:491–7.

14. Zhu C, Sawrey-Kubicek L, Beals E, Hughes RL, Rhodes CH, Sacchi R, Zivkovic AM. The HDL lipidome is widely remodeled by fast food versus Mediterranean diet in 4 days. Metabolomics 2019;15:114.

15. Oaks BM, Stewart CP, Laugero KD, Adu-Afarwuah S, Lartey A, Vosti SA, Ashorn P, Dewey KG. Maternal plasma cholesterol and duration of pregnancy: A prospective cohort study in Ghana. Matern Child Nutr 2017;13:10.1111/mcn.12418.

16. Sawrey-Kubicek L, Zhu C, Bardagjy AS, Rhodes CH, Sacchi R, Randolph JM, Steinberg FM, Zivkovic AM. Whole egg consumption compared with yolk-free egg increases the cholesterol efflux capacity of high-density lipoproteins in overweight, postmenopausal women. Am J Clin Nutr 2019;110:617–27.

17. Hong BV, Zhu C, Wong M, Sacchi R, Rhodes CH, Kang JW, Arnold CD, Adu-Afarwuah S, Lartey A, Oaks BM, et al. Lipid-Based Nutrient Supplementation Increases High-Density Lipoprotein (HDL) Cholesterol Efflux Capacity and Is Associated with Changes in the HDL Glycoproteome in Children. ACS Omega 2021;6:32022–31.

18. Davidson WS, Heink A, Sexmith H, Melchior JT, Gordon SM, Kuklenyik Z, Woollett L, Barr JR, Jones JI, Toth CA, et al. The effects of apolipoprotein B depletion on HDL subspecies composition and function. J Lipid Res 2016;57:674–86.

19. Zhu C, Wong M, Li Q, Sawrey-Kubicek L, Beals E, Rhodes CH, Sacchi R, Lebrilla CB, Zivkovic AM. Site-Specific Glycoprofiles of HDL-Associated ApoE are Correlated with HDL Functional Capacity and Unaffected by Short-Term Diet. J Proteome Res 2019;18:3977–84.

20. Thompson PD, Cullinane EM, Sady SP, Flynn MM, Bernier DN, Kantor MA, Saritelli AL, Herbert PN. Modest changes in high-density lipoprotein concentration and metabolism with prolonged exercise training. Circulation American Heart Association; 1988;78:25–34.

21. Rousset X, Vaisman B, Amar M, Sethi AA, Remaley AT. Lecithin: cholesterol acyltransferase--from biochemistry to role in cardiovascular disease. Curr Opin Endocrinol Diabetes Obes 2009;16:163–71.

22. Petropoulou P-I, Berbée JFP, Theodoropoulos V, Hatziri A, Stamou P, Karavia EA, Spyridonidis A, Karagiannides I, Kypreos KE. Lack of LCAT reduces the LPS-neutralizing

capacity of HDL and enhances LPS-induced inflammation in mice. Biochim Biophys Acta Netherlands; 2015;1852:2106–15.

23. Nguyen M, Gautier T, Reocreux G, Pallot G, Maquart G, Bahr P-A, Tavernier A, Grober J, Masson D, Bouhemad B, et al. Increased Phospholipid Transfer Protein Activity Is Associated With Markers of Enhanced Lipopolysaccharide Clearance in Human During Cardiopulmonary Bypass. Front Cardiovasc Med [Internet] 2021;8. Available from: https://www.frontiersin.org/article/10.3389/fcvm.2021.756269

24. Reisinger AC, Schuller M, Sourij H, Stadler JT, Hackl G, Eller P, Marsche G. Impact of Sepsis on High-Density Lipoprotein Metabolism. Front Cell Dev Biol [Internet] 2022;9. Available from: https://www.frontiersin.org/article/10.3389/fcell.2021.795460

25. Blauw LL, Wang Y, Willems van Dijk K, Rensen PCN. A Novel Role for CETP as Immunological Gatekeeper: Raising HDL to Cure Sepsis? Trends Endocrinol Metab 2020;31:334–43.

26. Kolovou GD, Anagnostopoulou KK, Kostakou PM, Mikhailidis DP. Cholesterol ester transfer protein (CETP), postprandial lipemia and hypolipidemic drugs. Curr Med Chem United Arab Emirates; 2009;16:4345–60.

27. Iglesias A, Montelongo A, Herrera E, Lasunción MA. Changes in cholesteryl ester transfer protein activity during normal gestation and postpartum. Clin Biochem 1994;27:63–8.

28. Gillett MP, Silva TB. Relationship between plasma lecithin:cholesterol acyltransferase activity and plasma triglyceride concentration in non-pregnant and pregnant Brazilian women. Scand J Clin Lab Investig Suppl Norway; 1978;150:118–23.

29. Scholler M, Wadsack C, Lang I, Etschmaier K, Schweinzer C, Marsche G, Dieber-Rotheneder M, Desoye G, Panzenboeck U. Phospholipid Transfer Protein in the Placental Endothelium Is Affected by Gestational Diabetes Mellitus. J Clin Endocrinol Metab 2012;97:437–45.

30. Zhang J, Kris-Etherton PM, Thompson JT, Hannon DB, Gillies PJ, Heuvel JPV. Alpha-linolenic acid increases cholesterol efflux in macrophage-derived foam cells by decreasing stearoyl CoA desaturase 1 expression: evidence for a farnesoid-X-receptor mechanism of action. J Nutr Biochem 2012;23:400–9.

31. Oaks BM, Young RR, Adu-Afarwuah S, Ashorn U, Jackson KH, Lartey A, Maleta K, Okronipa H, Sadalaki J, Baldiviez LM, et al. Effects of a lipid-based nutrient supplement during pregnancy and lactation on maternal plasma fatty acid status and lipid profile: Results of two randomized controlled trials. Prostaglandins Leukot Essent Fatty Acids 2017/01/25 ed. Churchill Livingstone; 2017;117:28–35.

32. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, et al. Cholesterol Efflux Capacity, High-Density Lipoprotein Function, and Atherosclerosis. N Engl J Med Massachusetts Medical Society; 2011;364:127–35.

33. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, et al. HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events. N Engl J Med 2014;371:2383–93.

34. Shea S, Stein JH, Jorgensen NW, McClelland RL, Tascau L, Shrager S, Heinecke JW, Yvan-Charvet L, Tall AR. Cholesterol Mass Efflux Capacity, Incident Cardiovascular Disease, and Progression of Carotid Plaque. Arterioscler Thromb Vasc Biol 2019;39:89–96.

35. Mistry HD, Kurlak LO, Mansour YT, Zurkinden L, Mohaupt MG, Escher G. Increased maternal and fetal cholesterol efflux capacity and placental CYP27A1 expression in preeclampsia. J Lipid Res 2017/04/10 ed. The American Society for Biochemistry and Molecular Biology; 2017;58:1186–95.

36. Roland MCP, Godang K, Aukrust P, Henriksen T, Lekva T. Low CETP activity and unique composition of large VLDL and small HDL in women giving birth to small-for-gestational age infants. Sci Rep 2021;11:6213.

37. McEneny J, McKavanagh P, York E, Nadeem N, Harbinson M, Stevenson M, Ball P, Lusk L, Trinick T, Young IS, et al. Serum- and HDL3-serum amyloid A and HDL3-LCAT activity are influenced by increased CVD-burden. Atherosclerosis 2016;244:172–8.

38. Levels JHM, Pajkrt D, Schultz M, Hoek FJ, van Tol A, Meijers JCM, van Deventer SJH. Alterations in lipoprotein homeostasis during human experimental endotoxemia and clinical sepsis. Biochim Biophys Acta BBA - Mol Cell Biol Lipids 2007;1771:1429–38.

39. Dullaart RPF, Groen AK, Dallinga-Thie GM, Vries R de, Sluiter WJ, Tol A van. Fibroblast cholesterol efflux to plasma from metabolic syndrome subjects is not defective despite low high-density lipoprotein cholesterol. Eur J Endocrinol Bristol, UK: BioScientifica; 2008;158:53–60.

40. Vikstedt R, Metso J, Hakala J, Olkkonen VM, Ehnholm C, Jauhiainen M. Cholesterol efflux from macrophage foam cells is enhanced by active phospholipid transfer protein through generation of two types of acceptor particles. Biochemistry United States; 2007;46:11979–86.

41. Oram JF, Wolfbauer G, Vaughan AM, Tang C, Albers JJ. Phospholipid Transfer Protein Interacts with and Stabilizes ATP-binding Cassette Transporter A1 and Enhances Cholesterol Efflux from Cells*. J Biol Chem 2003;278:52379–85.

42. Scholler M, Wadsack C, Metso J, Chirackal Manavalan AP, Sreckovic I, Schweinzer C, Hiden U, Jauhiainen M, Desoye G, Panzenboeck U. Phospholipid Transfer Protein Is Differentially Expressed in Human Arterial and Venous Placental Endothelial Cells and Enhances Cholesterol Efflux to Fetal HDL. J Clin Endocrinol Metab 2012;97:2466–74.

43. Meroño T, Dauteuille C, Tetzlaff W, Martín M, Botta E, Lhomme M, Saez MS, Sorroche P, Boero L, Arbelbide J, et al. Oxidative stress, HDL functionality and effects of intravenous iron administration in women with iron deficiency anemia. Clin Nutr 2017;36:552–8.

44. Saeed O, Otsuka F, Polavarapu R, Karmali V, Weiss D, Davis T, Rostad B, Pachura K, Adams L, Elliott J, et al. Pharmacological Suppression of Hepcidin Increases Macrophage Cholesterol Efflux and Reduces Foam Cell Formation and Atherosclerosis. Arterioscler Thromb Vasc Biol American Heart Association; 2012;32:299–307.

45. Hämäläinen P, Saltevo J, Kautiainen H, Mäntyselkä P, Vanhala M. Hemoglobin level and lipoprotein particle size. Lipids Health Dis BioMed Central; 2018;17:10–10.

46. Sumberg J, Jatoe J, Kleih U, Flynn J. Ghana's evolving protein economy. Food Secur 2016;8:909–20.

47. Essumang DK, Dodoo DK, Adjei JK. Polycyclic aromatic hydrocarbon (PAH) contamination in smoke-cured fish products. J Food Compos Anal 2012;27:128–38.

48. Hasselberg AE, Aakre I, Scholtens J, Overå R, Kolding J, Bank MS, Atter A, Kjellevold M. Fish for food and nutrition security in Ghana: Challenges and opportunities. Glob Food Secur 2020;26:100380.

49. Abizari A-R, Azupogo F, Nagasu M, Creemers N, Brouwer ID. Seasonality affects dietary diversity of school-age children in northern Ghana. PloS One Public Library of Science; 2017;12:e0183206–e0183206.

# Chapter 4: High-Density Lipoprotein Changes in Alzheimer's Disease Are *APOE* Genotype-Specific

Brian V. Hong[1] , Jingyuan Zheng[1], Joanne K. Agus[1], Xinyu Tang[1], Carlito B. Lebrilla[2], Lee-Way Jin[3], Izumi Maezawa[3], Kelsey Erickson[3], Danielle J. Harvey[4], Charles S. DeCarli[5], Dan M. Mungas[5], John M. Olichney[5], Sarah T. Farias[5] and Angela M. Zivkovic[1]*

[1]Department of Nutrition, University of California-Davis, Davis, CA 95616, USA;

[2]Department of Chemistry, University of California-Davis, Davis, CA 95616, USA

[3]Department of Pathology and Laboratory Medicine, School of Medicine, University of California-Davis, Davis, CA 95817, USA

[4]Department of Public Health Sciences, University of California-Davis, Davis, CA 95616, USA

[5]Department of Neurology, School of Medicine, University of California-Davis, Davis, CA 95817, USA

* Correspondence: amzivkovic@ucdavis.edu

**ABSTRACT**

High-density lipoproteins (HDL) play a critical role in cholesterol homeostasis. Apolipoprotein E (*APOE)*, particularly the *E4* allele, is a significant risk factor for Alzheimer's disease but is also a key HDL-associated protein involved in lipid transport in both the periphery and central nervous systems. The objective was to determine the influence of the *APOE* genotype on HDL function and size in the context of Alzheimer's disease. HDL from 194 participants (non-demented controls, mild cognitive impairment, and Alzheimer's disease dementia) were isolated from the plasma. The HDL cholesterol efflux capacity (CEC), lecithin-cholesterol acyltransferase (LCAT) activity, and particle diameter were measured. Neuropsychological test scores, clinical dementia rating, and magnetic resonance imaging scores were used to determine if cognition is associated with HDL function and size. HDL CEC and LCAT activity were reduced in *APOE3E4* carriers compared to *APOE3E3* carriers, regardless of diagnosis. In *APOE3E3* carriers, CEC and LCAT activity were lower in patients. In *APOE3E4* patients, the average particle size was lower. HDL LCAT activity and particle size were positively correlated with the neuropsychological scores and negatively correlated with the clinical dementia rating. We provide evidence for the first time of *APOE* genotype-specific alterations in HDL particles in Alzheimer's disease and an association between HDL function, size, and cognitive function.

**Keywords**: Alzheimer's disease; APOE; cholesterol efflux capacity; HDL; LCAT

## INTRODUCTION

Alzheimer's disease is the leading cause of dementia and poses a considerable economic and public health burden [1]. Alzheimer's disease develops over the course of decades [2], and drugs targeting late-stage processes once dementia and brain volume loss have set in may be "too late". There is an urgent need for effective, long-term treatments that target the underlying pathophysiology of Alzheimer's disease to prevent cognitive function loss and the onset of dementia.

Increasing evidence suggests that high-density lipoprotein (HDL) particles, both in the central nervous system (CNS) and in the periphery, are implicated in Alzheimer's disease pathology. Alzheimer's disease patients typically have lower plasma HDL cholesterol (HDL-C) and apolipoprotein-A1 (ApoA-I) concentrations than controls [3,4], suggesting that a lack of HDL is detrimental. HDL-C concentrations measured during midlife are negatively correlated with the onset of late-life mild cognitive impairment (MCI) and dementia [5], suggesting that HDL are protective. However, the functional capacity of HDL is a stronger predictor of disease risk than HDL-C concentrations, since the measurement of HDL-C does not encompass the complex, destructive changes to HDL particles that can occur in disease states and in aging [6,7]. The main function of HDL particles is to mediate reverse cholesterol transport, though they perform numerous additional protective functions, including reducing inflammation, promoting endothelial function, and antioxidant effects, among others [8].

Although ApoA-I is not expressed in the CNS, the peripheral overexpression of human ApoA-I preserves cognitive function, reduces neuroinflammation, and protects mice from cerebral amyloid angiopathy [9], suggesting a role for peripheral HDL in the clearance of brain amyloid beta (Aβ). In a small study of 39 Alzheimer's disease patients and 20 healthy

participants (unknown *APOE* genotype), HDL isolated from plasma showed a diminished HDL cholesterol efflux capacity (CEC) and lecithin-cholesterol acyltransferase (LCAT) activity—the enzyme responsible for increasing the HDL cholesterol carrying capacity—and the level of LCAT activity was negatively correlated with the cognitive score [10]. Together, these studies suggest that circulating HDL is involved in the pathology of Alzheimer's disease. However, the relationship between the peripheral HDL functional capacity and Alzheimer's disease has not been fully investigated in a larger cohort of Alzheimer's disease patients, and the effects of the *APOE* genotype on this relationship have not been investigated.

In the current study, we determined whether HDL CEC and LCAT activity are altered in a large *APOE* genotyped cohort of elderly participants clinically diagnosed as either non-demented, MCI, or Alzheimer's disease dementia (AD). We also determined whether the HDL particle size, which is closely related to the HDL function, was altered in the same cohort. We further explored whether the HDL functional metrics and particle size were associated with the participants' cognitive, functional, and imaging scores.

## MATERIALS AND METHODS

### Participants

This study used plasma samples collected from the University of California, Davis Alzheimer's Disease Research Center (ADRC) Biorepository. The ADRC biorepository is nationally recognized for recruiting an ethnically diverse clinic-based and community based elderly cohort [11]. We aimed to select 200 samples from the ADRC biorepository to include non-demented (controls), MCI, and AD patients, with each diagnosis group having as close as possible to equal numbers of participants with the *APOE3E3* and *APOE3E4* genotypes and with each genotype X diagnosis group having as close as possible to equal

numbers of the sexes (males and females), with equal average ages. Only participants with an adequate sample volume available (500 µL) and body mass index (BMI) not greater than 40 kg/m2 were included. Participants were diagnosed following the ADRC criteria for a clinical diagnosis within one year of a blood draw, as previously described [12]. Dementia patients were included in the AD group if their etiologic diagnosis was classified as "probable" or "possible" AD. Five patients in the AD group had an etiologic diagnosis of frontotemporal dementia. Removing these patients did not change the overall findings; thus, they were included in the analysis. Samples from a total of 194 participants met the above criteria and were included in the final analysis. The study was approved by the Institutional Review Board of the University of California, Davis.

**HDL Isolation Method**

HDL particles from plasma were isolated by two-step sequential flotation densityultracentrifugation, followed by size exclusion chromatography, to yield highly purified HDL fractions, as previously described [13]. Following the manufacturer's instructions, the total HDL protein was measured using a Micro BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA, catalog 23235).

**Cholesterol Efflux Capacity**

HDL cholesterol efflux was measured using a commercially available kit (Abcam, Cambridge, UK, catalog ab19685) with modifications, as previously described [14]. J774A.1 (ATCC, Manassas, VA, USA, catalog TIB-67) were seeded at 100,000 cells in 96-well plates for 4 h in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum and 100 µg/mL penicillin and streptomycin. The cells were washed and labeled with fluorescent cholesterol, acyl-coenzyme A:cholesterol acyltransferase inhibitor, and cyclic adenosine

monophosphate for 4 h, followed by wash and incubation with 10 µg of HDL protein for another 4 h. The cellular supernatant was removed, and the remaining cells were lysed with M-PER cell lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA, catalog 78505). The supernatant and lysed fraction fluorescence were measured at 482/515 nm (emission/excitation) on a Synergy H1 plate reader (BioTek, Winooski, VT, USA). To account for the inter-plate variability, CEC values were normalized, with the CEC value of HDL isolated from pooled plasma collected from healthy volunteers on each plate to calculate a final CEC Index, as previously described [15].

**LCAT Activity**

A commercially available kit was used to measure the LCAT activity (Roar Biomedical, Millipore Sigma, Burlington, MA, USA, catalog mak107) in 5 µg of HDL (measured by the total protein) following the manufacturer's instructions. Measurements were collected on a Synergy H1 plate reader (BioTek, Winooski, VT, USA) and read at 340-nm excitation and two emission wavelengths at 390 nm and 470 nm, representing the hydrolyzed and intact substrate, respectively. Higher LCAT activity is indicated as increased λem390/λem470 nm ratios.

**HDL Particle Size**

The HDL particle size was assessed using negative-stained transmission electron microscopy (TEM) based on the published methods [16], in which over 3000 particles were imaged and sized from each individual participant sample. A schematic workflow of the particle size measurements by TEM is presented in Supplementary Figure S1. Briefly, isolated HDL samples were diluted to 30–100 µg/mL HDL protein concentrations with deionized water. A diluted HDL sample (4 µL) was loaded onto glow-discharged carboncoated grids (TedPella Inc., Redding, CA, USA) and left for sample attachment for 1 min. Extra sample was removed using filter paper. Uranyl formate solution (2%, pH 7.4) was then added onto the

grid and removed 5 times to achieve negative staining. Negatively stained sample grids were then air-dried for 5 min and loaded onto a specimen holder for TEM, according to the manufacturer's user manual (JEOL USA 1230 Transmission Electron Microscope, JEOL USA Inc., Peabody, MA, USA). Samples were viewed under the TEM using high tension = 100 kv and 40,000× magnification. Micrograph images were taken at random locations using an attached CCD camera (model) with an exposure time of 300 ms.

The software ImageJ [17] was used to characterize the HDL particle size from the TEM micrographs, following a previously published procedure [13], with minor modifications: Noise in the micrographs was first removed using the "Bandpass Filter" function, with "filter large = 100, filter small = 10, suppress = None, and tolerance = 5 autoscale saturate" parameters. The contrast of the cleaned micrograph was then set to "min = 50, max = 205". The threshold of the micrographs was then set using a premade "Intermodes dark" option. The particle area was then analyzed using the "Analyze Particles" function with "size = 20–7850, circularity = 0.30–1.00, display, exclude, include, and add" parameters. Particles that were captured by the function were then outlined onto the original TEM micrograph and were checked manually for accuracy.

**Cognitive Function Analysis**

The Spanish English Neuropsychological Assessment Scales (SENAS) evaluated participants' neuropsychological functional assessments, as previously described and validated elsewhere [18,19]. This study uses the SENAS cognitive domains: verbal memory, executive function, spatial, and semantic memory.

The Clinical Dementia Rating Scale (CDR) is a semi-structured interview administered by a clinician as a global measure of independent function [20]. Six cognitive domains

in memory, orientation, judgment and problem-solving, community affairs, home and hobbies, and personal care were assessed. The combination of the scores obtained ("sum of boxes") was used for analysis.

The white matter hyperintensities (WMH) measurements were acquired by magnetic resonance imaging (MRI), as previously described [21,22]. The total cranial volume was used to normalize the head size differences among participants.

**Statistical Analysis**

Data analyses were conducted using statistical software R version 4.1.1. (R Project for Statistical Computing, Vienna, Austria). Differences in the HDL functional metrics and particle sizes between AD patients, MCI patients, and controls were tested with one-way ANOVA. The null hypothesis of no difference between groups was rejected if $p < 0.05$. A post hoc Tukey's HSD was conducted for pairwise comparisons between groups when a significant difference was observed. For two group comparisons, a two-sample *t*-test assuming equal variance was used. For all pairwise comparisons, significance values were reported with $p < 0.05$. Statistically significant findings are indicated as shown: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. The distribution of the outcome variables and key baseline variables was inspected for normality using the Shapiro–Wilks test. The homogeneity of the variance was examined using Levene's test. Kruskal–Wallis tests were performed on non-normally distributed data between more than two groups. A chi-squared ($\chi 2$) test was used to compare the categorical variables.

To examine the association between HDL functional metrics and particle size with cognitive, functional, or MRI assessments, a correlation analysis, adjusting for the *APOE*

genotype, was conducted across all diagnoses using Spearman's correlation. For Spearman's correlation, the rho (r) values and 95% confidence intervals were reported.

We performed complete case analyses to describe the participant characteristics, HDL functional metrics, and particle size and to assess the association between HDL function and size with cognitive, functional, and MRI assessments. For the HDL particle size analysis, one out of the total of 194 samples was removed due to an insufficient number of total particles on the TEM slide at the time of analysis.

## RESULTS

### Participant Characteristics

Details of the participant characteristics and clinical parameters are summarized in Table 1. There were no significant differences observed in the ratio of male to female ($\chi2(2) = 4.10$, $p = 0.129$), proportion of ethnicity ($\chi2(6) = 10.97$, $p = 0.089$), body mass index (BMI) ($F(2, 181) = 2.00$, $p = 0.138$), or higher history of hypertension ($\chi2(2) = 1.56$, $p = 0.459$) among the diagnoses, and these characteristics were not significantly different between groups when stratified by the *APOE* genotype. One-way ANOVA revealed a significant difference in age ($F(2, 191) = 4.82$, $p = 0.009$), with significantly younger participants in the control group than the AD group (mean ± SD age, 75.5 ± 7.0 y vs. 78.9 ± 7.2 y, $p = 0.008$) but not significantly younger than the MCI group (78.0 ± 7.0 y, $p = 0.153$). When the groups were stratified by the *APOE* genotype, one-way ANOVA revealed that none of the age differences among the diagnoses were significantly different within the *APOE3E3* group ($F(2, 100) = 2.55$, $p = 0.080$) or the *APOE3E4* group ($F(2, 88) = 2.28$, $p = 0.108$). There was a significant difference in the prevalence of diabetes at the sample collection ($\chi2(2) = 22.94$, $p < 0.001$) and history of diabetes ($\chi2(2) = 28.50$, $p < 0.001$) among the groups,

with both of these characteristics being higher in the control participants (34% and 39%, respectively) compared to MCI patients (7.9% and 10%, respectively) and AD patients (4.9% and 5.6%, respectively). When stratified by the *APOE* genotype, the prevalence of diabetes at the sample collection was significantly different among the *APOE3E3* carriers ($\chi 2(2) = 19.30$, $p < 0.001$) but not in the *APOE3E4* carriers ($\chi 2(2) = 5.64$, $p = 0.060$). Post hoc comparisons in the *APOE3E3* carriers revealed that diabetes during sample collection was higher in the controls (39%) compared with MCI (9.5%) and AD (0%) patients. A history of hypercholesterolemia was significantly different among the diagnoses ($\chi 2(2) = 6.70$, $p = 0.035$), with a higher history of hypercholesterolemia in the controls compared with AD (74% vs. 54%). When stratified by the *APOE* genotype, a history of hypercholesterolemia was significantly different among the *APOE3E4* carriers ($\chi 2(2) = 8.52$, $p = 0.014$) but not in the *APOE3E3* carriers ($\chi 2(2) = 2.21$, $p = 0.331$). A post hoc comparison revealed that the *APOE3E4* controls had a significantly higher history of hypercholesterolemia (87%) than both *APOE3E4* MCI (56%) and AD (62%) patients.

**Table 1. Participant characteristics.**

| | | Control | MCI | AD | *F*(*df*, *df* error) or $\chi^2(df)$ | *P* value |
|---|---|---|---|---|---|---|
| *N*, combined | | 83 | 40 | 71 | n/a | n/a |
| | *APOE3E3* | 44 | 22 | 37 | n/a | n/a |
| | *APOE3E4* | 39 | 18 | 34 | n/a | n/a |
| Sex proportion, (male/female), combined | | 35:48 | 23:17 | 27:44 | $\chi^2(2) = 4.10$ | 0.129 |
| | *APOE3E3* | 18:26 | 12:10 | 15:22 | $\chi^2(2) = 1.34$ | 0.511 |
| | *APOE3E4* | 17:22 | 11:7 | 12:22 | $\chi^2(2) = 3.18$ | 0.203 |
| Age, years, mean ± SD, (*n*), combined | | 75.5 ± 7.0 (83) | 78.0 ± 7.0 (40) | 78.9 ± 7.2 (71) | $F(2, 191) = 4.82$ | **0.009**[ab] |
| | *APOE3E3* | 75.9 ± 7.0 (44) | 78.6 ± 7.7 (22) | 79.7 ± 8.4 (37) | $F(2, 100) = 2.55$ | 0.080 |
| | *APOE3E4* | 75.0 ± 7.1 (39) | 77.3 ± 6.2 (18) | 78.1 ± 5.7 (34) | $F(2, 88) = 2.28$ | 0.108 |
| BMI, kg/m$^2$, mean ± SD, (*n*), combined | | 28.1 ± 4.6 (81) | 27.1 ± 4.2 (39) | 26.6 ± 4.6 (64) | $F(2, 181) = 2.00$ | 0.138 |
| | *APOE3E3* | 28.6 ± 4.5 (43) | 27.8 ± 5.1 (21) | 26.2 ± 4.7 (32) | $F(2, 93) = 2.49$ | 0.089 |
| | *APOE3E4* | 27.5 ± 4.6 (38) | 26.3 ± 2.7 (18) | 27.0 ± 4.9 (32) | $F(2, 85) = 0.42$ | 0.658 |
| Ethnicity proportion, (African American/Asian/Hispanic/White), combined | | 18:2:19:44 | 4:1:4:31 | 7:1:11:52 | $\chi^2(6) = 10.97$ | 0.089 |
| Diabetes at sample collection, %, (*n*), combined | | 34% (76) | 7.9% (38) | 4.9% (61) | $\chi^2(2) = 22.94$ | **<0.001**[abc] |
| | *APOE3E3* | 39% (41) | 9.5% (21) | 0% (32) | $\chi^2(2) = 19.30$ | **<0.001**[abc] |
| | *APOE3E4* | 29% (35) | 5.9% (17) | 10% (29) | $\chi^2(2) = 5.64$ | 0.060 |
| History of diabetes, %, (*n*), combined | | 39% (83) | 10% (39) | 5.6% (71) | $\chi^2(2) = 28.50$ | **<0.001**[abc] |
| | *APOE3E3* | 39% (44) | 14% (21) | 0% (37) | $\chi^2(2) = 19.51$ | **<0.001**[abc] |
| | *APOE3E4* | 38% (39) | 5.6% (18) | 12% (34) | $\chi^2(2) = 8.52$ | **0.014**[abc] |
| History of hypercholesterolemia, %, (*n*), combined | | 74% (81) | 60% (40) | 54% (70) | $\chi^2(2) = 6.70$ | **0.035**[ab] |
| | *APOE3E3* | 62% (42) | 64% (22) | 47% (36) | $\chi^2(2) = 2.21$ | 0.331 |
| | *APOE3E4* | 87% (39) | 56% (18) | 62% (34) | $\chi^2(2) = 8.52$ | **0.014**[abc] |
| History of hypertension, %, (*n*), combined | | 72% (83) | 65% (40) | 76% (71) | $\chi^2(2) = 1.56$ | 0.459 |
| | *APOE3E3* | 75% (44) | 77% (22) | 76% (37) | $\chi^2(2) = 0.04$ | 0.980 |
| | *APOE3E4* | 69% (39) | 50% (18) | 76% (34) | $\chi^2(2) = 3.84$ | 0.147 |

Abbreviations: BMI = body mass index. ANOVA were performed for continuous variables (age and bmi), and chi-squared ($\chi^2$) were performed for categorical variables (sex proportion, ethnicity proportion, diabetes at sample collection, history of diabetes, history of hypercholesterolemia, and history of hypertension). If significance is reached by ANOVA or $\chi^2$, Tukey's or Bonferroni post hoc comparison was carried out, respectively.

[a]Significance across all three groups.

[b]Significance between control compared with AD.

[c]Significance between control compared with MCI.

**HDL CEC Index and LCAT Activity Differences**

Mean ± SD CEC index and LCAT activity is shown in Table 2. When participants were not stratified by *APOE* genotype there was no significant difference in HDL CEC index between control, MCI, and AD participants by one-way ANOVA (1.10 ± 0.16, 1.06 ± 0.14, and 1.12 ± 0.14, $F(2, 191) = 2.14$, $p = 0.120$, Fig. 1A). A one-way ANOVA revealed that there was a significant difference in HDL LCAT activity between at least two groups ($F(2, 191) = 3.87$, $p = 0.023$). Post hoc comparisons indicate HDL LCAT activity was significantly higher in the control group versus MCI (1.05 ± 0.09 vs 1.01 ± 0.07, $p = 0.030$), but not significantly higher than the AD group (1.03 ± 0.07, $p = 0.118$, Fig. 1B). There were no significant differences in LCAT activity between MCI and AD ($p = 0.661$). The CEC index (1.06 ± 0.16 vs 1.13 ± 0.14, $t(192) = 3.00$, $p = 0.003$) and LCAT activity (1.01 ± 0.07 vs 1.05 ± 0.08, $t(192) = 3.53$, $p < 0.001$) were significantly lower in the *APOE3E4* carriers relative to *APOE3E3* carriers (Fig. 1CD).

**Table 2. Comparison of HDL CEC index, LCAT activity, and particle size across diagnoses.**

| | Group | | | ANOVA | | | | Post hoc comparison *p* value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | MCI | AD | *F* | *df* | *df* error | *p* value | Control versus MCI | Control versus AD | MCI Versus AD |
| **CEC Index** | | | | | | | | | | |
| *APOE3E3+ APOE3E4* | 1.10 ± 0.16 | 1.06 ± 0.14 | 1.12 ± 0.14 | 2.14 | 2 | 191 | 0.120 | - | - | - |
| *APOE3E3* | 1.16 ± 0.13 | 1.07 ± 0.13 | 1.11 ± 0.15 | 3.26 | 2 | 100 | **0.042** | **0.042** | 0.246 | 0.551 |
| *APOE3E4* | 1.02 ± 0.16 | 1.03 ± 0.14 | 1.12 ± 0.14 | 4.38 | 2 | 88 | **0.015** | 0.964 | **0.016** | 0.114 |
| **LCAT Activity (390/470 nm)** | | | | | | | | | | |
| *APOE3E3+ APOE3E4* | 1.05 ± 0.09 | 1.01 ± 0.07 | 1.03 ± 0.07 | 3.87 | 2 | 191 | **0.023** | **0.030** | 0.118 | 0.661 |
| *APOE3E3* | 1.09 ± 0.09 | 1.03 ± 0.08 | 1.03 ± 0.07 | 7.00 | 2 | 100 | **0.001** | **0.012** | **0.004** | 0.995 |
| *APOE3E4* | 1.01 ± 0.07 | 1.00 ± 0.06 | 1.02 ± 0.07 | 0.91 | 2 | 88 | 0.410 | - | - | - |
| **HDL Particle Diameter (nm)** | | | | | | | | | | |
| *APOE3E3+ APOE3E4* | 9.06 ± 0.69 | 8.67 ± 0.63 | 8.61 ± 0.68 | 9.70 | 2 | 190 | **<0.001** | **0.008** | **<0.001** | 0.901 |
| *APOE3E3* | 8.99 ± 0.85 | 8.79 ± 0.58 | 8.72 ± 0.64 | 1.40 | 2 | 99 | 0.252 | - | - | - |
| *APOE3E4* | 9.15 ± 0.42 | 8.53 ± 0.67 | 8.50 ± 0.70 | 13.04 | 2 | 88 | **<0.001** | **0.001** | **<0.001** | 0.985 |

ANOVA were performed to compare group differences. If significance is reached by ANOVA, Tukey's post hoc comparison was carried out. Values are represented as mean ± standard deviation.

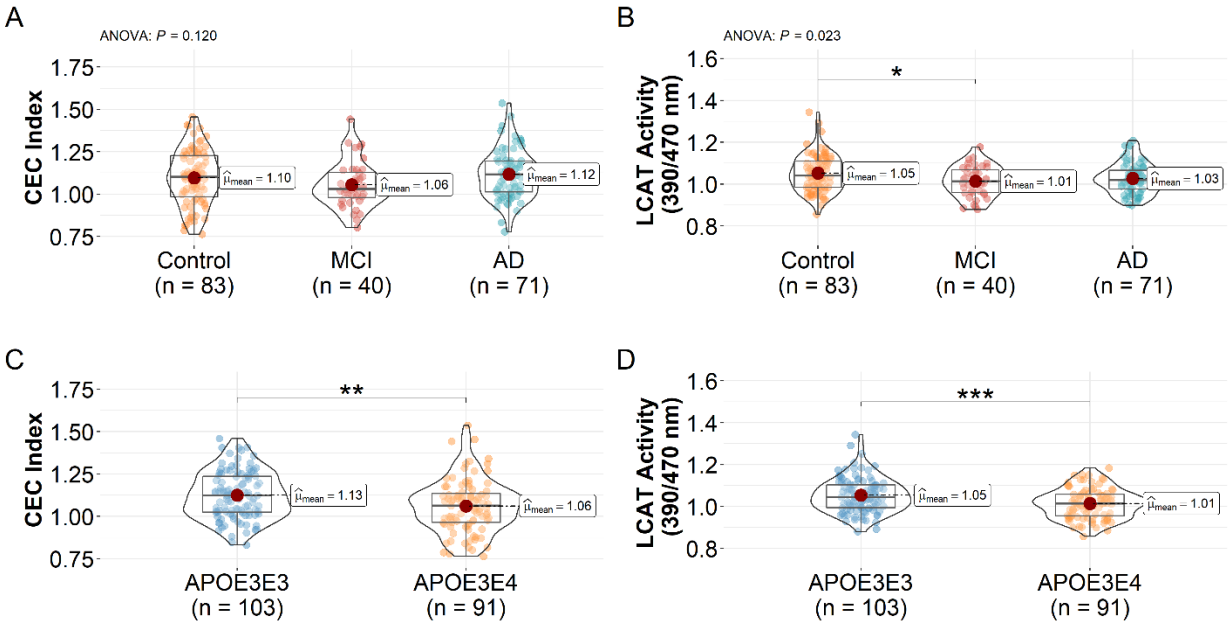


**Figure 1.** *APOE3E4* carriers have reduced HDL CEC index and LCAT activity. HDL (**A**) CEC index and (**B**) LCAT activity by diagnosis. One-way ANOVA followed by Tukey's multiple comparison tests was used. Decrease of (**C**) CEC index and (**D**) LCAT activity in the *APOE3E4* genotype using two-sample *t*-tests. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Abbreviations: AD = Alzheimer's disease dementia, CEC = cholesterol efflux capacity, HDL = high-density lipoproteins, LCAT = lecithin-cholesterol acyltransferase, MCI = mild cognitive impairment.

When participants were stratified by *APOE* genotype, one-way ANOVA showed a significant difference in HDL CEC index between at least two groups within *APOE3E3* carriers ($F(2, 100) = 3.26$, $p = 0.042$) and *APOE3E4* carriers ($F(2, 88) = 4.38$, $p = 0.015$). In the *APOE3E3* group, post hoc comparison showed HDL CEC index was lower in MCI patients relative to controls (1.07 ± 0.13 vs 1.16 ± 0.13, $P = 0.042$, Fig. 2A), but not in AD patients (1.11 ± 0.15) versus controls ($p = 0.246$). There was no significant difference in HDL CEC index between MCI and AD patients ($p = 0.551$). For *APOE3E4* carriers, AD patients displayed a higher HDL CEC index relative to controls (1.12 ± 0.14 vs 1.02 ± 0.16, $p = 0.016$, Fig. 2B), and a trend toward higher efflux in AD versus MCI patients (1.12 ± 0.14 vs 1.03 ± 0.14, $p = 0.114$). There was no significant difference in HDL CEC index between controls and MCI patients ($p = 0.964$).

In *APOE3E3* carriers, there was a significant difference in HDL LCAT activity between at least two groups by one-way ANOVA ($F(2, 100) = 7.00$, $p = 0.001$). Post hoc comparisons indicate HDL LCAT activity was reduced in MCI patients relative to controls (1.03 ± 0.08 vs 1.09 ± 0.09, $p = 0.012$, Fig. 2C) and reduced in AD patients versus controls (1.03 ± 0.07 vs 1.09 ± 0.09, $p = 0.004$), with no difference between MCI and AD patients ($p = 0.995$). When we compared across diagnosis groups within the *APOE3E4* carriers, there was no significant difference in LCAT activity by one-way ANOVA ($F(2, 88) = 0.91$, $P = 0.410$, Fig. 2D).

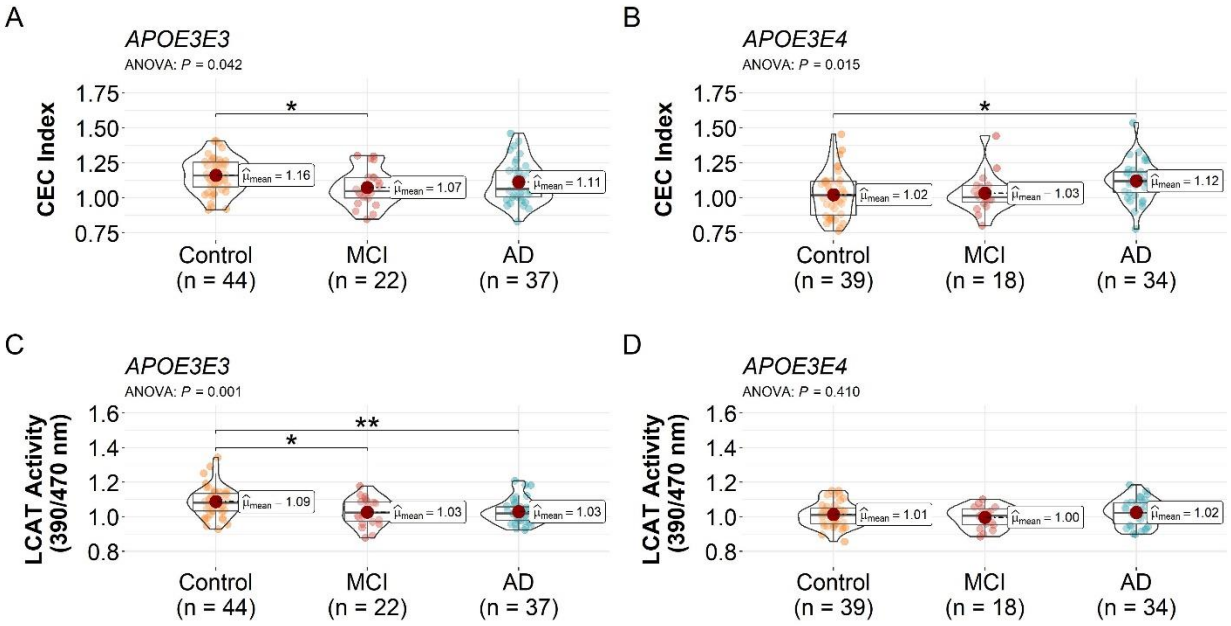


**Figure 2.** *APOE*-specific alteration in the HDL CEC index and LCAT activity amongst the control, MCI, and AD participants. The HDL CEC index within the (A) *APOE3E3* and (B) *APOE3E4* carriers by diagnosis. Decrease of LCAT activity in (C) *APOE3E3* patients but not (D) *APOE3E4* patients. One-way ANOVA followed by Tukey's multiple comparison tests was used. * $p < 0.05$ and ** $p < 0.01$. Abbreviations: AD = Alzheimer's disease dementia, CEC = cholesterol efflux capacity, HDL = highdensity lipoproteins, LCAT = lecithin-cholesterol acyltransferase, MCI = mild cognitive impairment.

HDL CEC index and LCAT activity were positively correlated in *APOE3E3* carriers (r = 0.43, 95% CI (0.26, 0.58), $p < 0.001$, Supplementary Figure S2A), whereas no correlation was observed in *APOE3E4* carriers (r = 0.16, 95% CI (-0.04, 0.37), $p = 0.136$). When participants were analyzed together regardless of diagnosis or *APOE* genotype, HDL LCAT activity and CEC index were positively correlated (r = 0.34, 95% CI (0.21, 0.47), $p < 0.001$, Supplementary Figure. S2B) and remained correlated after adjusting for *APOE* genotype (r = 0.31, 95% CI (0.17, 0.42], $p < 0.001$).

**HDL Particle Size Differences**

The mean ± SD HDL particle size is shown in Table 2. Without stratification for the *APOE* genotype, one-way ANOVA revealed a significant difference in at least two groups ($F(2, 190) = 9.70$, $p < 0.001$). Post hoc comparisons showed that the HDL particle sizes in the control group were significantly larger than the MCI (9.06 ± 0.69 nm vs. 8.67 ± 0.63 nm, $p = 0.008$) and the AD group (8.61 ± 0.68 nm, $p < 0.001$, Figure 3A). When stratified by the *APOE* genotype, there were no significant differences among the control, MCI, and AD groups in the *APOE3E3* carriers by one-way ANOVA (8.99 ± 0.85 nm, 8.79 ± 0.58 nm, and 8.72 ± 0.64 nm, respectively, $F(2, 99) = 1.40$, $p = 0.252$, Figure 3B). In the *APOE3E4* carriers, there was a significant difference in HDL particle sizes between at least two groups ($F(2, 88) = 13.04$, $p < 0.001$). Post hoc comparisons indicate that the HDL particle size in the control group was larger than the MCI (9.15 ± 0.42 nm vs. 8.53 ± 0.67 nm, $p = 0.001$) and the AD group (8.50 ± 0.70 nm, $p < 0.001$, Figure 3C). There was no significant difference in the HDL particle size between *APOE3E3* and *APOE3E4* carriers when the diagnosis was not taken into account (8.85 ± 0.73 nm vs. 8.78 ± 0.66 nm, $t(191) = 0.64$, $p = 0.520$).

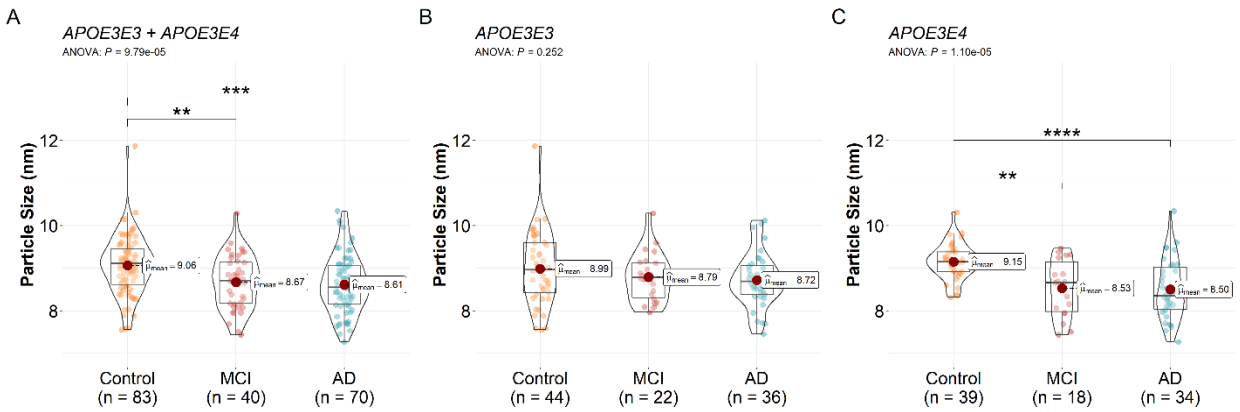


**Figure 3.** High-density lipoproteins particle size is reduced in MCI and AD patients. Mean particle diameter (nm) between (**A**) the controls, mild cognitive impairment (MCI), and Alzheimer's disease dementia (AD) patients and controls, MCI, and AD patients stratified by the (**B**) *APOE3E3* and (**C**) *APOE3E4* genotypes. One-way ANOVA followed by Tukey's multiple comparison tests was used. ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

**Correlation between HDL Function, Size, and Cognitive Measures**

Details of the participants' cognitive, functional, and imaging scores are summarized in Table 3. As expected, and by definition, the cognitive, functional, and imaging scores, were significantly differently among the diagnosis groups. The LCAT activity was positively associated with the verbal memory score (r = 0.17, 95% CI (0.01, 0.33), $p = 0.037$) and negatively associated with the CDR (r = -0.20, 95% CI (-0.37, -0.03), $p = 0.019$) and both the verbal memory score (r = 0.18, 95% CI (0.01, 0.33), $p = 0.033$) and CDR (r = -0.20, 95% CI (-0.35, -0.02), $p = 0.025$) remained statistically significant after adjusting for the *APOE* genotype (Table 4). None of the measured cognitive, functional, and imaging scores were significantly correlated with the CEC index. The particle size was positively correlated with the verbal memory score (r = 0.31, 95% CI (0.15, 0.45), $p < 0.001$) and executive function score (r = 0.21, 95% CI (0.04, 0.36), $p = 0.013$) and was negatively correlated with both the CDR (r = -0.31, 95% CI (-0.46, -0.14), $p < 0.001$) and WMH (r = -0.17, 95% (-0.33, 0.00), $p = 0.049$). The HDL particle size correlation with the verbal memory score (r = 0.31, 95% CI (0.15, 0.45), $p < 0.001$), executive function score (r = 0.21, 95% CI (0.05, 0.34), $p = 0.007$), and CDR (r = -0.31, 95% CI (-0.45, -0.14), $p < 0.001$) remained statistically significant after adjusting for the *APOE* genotype but not the correlation with WMH (r = -0.17, 95% (-0.32, 0.02), $p = 0.053$, Table 4). Regardless of the *APOE* genotype or diagnosis status, particle size was not correlated with the CEC index (r = -0.03, 95% CI (-0.17, 0.12), $p = 0.685$) or LCAT activity (r = 0.02, 95% CI (-0.12, 0.17), $p = 0.761$).

**Table 3.** Cognitive, functional, and MRI imaging score across diagnoses.

| | Variable | Group | | | Kruskal-Wallis test | | |
|---|---|---|---|---|---|---|---|
| | | **Control** $N = 83$ | **MCI** $N = 40$ | **AD** $N = 71$ | $\chi^2$ | *df* | ***P* value** |
| Cognitive[a] | Verbal memory score, median [25th, 75th], (*n*) | 0.24 [-0.39, 0.67] (69) | -0.97 [-1.37, -0.73] (35) | -1.41 [-1.90, -1.06] (40) | 87.72 | 2 | <0.001 |
| | Executive function score, median [25th, 75th], (*n*) | -0.03 [-0.27, 0.44] (68) | -0.37 [-0.60, -0.07] (35) | -0.96 [-1.52, -0.55] (44) | 54.29 | 2 | <0.001 |
| | Semantic memory score, median [25th, 75th], (*n*) | 0.50 [-0.08, 0.91] (68) | 0.23 [-0.12, 0.66] (35) | -0.50 [-1.01, 0.17] (43) | 32.95 | 2 | <0.001 |
| | Spatial score, Median [25th, 75th], (*n*) | 0.42 [-0.18, 0.83] (67) | 0.14 [-0.21, 0.48] (35) | -0.86 [-1.36, -0.05] (37) | 32.09 | 2 | <0.001 |
| Functional[a] | CDR sum of boxes, median [25th, 75th], (*n*) | 0.00 [0.00, 0.50] (59) | 3.00 [1.50, 3.50] (33) | 5.00 [3.38, 7.00] (40) | 94.79 | 2 | <0.001 |
| Imaging[a] | White matter hyperintensities volume, median [25th, 75th], (*n*) | 0.00 [0.00, 0.01] (65) | 0.01 [0.01, 0.02] (33) | 0.01 [0.00, 0.01] (40) | 18.27 | 2 | <0.001 |

[a]White matter hyperintensities volume was normalized to total intracranial volume.

**Table 4.** Correlation analysis across diagnoses adjusting for *APOE* genotype between HDL function and size with either cognitive, functional, and imaging scores.

| | Cognitive | | | | Functional | Imaging[a] |
|---|---|---|---|---|---|---|
| **Characteristic** | **Verbal memory score** | **Executive function score** | **Semantic memory score** | **Spatial score** | **CDR sum of boxes** | **White matter hyperintensities volume** |
| *N* | 144 | 147 | 146 | 139 | 132 | 138 |
| | | | **CEC Index** | | | |
| r | 0.018 | 0.013 | -0.100 | -0.010 | 0.035 | 0.123 |
| 95% CI | (-0.15, 0.19) | (-0.15, 0.18) | (-0.26, 0.07) | (-0.27, 0.07) | (-0.14, 0.21) | (-0.05, 0.29) |
| *P* value | 0.826 | 0.877 | 0.234 | 0.245 | 0.693 | 0.149 |
| Adjusted[b] r | 0.019 | 0.030 | -0.071 | -0.083 | 0.050 | 0.121 |
| Adjusted[b] 95% CI | (-0.14, 0.19) | (-0.15, 0.19) | (-0.23, 0.09) | (-0.24, 0.08) | (-0.12, 0.23) | (-0.04, 0.29) |
| Adjusted[b] *P* value | 0.824 | 0.720 | 0.397 | 0.334 | 0.571 | 0.160 |
| | | | **LCAT Activity (390/470 nm)** | | | |
| r | **0.174** | 0.134 | -0.125 | -0.095 | **-0.203** | -0.050 |
| 95% CI | **(0.01, 0.33)** | (-0.03, 0.29) | (-0.29, 0.04) | (-0.26, 0.08) | **(-0.37, -0.03)** | (-0.22, 0.12) |
| *P* value | **0.037** | 0.105 | 0.132 | 0.265 | **0.019** | 0.562 |
| Adjusted[b] r | **0.178** | 0.151 | -0.100 | -0.079 | **-0.196** | -0.057 |
| Adjusted[b] 95% CI | **(0.01, 0.33)** | (-0.02, 0.29) | (-0.27, 0.07) | (-0.26, 0.07) | **(-0.35, -0.02)** | (-0.21, 0.10) |
| Adjusted[b] *P* value | **0.033** | 0.069 | 0.231 | 0.358 | **0.025** | 0.512 |
| | | | **Particle Size (nm)** | | | |
| r | **0.329** | **0.226** | 0.080 | 0.058 | **-0.372** | -0.153 |
| 95% CI | **(0.17, 0.48)** | **(0.06, 0.38)** | (-0.09, 0.25) | (-0.12, 0.23) | **(-0.52, -0.21)** | (-0.32, 0.02) |
| *P* value | **<0.001** | **0.008** | 0.351 | 0.511 | **<0.001** | 0.08 |
| Adjusted[b] r | **0.329** | **0.226** | 0.083 | 0.060 | **-0.372** | -0.157 |
| Adjusted[b] 95% CI | **(0.17, 0.47)** | **(0.08, 0.37)** | (-0.07, 0.24) | (-0.12, 0.23) | **(-0.51, -0.22)** | (-0.32, 0.02) |
| Adjusted[b] *P* value | **<0.001** | **0.007** | 0.336 | 0.500 | **<0.001** | 0.074 |

Spearman's correlation coefficients (R) of CEC index and LCAT activity with cognitive and magnetic resonance imaging assessments across diagnoses (control, MCI, and AD).
[a]White matter hyperintensities volume were normalized to total intracranial volume.
[b]Adjusted for *APOE* genotype.

## DISCUSSION

It is becoming increasingly apparent that disruption in the cholesterol metabolism can influence Alzheimer's disease pathology [23], and HDL particles have a critical role in maintaining the metabolic and homeostatic processes of regulating cellular cholesterol concentrations. HDL particles in the circulation have been recognized for their potential involvement in Alzheimer's disease, and the current evidence has linked peripheral HDL to cerebrovascular health and Alzheimer's disease [6]. However, HDL-C alone is a poor indicator of the disease risk [24], whereas the functionality of the HDL particles may be a better indicator of disease risk, with CEC emerging as useful in identifying individuals at risk for cardiovascular disease across multiple cohorts [25–27]. Here, our study revealed *APOE* genotype-specific alterations in peripheral HDL CEC and LCAT activity, two critical functional metrics in the reverse cholesterol transport pathway.

Limited studies have measured HDL CEC and, to a much lesser degree, LCAT activity in Alzheimer's disease patients. Khalil et al. [10] found impairment in the peripheral HDL CEC and LCAT activity in 39 Alzheimer's disease patients compared to 20 healthy controls, but the *APOE* genotype was not assessed. Both Yassine et al. [28] and Marchi et al. [29] reported a reduction in cerebrospinal fluid-mediated CEC in Alzheimer's disease patients. Such findings suggest a global decrease in the ability to efflux and remove excess cholesterol in Alzheimer's disease. However, in our study of *APOE* genotyped controls, as well as MCI and AD patients, we did not find statistically significant alterations in peripheral HDL CEC in the MCI or AD patients without stratifying for the *APOE* genotype. To our knowledge, we report for the first time that both the CEC and LCAT activity are overall lower in *APOE3E4* carriers compared to *APOE3E3* carriers regardless of the AD diagnosis, highlighting the importance of the *APOE*

genotype in the ability to efflux and transport cholesterol. *APOE3E3* MCI and AD patients exhibited lower LCAT activity, and *APOE3E3* MCI patients had significantly lower HDL CEC than the controls, while the HDL particles of *APOE3E3* AD patients exhibited a wider variability, and thus, the decrease in HDL CEC did not reach statistical significance.

On the other hand, paradoxically, *APOE3E4* AD patients had higher HDL CEC than the controls. There are three plausible explanations for this observation. First, given the fact that *APOE4* carriers are known to be at a higher risk for the earlier onset of dementia [30,31], it is possible that the *APOE3E4* carriers have higher HDL CEC as a positive compensatory response to a poorer efflux capacity over the entire lifetime. Second, *APOE3E4* AD patients had a lower prevalence of metabolic disease (diabetes or hypercholesterolemia), which is known to be linked with poorer HDL function [32,33]. Third, we observed that *APOE3E4* AD patients have more small HDL particles, and because we standardized the quantity of HDL used in the efflux experiment based on the HDL protein content, it is likely that a higher number of total HDL particles was applied to the assay compared to controls. Thus, the observed increase in HDL CEC in *APOE3E4* AD patients may simply be an observation of more total particles being able to efflux more total cholesterol rather than an actual higher cholesterol efflux capacity per particle. Most experiments measuring HDL CEC do so with apolipoprotein-B (ApoB) precipitated plasma and therefore apply the HDL dose as a percentage by volume in the cell media [25,34]. With this approach, there would also be a higher number of smaller particles applied to the assay if there were more total particles in the plasma. In our study, we isolated and purified HDL particles from plasma and applied equal amounts of HDL as the total protein. The disadvantage of measuring CEC in ApoB precipitated plasma is that this is not a direct measurement of the CEC of the HDL particles per se but of everything that remains in the

plasma compartment after the ApoB-containing particles have been removed, thus the CEC measurement includes proteins and other components in plasma that could influence the ability to efflux cholesterol. The advantage of our approach is that we are measuring the CEC of the HDL particles themselves, rather than a combination of factors. One limitation of our approach for measuring the particle size by TEM is that this method does not capture all the particles in plasma and therefore does not enable quantification of the total HDL particle number. In the future, as methods become available to determine the particle count while simultaneously isolating HDL particles, the CEC assay can be dosed by equal particle number to compare the CEC on a per particle basis. Further studies are also needed to determine whether there are differences in the total particle numbers and particle size distributions in MCI and AD patients by the *APOE* genotype and to determine the HDL function and size in the other *APOE* genotypes (i.e., *APOE3E2*, *APOE4E4*, *APOE4E2*, and *APOE2E2*).

We did not find statistically significant differences in LCAT activity among the *APOE3E4* carriers. We observed overall lower LCAT activity in *APOE3E4* vs. *APOE3E3* carriers; thus, the lack of difference in LCAT activity among the *APOE3E4* carriers may be due to the fact that all individuals who are carriers of *APOE4* may already have diminished LCAT activity compared to non-*APOE4* carriers, and this reduction is not further enhanced in patients with dementia compared to the controls. Furthermore, the LCAT abundance in HDL was not measured. Thus, changes in the LCAT activity may be due to a lower abundance of LCAT protein in *APOE4* carriers, which could reflect overall lower LCAT activity.

The HDL size reflects the remodeling stage of HDL and is highly associated with the HDL composition and function. For example, it has been documented that certain HDL-associated proteins are either exclusively associated with or enriched in certain size-based HDL

subclasses [13,35]. Notably, ApoE tends to associate exclusively with larger HDL particles [13]. Smaller HDL particle sizes are also associated with metabolic syndrome and chronic kidney disease [36]. We found that the HDL particle size was significantly smaller in AD and MCI patients compared to the controls overall and in the *APOE3E4* carriers but not in the *APOE3E3* carriers. Future studies are needed to better understand the relationships between the overall metabolic status and HDL particle structure and function in the context of Alzheimer's disease and the *APOE* genotype.

Notably, we observed that HDL LCAT activity and particle size were positively correlated with the verbal memory score and negatively correlated with CDR, while the HDL particle size was additionally positively correlated with the executive function. Together, these findings suggest that disturbances in the HDL structure and function are associated with cognitive function and may be involved in Alzheimer's disease pathology. In elderly participants, verbal memory and expression strongly predict the progression from normal cognitive function to MCI before the appearance of clinical symptoms [37]. Subtle changes in the functional measurements in participants with higher CDR scores are associated with an increased risk of converting from normal cognition to MCI [38].

Although it is not yet clear whether and how peripheral HDL particles cross the blood–brain barrier, studies have found high correlations between plasma and cerebrospinal fluid concentrations of HDL-associated apoproteins that are known not to be expressed in the CNS [39,40]. Additionally, the peripheral overexpression of human ApoA-I improved the cognitive function, reduced neuroinflammation, and protected mice from cerebral amyloid angiopathy [9]. Future studies are needed to understand the mechanisms by which peripheral HDL particles and/or their components affect the brain.

The study's strengths include the measure of two critical functions of HDL in the reverse cholesterol transport pathway and the measurement of the HDL particle size by TEM in HDL from plasma samples obtained from a large multi-ethnic cohort of *APOE*genotyped, well characterized, clinically diagnosed, and/or pathologically confirmed elderly participants from the UC Davis ADRC biorepository. To our knowledge, we provide evidence for the first time of an *APOE* genotype-dependent difference in HDL functional capacity, size, and dementia and also an association between HDL functional capacity, size, and cognitive function. However, the use of cross-sectional samples limits our ability to detect changes in HDL function and size across the continuum of Alzheimer's disease and changes in these parameters over time. Furthermore, in this study, HDL CEC was measured using the J774A.1 cell line. Future studies measuring the CEC using neuronal cells, such as neuroblastoma (SK-N-SK) cells, microglia, and other brain relevant cell types, are needed. Another limitation is that, in this study, information on whether the participants were consuming antidiabetic agents or statins, which are commonly prescribed in AD patients and which may influence the HDL functional capacity and size, was not available, although the effects of statins on CEC remain uncertain, as reviewed elsewhere [41]. Future studies are needed to further investigate the underlying compositional and structural differences that explain the observed differences in the HDL functional capacity, particularly with regards to the *APOE* genotype. Many modifications of HDL particles, including oxidation, glycation, loss of functional components, and gain of deleterious components, have been found to influence the HDL functional capacity, even beyond the ability to efflux cholesterol [42–44].

## CONCLUSION

Our findings further support earlier observations that HDL particles are implicated in Alzheimer's disease pathology and highlight, for the first time, that there is an *APOE* genotype

dependent relationship that merits further study. Notably, our results suggest that the mechanisms of HDL deficiency in *APOE4* carriers vs. non-carriers are different, highlighting the need for further research on HDL metabolism and function in *APOE* genotyped individuals to elucidate the potential precision medicine-based approaches to improve the HDL functionality in individuals at risk for Alzheimer's disease tailored to their *APOE* genotype. It will be important to design future studies to determine which HDL compositional and structural changes underlie the loss of function that contributes to Alzheimer's disease pathology and how *APOE* genotype shapes these processes, so that potential therapeutic strategies to improve HDL functionality can be tested for their effectiveness in the prevention or treatment of cognitive decline.

**Supplementary Materials**

The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/biomedicines10071495/s1: Figure S1: Schematic working flow for particle selection and size analysis on sample TEM images. Figure S2: Spearman's correlation between HDL CEC and LCAT activity.

**Author Contributions**

Conceptualization, B.V.H., C.B.L., L.-W.J. and A.M.Z.; Data curation, D.J.H.; Formal analysis, B.V.H., J.Z., X.T. and A.M.Z.; Funding acquisition, C.B.L., L.-W.J. and A.M.Z.; Investigation, B.V.H., J.Z., J.K.A. and A.M.Z.; Methodology, B.V.H., J.Z., J.K.A., C.B.L., L.-W.J. and A.M.Z.; Project administration, A.M.Z.; Resources, C.B.L., L.-W.J., I.M., K.E., D.J.H., C.S.D., D.M.M., J.M.O., S.T.F. and A.M.Z.; Supervision, A.M.Z.; Visualization, B.V.H. and X.T.; Writing—original draft, B.V.H., J.Z. and A.M.Z.; and Writing—review and editing, B.V.H., J.Z., J.K.A., X.T., L.-W.J., D.J.H., C.S.D., D.M.M., J.M.O., S.T.F. and A.M.Z. All authors have read and agreed to the published version of the manuscript.

**FUNDING**

The project described was supported by the National Institute on Aging of the National Institute of Health (R01AG062240), the UC Davis Alzheimer's Disease Research Center (P30AG072972), and the USDA National Institute of Food and Agriculture Hatch Fund Project (CA-D-NUT-2242-H).

**INSTITUTIONAL REVIEW BOARD STATEMENT**

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the University of California, Davis (protocol code: 227656-9, date of approval: 27 March 2017).

**INFORMED CONSENT STATEMENT**

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

**DATA AVAILABILITY STATEMENT**

The data used in the study are available from the corresponding author upon request.

**ACKNOWLEDGMENTS**

We thank all of the co-investigators and collaborators involved in recruiting and evaluating participants at the UC Davis Alzheimer's Disease Research Center and for collecting and providing plasma samples for this study.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

## REFERENCES


1. Alzheimer's Association. 2021 Alzheimer's Disease Facts and Figures. *Alzheimers Dement.* **2021**, *17*, 327–406. [CrossRef]
2. Sperling, R.A.; Aisen, P.S.; Beckett, L.A.; Bennett, D.A.; Craft, S.; Fagan, A.M.; Iwatsubo, T.; Jack, C.R., Jr.; Kaye, J.; Montine, T.J.; et al. Toward Defining the Preclinical Stages of Alzheimer's Disease: Recommendations from the National Institute on Aging-Alzheimer's Association Workgroups on Diagnostic Guidelines for Alzheimer's Disease. *Alzheimers Dement. J. Alzheimers Assoc.* **2011**, *7*, 280–292. [CrossRef]
3. Zuin, M.; Cervellati, C.; Trentini, A.; Passaro, A.; Rosta, V.; Zimetti, F.; Zuliani, G. Association between Serum Concentrations of Apolipoprotein A-I (ApoA-I) and Alzheimer's Disease: Systematic Review and Meta-Analysis. *Diagnostics* **2021**, *11*, 984. [CrossRef]
4. Merched, A.; Xia, Y.; Visvikis, S.; Serot, J.M.; Siest, G. Decreased High-Density Lipoprotein Cholesterol and Serum Apolipoprotein AI Concentrations Are Highly Correlated with the Severity of Alzheimer's DiseaseI. *Neurobiol. Aging* **2000**, *21*, 27–30. [CrossRef]
5. Svensson, T.; Sawada, N.; Mimura, M.; Nozaki, S.; Shikimoto, R.; Tsugane, S. The Association between Midlife Serum HighDensity Lipoprotein and Mild Cognitive Impairment and Dementia after 19 Years of Follow-Up. *Transl. Psychiatry* **2019**, *9*, 26. [CrossRef]
6. Button, E.B.; Robert, J.; Caffrey, T.M.; Fan, J.; Zhao, W.; Wellington, C.L. HDL from an Alzheimer's Disease Perspective. *Curr. Opin. Lipidol.* **2019**, *30*, 224–234. [CrossRef]

7. Holzer, M.; Trieb, M.; Konya, V.; Wadsack, C.; Heinemann, A.; Marsche, G. Aging Affects High-Density Lipoprotein Composition and Function. *Biochim. Biophys. Acta* **2013**, *1831*, 1442–1448. [CrossRef]
8. Soran, H.; Hama, S.; Yadav, R.; Durrington, P.N. HDL Functionality. *Curr. Opin. Lipidol.* **2012**, *23*, 353–366. [CrossRef]
9. Lewis, T.L.; Cao, D.; Lu, H.; Mans, R.A.; Su, Y.R.; Jungbauer, L.; Linton, M.F.; Fazio, S.; LaDu, M.J.; Li, L. Overexpression of Human Apolipoprotein A-I Preserves Cognitive Function and Attenuates Neuroinflammation and Cerebral Amyloid Angiopathy in a Mouse Model of Alzheimer Disease. *J. Biol. Chem.* **2010**, *285*, 36958–36968. [CrossRef]
10. Khalil, A.; Berrougui, H.; Pawelec, G.; Fulop, T. Impairment of the ABCA1 and SR-BI-Mediated Cholesterol Efflux Pathways and HDL Anti-Inflammatory Activity in Alzheimer's Disease. *Mech. Ageing Dev.* **2012**, *133*, 20–29. [CrossRef]
11. Hinton, L.; Carter, K.; Reed, B.R.; Beckett, L.; Lara, E.; DeCarli, C.; Mungas, D. Recruitment of a Community-Based Cohort for Research on Diversity and Risk of Dementia. *Alzheimer Dis. Assoc. Disord.* **2010**, *24*, 234–241. [CrossRef] [PubMed]
12. Miller, J.W.; Harvey, D.J.; Beckett, L.A.; Green, R.; Farias, S.T.; Reed, B.R.; Olichney, J.M.; Mungas, D.M.; DeCarli, C. Vitamin D Status and Rates of Cognitive Decline in a Multiethnic Cohort of Older Adults. *JAMA Neurol.* **2015**, *72*, 1295–1303. [CrossRef]
13. Zheng, J.J.; Agus, J.K.; Hong, B.V.; Tang, X.; Rhodes, C.H.; Houts, H.E.; Zhu, C.; Kang, J.W.; Wong, M.; Xie, Y.; et al. Isolation of HDL by Sequential Flotation Ultracentrifugation Followed by Size Exclusion Chromatography Reveals Size-Based

Enrichment of HDL-Associated Proteins. *Sci. Rep.* **2021**, *11*, 16086. [CrossRef] [PubMed]

14. Hong, B.V.; Zhu, C.; Wong, M.; Sacchi, R.; Rhodes, C.H.; Kang, J.W.; Arnold, C.D.; Adu-Afarwuah, S.; Lartey, A.; Oaks, B.M.; et al. Lipid-Based Nutrient Supplementation Increases High-Density Lipoprotein (HDL) Cholesterol Efflux Capacity and Is Associated with Changes in the HDL Glycoproteome in Children. *ACS Omega* **2021**, *6*, 32022–32031. [CrossRef] [PubMed]
15. Minami-Takano, A.; Iwata, H.; Miyosawa, K.; Shiozawa, T.; Hayashi, H.; Funamizu, T.; Ishii, K.; Nozaki, Y.; Tabuchi, H.; Sekita, G.; et al. The Association between Impairment of HDL Cholesterol Efflux Capacity and Atrial Remodeling in Atrial Fibrillation. *Sci. Rep.* **2021**, *11*, 3547. [CrossRef] [PubMed]
16. Zhang, L.; Tong, H.; Garewal, M.; Ren, G. Optimized Negative-Staining Electron Microscopy for Lipoprotein Studies. *Biochim. Biophys. Acta* **2013**, *1830*, 2150–2159. [CrossRef]
17. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; et al. Fiji: An Open-Source Platform for Biological-Image Analysis. *Nat. Methods* **2012**, *9*, 676–682. [CrossRef]
18. Mungas, D.; Reed, B.R.; Marshall, S.C.; González, H.M. Development of Psychometrically Matched English and Spanish Language Neuropsychological Tests for Older Persons. *Neuropsychology* **2000**, *14*, 209–223. [CrossRef]
19. Mungas, D.; Reed, B.R.; Crane, P.K.; Haan, M.N.; González, H. Spanish and English Neuropsychological Assessment Scales (SENAS): Further Development and Psychometric Characteristics. *Psychol. Assess.* **2004**, *16*, 347–359. [CrossRef]

20. Daly, E.; Zaitchik, D.; Copeland, M.; Schmahmann, J.; Gunther, J.; Albert, M. Predicting Conversion to Alzheimer Disease Using Standardized Clinical Information. *Arch. Neurol.* **2000**, *57*, 675–680. [CrossRef]

21. DeCarli, C.; Maisog, J.; Murphy, D.G.; Teichberg, D.; Rapoport, S.I.; Horwitz, B. Method for Quantification of Brain, Ventricular, and Subarachnoid CSF Volumes from MR Images. *J. Comput. Assist. Tomogr.* **1992**, *16*, 274–284. [CrossRef] [PubMed]

22. DeCarli, C.; Miller, B.L.; Swan, G.E.; Reed, T.; Wolf, P.A.; Garner, J.; Jack, L.; Carmelli, D. Predictors of Brain Morphology for the Men of the NHLBI Twin Study. *Stroke* **1999**, *30*, 529–536. [CrossRef] [PubMed]

23. Liu, Y.; Thalamuthu, A.; Mather, K.A.; Crawford, J.; Ulanova, M.; Wong, M.W.K.; Pickford, R.; Sachdev, P.S.; Braidy, N. Plasma Lipidome Is Dysregulated in Alzheimer's Disease and Is Associated with Disease Risk Genes. *Transl. Psychiatry* **2021**, *11*, 344. [CrossRef]

24. Rader, D.J.; Tall, A.R. Is It Time to Revise the HDL Cholesterol Hypothesis? *Nat. Med.* **2012**, *18*, 1344–1346. [CrossRef] [PubMed]

25. Rohatgi, A.; Khera, A.; Berry, J.D.; Givens, E.G.; Ayers, C.R.; Wedin, K.E.; Neeland, I.J.; Yuhanna, I.S.; Rader, D.R.; de Lemos, J.A.; et al. HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events. *N. Engl. J. Med.* **2014**, *371*, 2383–2393. [CrossRef] [PubMed]

26. Shea, S.; Stein, J.H.; Jorgensen, N.W.; McClelland, R.L.; Tascau, L.; Shrager, S.; Heinecke, J.W.; Yvan-Charvet, L.; Tall, A.R. Cholesterol Mass Efflux Capacity, Incident Cardiovascular Disease, and Progression of Carotid Plaque. *Arterioscler. Thromb. Vasc. Biol.* **2019**, *39*, 89–96. [CrossRef]

27. Khera, A.V.; Demler, O.V.; Adelman, S.J.; Collins, H.L.; Glynn, R.J.; Ridker, P.M.; Rader, D.J.; Mora, S. Cholesterol Efflux Capacity, High-Density Lipoprotein Particle Number, and Incident Cardiovascular Events: An Analysis From the JUPITER Trial (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin). *Circulation* **2017**, *135*, 2494–2504. [CrossRef]

28. Yassine, H.N.; Feng, Q.; Chiang, J.; Petrosspour, L.M.; Fonteh, A.N.; Chui, H.C.; Harrington, M.G. ABCA1-Mediated Cholesterol Efflux Capacity to Cerebrospinal Fluid Is Reduced in Patients With Mild Cognitive Impairment and Alzheimer's Disease. *J. Am. Heart Assoc.* **2016**, *5*, e002886. [CrossRef]

29. Marchi, C.; Adorni, M.P.; Caffarra, P.; Ronda, N.; Spallazzi, M.; Barocco, F.; Galimberti, D.; Bernini, F.; Zimetti, F. ABCA1- and ABCG1-Mediated Cholesterol Efflux Capacity of Cerebrospinal Fluid Is Impaired in Alzheimer's Disease. *J. Lipid Res.* **2019**, *60*, 1449–1456. [CrossRef]

30. Elias-Sonnenschein, L.S.; Viechtbauer, W.; Ramakers, I.H.G.B.; Verhey, F.R.J.; Visser, P.J. Predictive Value of APOE-E4 Allele for Progression from MCI to AD-Type Dementia: A Meta-Analysis. *J. Neurol. Neurosurg. Psychiatry* **2011**, *82*, 1149 1156. [CrossRef]

31. Blacker, D.; Haines, J.L.; Rodes, L.; Terwedow, H.; Go, R.C.; Harrell, L.E.; Perry, R.T.; Bassett, S.S.; Chase, G.; Meyers, D.; et al. ApoE-4 and Age at Onset of Alzheimer's Disease: The NIMH Genetics Initiative. *Neurology* **1997**, *48*, 139–147. [CrossRef] [PubMed]

32. Femlak, M.; Gluba-Brzózka, A.; Ciałkowska-Rysz, A.; Rysz, J. The Role and Function of HDL in Patients with Diabetes Mellitus and the Related Cardiovascular Risk. *Lipids Health Dis.* **2017**, *16*, 207. [CrossRef] [PubMed]

33. Escolà-Gil, J.C.; Rotllan, N.; Julve, J.; Blanco-Vaca, F. Reverse Cholesterol Transport Dysfunction Is a Feature of Familial Hypercholesterolemia. *Curr. Atheroscler. Rep.* **2021**, *23*, 29. [CrossRef] [PubMed]

34. Khera, A.V.; Cuchel, M.; de la Llera-Moya, M.; Rodrigues, A.; Burke, M.F.; Jafri, K.; French, B.C.; Phillips, J.A.; Mucksavage, M.L.; Wilensky, R.L.; et al. Cholesterol Efflux Capacity, High-Density Lipoprotein Function, and Atherosclerosis. *N. Engl. J. Med.* **2011**, *364*, 127–135. [CrossRef]

35. Davidson, W.S.; Silva, R.A.G.D.; Chantepie, S.; Lagor, W.R.; Chapman, M.J.; Kontush, A. Proteomic Analysis of Defined HDL Subpopulations Reveals Particle-Specific Protein Clusters: Relevance to Antioxidative Function. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 870–876. [CrossRef]

36. Kaysen, G.A. Metabolic Syndrome and Renal Failure: Similarities and Differences. *Panminerva Med.* **2006**, *48*, 151–164.

37. Oulhaj, A.; Wilcock, G.K.; Smith, A.D.; de Jager, C.A. Predicting the Time of Conversion to MCI in the Elderly: Role of Verbal Expression and Learning. *Neurology* **2009**, *73*, 1436–1442. [CrossRef]

38. Chen, Y.; Denny, K.G.; Harvey, D.; Farias, S.T.; Mungas, D.; DeCarli, C.; Beckett, L. Progression from Normal Cognition to Mild Cognitive Impairment in a Diverse Clinic-Based and Community-Based Elderly Cohort. *Alzheimers Dement. J. Alzheimers Assoc.* **2017**, *13*, 399–405. [CrossRef]

39. Martinez, A.E.; Weissberger, G.; Kuklenyik, Z.; He, X.; Meuret, C.; Parekh, T.; Rees, J.C.; Parks, B.A.; Gardner, M.S.; King, S.M.; et al. The Small HDL Particle Hypothesis of Alzheimer's Disease. *Alzheimers Dement.* **2022**. [CrossRef]
40. Van Valkenburgh, J.; Meuret, C.; Martinez, A.E.; Kodancha, V.; Solomon, V.; Chen, K.; Yassine, H.N. Understanding the Exchange of Systemic HDL Particles Into the Brain and Vascular Cells Has Diagnostic and Therapeutic Implications for Neurodegenerative Diseases. *Front. Physiol.* **2021**, *12*, 700847. [CrossRef]
41. Yamamoto, S.; Narita, I.; Kotani, K. The Macrophage and Its Related Cholesterol Efflux as a HDL Function Index in Atherosclerosis. *Clin. Chim. Acta* **2016**, *457*, 117–122. [CrossRef] [PubMed]
42. Shao, B.; Cavigiolio, G.; Brot, N.; Oda, M.N.; Heinecke, J.W. Methionine Oxidation Impairs Reverse Cholesterol Transport by Apolipoprotein A-I. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 12224–12229. [CrossRef] [PubMed]
43. Hedrick, C.C.; Thorpe, S.R.; Fu, M.X.; Harper, C.M.; Yoo, J.; Kim, S.M.; Wong, H.; Peters, A.L. Glycation Impairs High-Density Lipoprotein Function. *Diabetologia* **2000**, *43*, 312–320. [CrossRef] [PubMed]
44. Kashyap, S.R.; Osme, A.; Ilchenko, S.; Golizeh, M.; Lee, K.; Wang, S.; Bena, J.; Previs, S.F.; Smith, J.D.; Kasumov, T. Glycation Reduces the Stability of ApoAI and Increases HDL Dysfunction in Diet-Controlled Type 2 Diabetes. *J. Clin. Endocrinol. Metab.* **2018**, *103*, 388–396. [CrossRef]