



Dietary fat and carbohydrate affect the metabolism of protein-based high-density lipoprotein subspecies

Frank M. Sacks^{a,b} and Allison B. Andraski^a

Purpose of review

Dietary fat compared to carbohydrate increases the plasma concentration of high-density lipoprotein (HDL)-cholesterol. However, neither the mechanism nor its connection to cardiovascular disease is known.

Recent findings

Protein-based subspecies of HDL, especially those containing apolipoprotein E (apoE) or apolipoprotein C3 (apoC3), offer a glimpse of a vast metabolic system related to atherogenicity, coronary heart disease (CHD) and other diseases. ApoE stimulates several processes that define reverse cholesterol transport through HDL, specifically secretion of active HDL subspecies, cholesterol efflux to HDL from macrophages involved in atherogenesis, size enlargement of HDL with cholesterol ester, and rapid clearance from the circulation. Dietary unsaturated fat stimulates the flux of HDL that contains apoE through these protective pathways. Effective reverse cholesterol transport may lessen atherogenesis and prevent disease. In contrast, apoC3 abrogates the benefit of apoE on reverse cholesterol transport, which may account for the association of HDL that contains apoC3 with dyslipidemia, obesity and CHD.

Summary

Dietary unsaturated fat and carbohydrate affect the metabolism of protein-defined HDL subspecies containing apoE or apoC3 accelerating or retarding reverse cholesterol transport, thus demonstrating new mechanisms that may link diet to HDL and to CHD.

Keywords

apolipoprotein C3, apolipoprotein E, diet, high-density lipoprotein, metabolism

INTRODUCTION

There are two starting points for this review. One is the clinical effect on high-density lipoprotein (HDL)-cholesterol of dietary fat and carbohydrate. The other is the multiplicity of proteins on HDL, organized into subspecies. Do the subspecies help to interpret the dietary effects? Do metabolic studies with subspecies lead to improved understanding of clinical relevance of the subspecies HDL system?

EFFECTS OF DIETARY FAT ON HIGH-DENSITY LIPOPROTEIN CONCENTRATION AND APOA1 METABOLISM

Most studies of diet and HDL have focused on the effect of dietary macronutrients – carbohydrate, fat, and protein – on HDL-cholesterol levels and the metabolism of total plasma apoA1.

Dietary fat when it replaces carbohydrate increases HDL-cholesterol

Saturated, monounsaturated, and *n*-6 polyunsaturated fats have this effect (Fig. 1), saturated more so

^aDepartment of Nutrition, Harvard T.H. Chan School of Public Health and ^bChanning Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Correspondence to Frank M. Sacks, MD, Department of Nutrition, Harvard T.H. Chan School of Public Health, Building 1, Room 203, 665 Huntington Ave, Boston, MA 02115, USA. Tel: +617 432 1420; fax: +617-432-3101; e-mail: fsacks@hsph.harvard.edu

Curr Opin Lipidol 2022, 33:1–15

DOI:10.1097/MOL.0000000000000809

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KEY POINTS

- Dietary fat increases the concentration of HDL-cholesterol, but the underlying mechanisms have not been well understood nor the relations to atherogenesis and CHD.
- HDL is composed of protein-defined subspecies that vary in function, diet responsiveness, and relation to CHD.
- HDL that contains apoC3 is associated with future risk of CHD, whereas HDL that contains apoE is associated with lower risk.
- Studies of HDL subspecies metabolism showed that HDL that contains apoE is especially active in pathways in reverse cholesterol transport, and these pathways are enhanced by unsaturated fat, whereas HDL that contains apoC3 abrogates the beneficial effect of HDL that contains apoE.
- Unsaturated fat, when replacing carbohydrate, decreased the catabolic rate of apoE and the secretion rate of apoC3, on specific HDL sizes, leading to more apoE and less apoC3 - a state more favorable to reverse cholesterol transport and protection against CHD.

than monounsaturated or polyunsaturated fat [1,2]. Carbohydrate lowers HDL-cholesterol by about 5 mg/dL per 10 percentage points of total daily calories exchanged with fat [2]. The HDL-cholesterol lowering effect of carbohydrate occurs whether the carbohydrate is refined or whole grain, or low or high

glycemic index [3]. Because fat and carbohydrate are macronutrients, supplying energy, another reference point is needed to determine direct effects of increasing dietary fat. Using protein as the reference, dietary fat replacing protein raises HDL-cholesterol even more than fat replacing carbohydrate [4]. Therefore, it is fair to conclude that dietary fat directly increases HDL-cholesterol regardless of whether it replaces carbohydrate or protein. However, the metabolic basis is not well established.

Dietary fat increases the synthetic rate and decreases the fractional catabolic rate of apoA1

Historically, kinetic studies of HDL metabolism exogenously labeled apoA1 with radio-iodine and modeled the disappearance (decay) curves and the apoA1 pool size to derive a fractional catabolic rate (FCR) and secretion rate of the entire HDL apoA1 plasma pool. Another technique endogenously labeled apoA1 in vivo by infusing nonradioactive (stable) isotopes of amino acids such as tri-deuterated leucine. Up to now, there have been 10 studies that we could find on the metabolism of HDL apoA1 that compare fat and carbohydrate, or different types of fat (Tables 1 and 2) [5–14]. Seven of them compared a high saturated fat diet with a high carbohydrate diet (Table 1) [5–11], and 3 compared high saturated with high cis or trans unsaturated fat (Table 2) [12–14].

A high-fat diet compared to a high carbohydrate liquid diet decreased FCR of apoA1 in 4 healthy

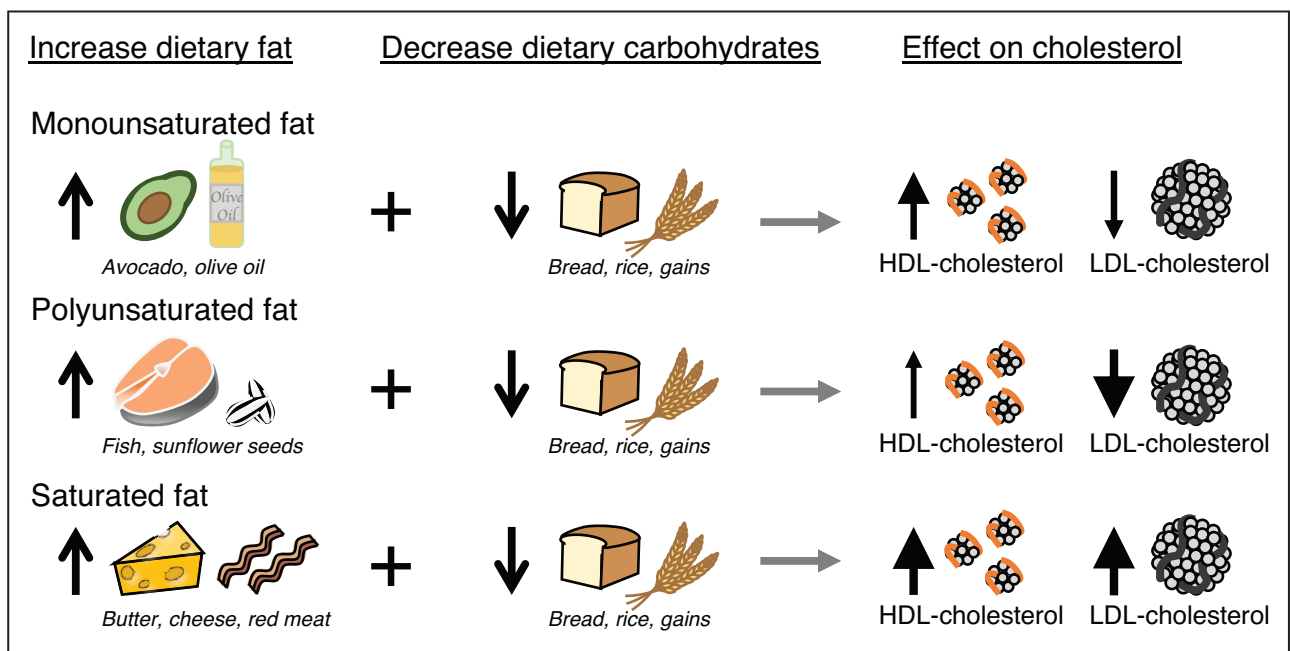


FIGURE 1. Effects of dietary fat and carbohydrate on plasma cholesterol levels. LDL, low-density lipoprotein.

Table 1. Previous studies overviewing the effect of dietary fat and carbohydrate on HDL apoA1 metabolism

Publication	Label	Participant criteria	Study design	Diet regimen	Effects on apoA1		
					Pool size	FCR	Secretion rate
Blum <i>et al.</i> J Clin Invest. 1977	Exogenous 125-I-HDL	8 normal, healthy participants (3 males, 5 females) ages 18 to 26 years	Baseline diet for 2 weeks ($n=8$), followed by high carb diet for 2 weeks ($n=4$)	Baseline/high-fat diet: 40% fat, 40% carbs High carb diet: <5g fat, 80% carbs	No difference between high carb and high fat	Increases on high carb compared to high fat	No difference between high carb and high fat
Nestle <i>et al.</i> Metabolism. 1981	Exogenous 125-I-HDL	7 male vegetarians and 6 male omnivores matched for age and weight	Consumed vegetarian or omnivorous diet for 1 month	Vegetarian diet / high carb: 26% fat (poly/sat ratio 1.6), 60% carb, 14% protein, <100 mg cholesterol Omnivorous diet / high fat: 36–43% fat (poly/sat ratio 0.2–0.4), 42–49% carbs, 15% protein, 500–700 mg cholesterol	Tends to be lower in vegetarians (high carb) compared to omnivores (high fat)	Higher in vegetarians (high carb) compared to omnivores (high fat)	No difference between vegetarians (high carb) and omnivores (high fat)
Brinton <i>et al.</i> J Clin Invest. 1990	Exogenous 125-I-apoA1	13 healthy participants (5 males, 8 females) with fasting HDL-C >30mg/dl	The two diets were fed in variable order, participants consumed both diets for 4 weeks each	High saturated fat / low carb diet: 41.9% fat (23.6% sat, 16.5% mono, 1.8% poly), 42.6% carb, 15.5% protein, 215 mg cholesterol Low saturated fat / high carb diet: 8.6% fat (2.1% sat, 4.2% mono, 2.3% poly), 75.5% carb, 16% protein, 40 mg cholesterol	Decreases when high carb replaces high sat fat	Increases when high carb replaces high sat fat	Decreases when high carb replaces high sat fat
Velez-Carrasco <i>et al.</i> Arterioscler Thromb Vasc Biol. 1999	Endogenous D3-Leucine	21 normal, healthy volunteers (14 males, 7 postmenopausal females), age range 41 to 74, mean BMI 27 ± 4 kg/m ²	6 weeks on baseline/high sat fat diet ($n=21$), followed by 24 weeks ($n=11$) or 6 weeks ($n=10$) on high carb diet	Baseline/high saturated fat diet: 36% fat (14% sat, 15% mono, 7% poly), 49% carbs, 15% protein, 150 mg cholesterol NCEP Step 2 diet / high carb diet: 25% fat (4% sat, 11% mono, 10% poly), 60% carb, 15% protein, 45 mg cholesterol	Decreases when carb replaces sat fat	No difference when carb replaces sat fat	Decreases when carb replaces sat fat
Desroches <i>et al.</i> J Lipid Res. 2004	Endogenous D3-Leucine	18 men with relatively normal lipid profiles and moderate obesity (BMI > 27 kg/m ² for two-thirds of participants)	18 men randomly assigned to either the low fat/high carb ($n=10$) or high mono ($n=8$) diet. Each diet was consumed for 6–7 weeks.	Low fat / high carbohydrate diet: 25.8% fat (6% sat, 13.3% mono, 5.1% poly), 58.3% carb, 15.9% protein, 105.8 mg cholesterol High mono diet: 40.1% fat (8.2% sat, 22.5% mono, 7.6% poly), 44.7% carbs, 15.2% protein, 110.1 mg cholesterol	No difference between high mono and low fat/high carb diets	Increases on the high mono diet compared to the low fat/high carb diets	Increases on the high mono diet compared to the low fat/high carb diets

Table 1 (Continued)

Publication	Label	Participant criteria	Study design	Diet regimen	Effects on apoA1		
					Pool size	FCR	Secretion rate
Ooi <i>et al.</i> J Lipid Res. 2012	Endogenous D3-Leucine	20 participants (7 males, 13 postmenopausal females), age >40 years	Consumed baseline diet for 6 weeks (n = 20), randomized to high fish (n = 10) or low fish (n = 10) diet for 24 weeks	Baseline / high sat, low carb diet: 35.4% fat (14.1% sat, 14.5% mono, 6.9% poly), 49.4% carbs, 15% protein High fish / low sat, high carb diet: 24.6% fat (4.5% sat, 11.6% mono, 10.3% poly - 1.23 g/day EPA and DHA), 56.1% carbs, 17.2% protein Low fish / low sat, high carb diet: 25.5% fat (4% sat, 10.8% mono, 10.5% poly - 0.27g/day EPA and DHA), 58.2% carbs, 16.3% protein	Decreases on high-fat and low-fat (low sat, high carb) diets compared to baseline (high sat, low carb); No difference between high fish and low fish diets	No difference between high fish sat/low fish (low sat, high carb) diets and baseline (high sat, low carb); No difference between high fish and low fish diets	Decreases on high fish and low fish (low sat fat, high carb) diets compared to baseline (high sat, low carb); No difference between high fish and low fish diets
Labonte <i>et al.</i> Br J Nutr. 2013	Endogenous D3-Leucine	16 participants (12 males, 4 postmenopausal females) with dyslipidemia (LDL-C >74 mg/dl)	Consumed baseline diet for 4 weeks (n = 16), randomized to high mono (n = 8) or low mono (n = 8) diet for 4 weeks	Baseline diet: 27.5% fat (4.6% sat, 10.6% mono, 9.9% poly), 52% carb, 20.3% protein. High mono / low carb diet: 45.5% fat (6.7% sat, 25.8% mono, 11.7% poly), 33.8% carb, 20.8% protein Low mono / high carb diet: 29.1% fat (4.6% sat, 12.9% mono, 10.9% poly), 49.4% carb, 21.5% protein	Increases with high mono diet compared to low mono diet; No difference between high mono and baseline; Tends to decrease on low mono compared to baseline (P = 0.055)	No difference between high and low mono diets; Decreases with high mono diet compared to baseline	no difference with high mono diet compared to low mono diet; No difference between high/low mono diets and baseline

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2. Previous studies overviewing the effect of different dietary fats on HDL apoA1 metabolism

Publication	Label	Participant criteria	Study design	Diet regimen	Effects on apoA1		
					Pool size	FCR	Secretion rate
Shepherd. J Clin Invest. 1978	Exogenous 125-I-apoA1	4 normal, healthy males	4 participants consumed a high saturated fat diet followed by a high polyunsaturated fat diet for 5 weeks each	High saturated fat diet: 40% fat (poly/saturate ratio 0.25), 40% carb, 20% protein, 400 mg cholesterol High polyunsaturated fat diet: 40% fat (poly/saturated ratio 4.0), 40% carb, 20% protein, 400 mg cholesterol	Decreases when poly replaces saturated fat	No difference when poly replaces saturated fat	Decreases when poly replaces saturated fat
Matthan <i>et al.</i> Arterioscler Thromb Vasc Biol. 2004	Endogenous D3-Leucine	8 postmenopausal women with high cholesterol (LDL-C >130 mg/dl)	Participants consumed each of the 3 diets in random order for 5 weeks each	High saturated fat (butter) diet: 29.1% fat (16.7% sat, 8.1% mono, 2.4% poly, 1.3% trans), 54% carbs, 16.9% protein, 121 mg cholesterol High hydrogenated fat (margarine) diet: 29.7% fat (8.5% sat, 8.5% mono, 6.3% poly, 6.7% trans), 53.5% carbs, 16.7% protein, 67 mg cholesterol High polyunsaturated fat (soybean oil) diet: 28.5% fat (7.3% sat, 8.1% mono, 12.5% poly, 0.6% trans), 55.8% carb, 15.7% protein, 66 mg cholesterol	Decreases when hydrogenated fat replaces saturated fat; No difference when poly replaces saturated or hydrogenated fats	Increases when hydrogenated fat replaces saturated fat; No difference when poly replaces saturated and hydrogenated fats	No difference when hydrogenated fat replaces saturated fat; No difference when poly replaces saturated or hydrogenated fats
Richard <i>et al.</i> Nutr J. 2013	Endogenous D3-Leucine	26 men, 18 to 65 years, with metabolic syndrome	Baseline diet for 5 weeks (n = 26), followed by Mediterranean diet for 5 weeks (n = 26)	Baseline / high sat fat diet: 34% fat (13% sat, 13.2% mono, 5.2% poly, 2% trans), 48.4% carbs, 17% protein, 414 mg cholesterol Mediterranean / high mono diet: 32% fat (6.7% sat, 18.1% mono, 4.7% poly, 0.3% trans), 50% carbs, 17% protein, 367 mg cholesterol	Decreases on Med (high mono) diet compared to baseline (high sat)	No difference between Med (high mono) diet and baseline (high sat)	Tends to decrease on Med (high mono) diet compared to baseline (high sat) (P = 0.07)

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

adults (Table 1) [5]. A study of 7 vegetarians eating a low-fat diet with 6 omnivores eating a high saturated fat diet found that the omnivores had higher HDL-cholesterol levels and lower apoA1 FCR [6]. Dietary saturated fat compared to carbohydrate increased HDL apoA1 levels by increasing the secretion rate and reducing the FCR in 13 healthy adults [7]. This study combined the data of all participants and diets in correlation analysis that suggested that change in FCR was a more important parameter than change in secretion rate to alter the plasma apoA1 level. Another study in 21 healthy adults allocated to a typical high saturated fat diet or a low-fat, high carbohydrate diet, found that increase in the apoA1 secretion rate rather than decrease in FCR accounted for the increase in apoA1 HDL pool size of higher dietary fat [8]. In 18 men with moderate obesity assigned to a high monounsaturated fat diet or a low-fat, high carbohydrate fat diet, both FCR and secretion rate were higher on the monounsaturated fat than carbohydrate diet [9]. In this study, HDL apoA1 pool size was not affected by the diet difference. Another study in 20 healthy adults measured HDL kinetics on a baseline high saturated fat diet after which they were allocated to low fat high carbohydrate, either low or high in fish. ApoA1 pool size decreased with high carbohydrate, irrespective of high or low fish intake. This decrease was accounted for by a decrease in apoA1 secretion. The FCR was not affected [11]. A study of 16 dyslipidemic adults assigned to either a high or low monounsaturated fat diet in which carbohydrate replaced monounsaturated fat reported that the HDL apoA1 pool size was increased by the high monounsaturated fat diet. Nonetheless, no significant changes were reported in FCR or secretion rate comparing high to low monounsaturated fat, although the FCR was significantly decreased from the baseline diet (higher in carbohydrate) to high monounsaturated fat [10].

All told, 4 of 7 studies reported that high saturated or monounsaturated fat compared to carbohydrate decreased the FCR of HDL apoA1 [5–7,10], and 4 studies found increases in the secretion rate [7–9,11] (Table 1). In one study, both FCR and secretion rate increased on high monounsaturated fat compared to carbohydrate [9]. Interesting but uncertain in importance is that the 3 of the 4 studies that reported a decrease in FCR with high fat used radio-labeling of HDL proteins or apoA1 [5–7], whereas the 4 studies that reported no change or paradoxically an increased FCR with high fat used endogenous labeling of apoA1 with infusion of a deuterated amino acid [8–11].

As mentioned, a usual dietary amount of *n*–6 polyunsaturated fat replacing saturated fat mildly

lowers HDL-cholesterol (Fig. 1) [1,2]. Researchers stressed this system by feeding a large amount of *n*–6 polyunsaturated fat or saturated fat to 4 healthy men (Table 2). The results were that polyunsaturated fat lowered HDL apoA1 by 21% by decreasing the synthetic rate and not affecting the FCR [12]. Another study compared saturated fat, polyunsaturated fat from soybean oil, and hydrogenated vegetable oil. The hydrogenated oil compared to saturated fat decreased plasma apoA1 by increasing the FCR whereas not affecting the secretion rate of apoA1 [13]. In this study, natural, unhydrogenated polyunsaturated fat did not affect apoA1 parameters in comparison to the other fats. Finally, a trial comparing saturated with monounsaturated fat in men with metabolic syndrome reported that the monounsaturated fat diet decreased the pool size and tended to decrease secretion rate of HDL apoA1 [14].

Altogether, these first-generation metabolism studies showed that changes in either catabolic or secretion rate underlie established dietary effects on plasma HDL-cholesterol levels (Tables 1 and 2). However, it is unclear how to interpret changes in FCR or secretion rate of HDL in terms of atheroprotective or atherogenic effects.

The diet and high-density lipoprotein paradox, and how to interpret changes in catabolic and secretion rates?

Dietary saturated fat is well established as a cause of atherosclerosis and coronary heart disease (CHD). The one established mechanism is an increase in low-density lipoprotein (LDL)-cholesterol, an established cause of CHD (Fig. 1), although there may be additional mechanisms [1].

HDL, the particle, is thought to protect against atherosclerosis by activating cholesterol transporters inside cells involved in atherosclerosis and transferring the cholesterol to the liver where it may be excreted into the biliary system, a process known as reverse cholesterol transport [15–17]. Hypothetically, a high cholesterol concentration of HDL may indicate more cholesterol transport from macrophages to HDL, a healthy metabolism, but it also could mean a lower rate of transfer of cholesterol out of HDL, perhaps atherogenic. We conclude that change in the plasma concentration of HDL-cholesterol or apoA1 is uninterpretable mechanistically, with respect to the robustness of reverse cholesterol transport, other atherogenic or antiatherogenic metabolism, development of atherosclerosis, and CHD. Additional mechanistic approaches are indicated to make progress on the HDL problem and to interpret dietary effects.

A NEW MODEL OF HIGH-DENSITY LIPOPROTEIN METABOLISM ACROSS HIGH-DENSITY LIPOPROTEIN SIZE

HDL circulates in nearly a 2-fold range of sizes. Before the first metabolic studies in humans of HDL size-based subfractions, a leading hypothesis was that the liver and intestine secrete very small discoidal HDL, which gradually enlarges by taking up cholesterol during its 2–4-day residence in circulation (Fig. 2, left panel). However, the concept that HDL has a discoidal primordial particle that is secreted into the circulation has not been confirmed by results of kinetic studies of the size-based subfractions. Direct kinetic studies of human HDL apoA1 within size categories revealed an entirely different model structure. HDL appears directly in the circulation in all sizes from very small discoidal to large spherical particles, and the majority of the very small discoidal particles originate from a medium-size HDL particle, not from liver secretion (Fig. 2, right panel) [18].

This *in vivo* model is consistent with studies of HDL assembly and secretion in cultured cells, showing a range of primordial particles, their size the result of cholesterol and phospholipid content of the cells in membrane domains that are used to synthesize HDL [19–23]. When cholesterol and phospholipid content is high, then large HDL particles are formed and secreted; and vice versa.

Once secreted into the circulation, most of the nascent HDL of all sizes remains in circulation within its secreted size for 2–4 days before it is

catabolized and irreversibly removed from the circulation [18]. However, compartmental modeling consistently resolved additional secondary pathways of two types; one is expansion of discoidal to large spherical particles as in the canonical metabolic structure (Fig. 2, right panel); and the other is remodeling of medium-size HDL to generate very small discoidal HDL (Fig. 2, right panel), processes found *in vitro* [24–26]. These pathways are mainly found in a species of HDL that contains apolipoprotein E (apoE), a minor fraction of the total HDL [27].

PROTEIN-BASED HIGH-DENSITY LIPOPROTEIN SUBSPECIES CONTAINING APOLIPOPROTEIN E AND/OR APOLIPOPROTEIN C3, THEIR CLINICAL RELEVANCE, METABOLISM, AND DIETARY EFFECTS

Progress on lipoprotein speciation of apoB lipoproteins [28] preceded that of HDL and serves as a guide for hypothesis formation pertaining to HDL.

Apolipoprotein B lipoprotein speciation

The apoB lipoproteins are secreted and circulate in subspecies that contain apoE or apolipoprotein C3 (apoC3), both, or neither [29,30]. Subspecies of very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and LDL that have apoE are metabolized to small VLDL and IDL, and leave the circulation rapidly and, most importantly, they are not converted to atherogenic slowly metabolized

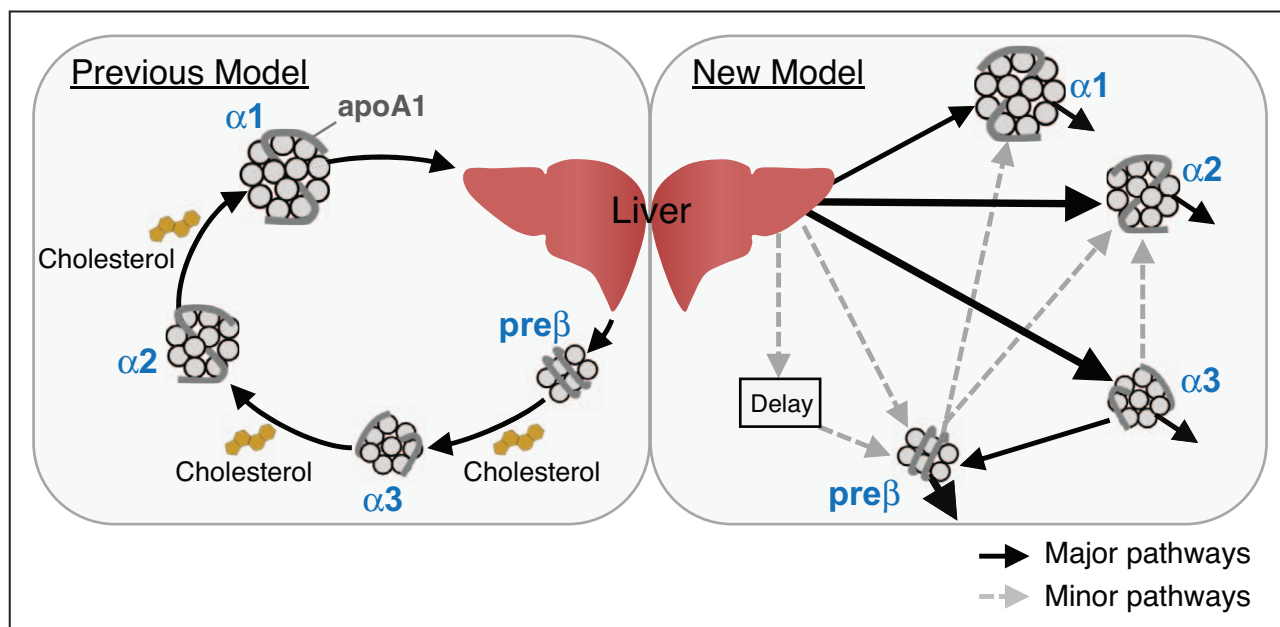


FIGURE 2. HDL metabolism across the HDL sizes: new model. HDL from small to large size: pre β , α 3, α 2, α 1. Figure summarizing data from Mendivil *et al.* *Arterioscler Thromb Vasc Biol.* 2016. HDL, high-density lipoprotein.

LDL. In contrast, those having apoC3 are mostly converted to LDL. VLDL and IDL that have both apoE and apoC3 have kinetics that are more like apoC3 than apoE, suggesting that apoC3 abrogates the beneficial properties of apoE on metabolism. ApoC3 fosters whereas apoE prevents atherogenic metabolism of triglyceride-rich lipoproteins that produce LDL. Finally, these apoB subspecies containing apoC3 predict CHD [31], and the higher risk is lessened by a high content of apoE [32]. Therefore, findings in kinetic and prospective epidemiological studies on the apoB lipoprotein subspecies having apoE and/or apoC3 were an impetus to delve into the effect of these proteins on HDL.

Discovery of protein-based subspecies of high-density lipoprotein, and their clinical relevance

ApoE was recognized as a component of cholesterol-rich HDL, and HDL with apoE bound avidly to liver apoE receptors, accelerating their removal from the circulation [33]. It was therefore logical to hypothesize that HDL in healthy humans has protein-based subspecies containing or lacking apoE and apoC3, and these proteins influence HDL function and relation to disease. The HDL subspecies were prepared from whole plasma using anti-apoE or anti-apoC3 immunoaffinity chromatography, and size separation by gradient gel electrophoresis. HDL that contains apoE or apoC3 each comprises 5–10% of total HDL apoA1 [34]. The two subspecies overlap such that about 50% of HDL that contains apoE also contains apoC3 and vice versa (Fig. 3a). The overlap has clinical meaning as described later. These subspecies are present throughout the size range of HDL, although more HDL with apoE is present in larger than smaller sizes and more HDL with apoC3 is present in smaller than larger sizes (Fig. 3b) [34]. The subspecies that has apoE has different metabolism from the majority of HDL that does not have apoE [27]. Several population samples suggested clinical relevance of the subspecies since HDL that contains apoC3 is associated with cardiovascular disease (CVD) risk factors including obesity, diabetes, and triglyceride levels [34,35]. Finally, HDL that contains apoC3 is predictive of CHD in 4 cohorts in men and women [36], and its presence with apoE abrogates the lower risk of CHD associated with apoE (Fig. 3c) [27].

Protein-defined high-density lipoprotein subspecies, in addition to apolipoprotein E and apolipoprotein C3, predicting risk of coronary heart disease

Many proteins reside on HDL, and they have specific functions that may be related to CHD. A set of 15

protein-defined subspecies were identified, characterized by proteomics, and studied as to risk of CHD [37,38^{***}]. The subspecies were HDL that contains apoA4, apoC1, apoC2, apoC3, apoE, apoJ, alpha-1-antitrypsin, alpha-2-macroglobulin (A2M), plasminogen (PLMG), fibrinogen (FBG), ceruloplasmin (CP), haptoglobin (HP), paraoxonase-1 (PON1), apoL1, or complement C3 (CoC3). A prospective study in 4 US cohorts included 932 CHD cases over 10 to 25-year follow-up. HDL that contains alpha-2 macroglobulin, complement C3, haptoglobin, or plasminogen were each associated with higher risk of CHD; and HDL containing apoC1 or apoE were associated with lower risk. These findings indicate that HDL subspeciation may reveal HDL functions that are involved in CHD and perhaps other conditions.

Obesity, overweight, physical inactivity and alcoholic beverage drinking affect high-density lipoprotein subspecies

An obese group was compared to normal weight participants matched on sex and age [34]. There were 20 participants in each group. Total apoA1 was similar in the two groups, 118–119 mg/dL. The obese group had about 2-fold higher apoA1 concentrations of the minor subspecies, HDL that contains apoE, HDL that contains apoC3, and HDL that contains apoE and apoC3; and lower concentration of the major HDL subspecies that do not have either protein (Fig. 3a). The obese group also had higher triglycerides, fasting plasma insulin, and HOMA index of insulin resistance [34].

A cross-sectional analysis of a population sample of 3631 Danish participants of the Diet, Cancer, and Health Study studied HDL that contains apoC3 [35]. ApoE was not studied. Adiposity, measured by waist circumference, body fat mass, and body mass index, was associated with higher HDL that contains apoC3. Physical inactivity and higher alcoholic beverage drinking were also associated with higher HDL that contains apoC3. Adherence to a self-selected Mediterranean diet was not associated with HDL that contains apoC3, using a scoring system for dietary pattern components. Of the components, only legumes were associated with HDL that contains apoC3, a larger intake corresponded to a lower concentration of HDL that contains apoC3 [35].

Dietary fat and carbohydrate affect the metabolism of high-density lipoprotein that contains apolipoprotein E

The effect of diet on the HDL subspecies defined by apoE was studied in the new model of HDL

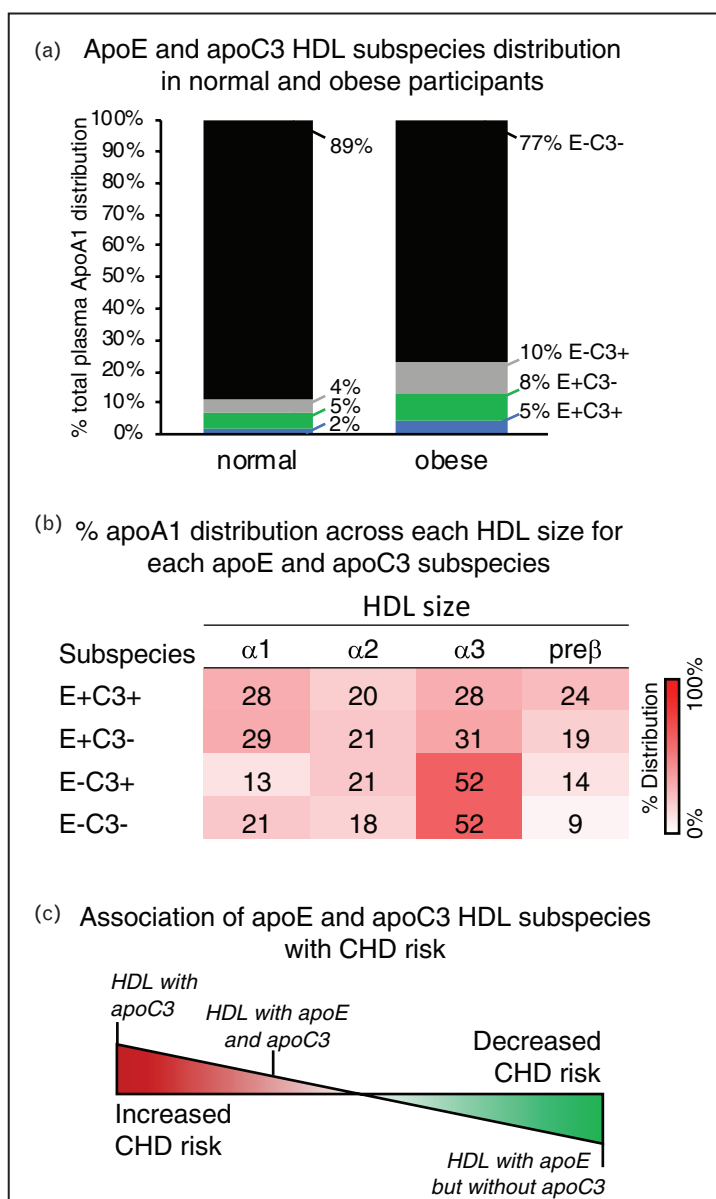


FIGURE 3. Distribution and disease risk of HDL subspecies containing apoE and/or apoC3. (a) Plasma apoA1 distribution across HDL subspecies that contain apoE and apoC3 (E+C3+), apoE but not apoC3 (E+C3-), apoC3 but not apoE (E-C3+), and neither apoE or apoC3 (E-C3-) in normal and obese people. (b) Percentage apoA1 distribution in each HDL subspecies from large ($\alpha 1$) to small (pre β) HDL sizes. (a,b) Figure summarizing data from Talayero *et al.* J Lipid Res. 2014. (c) Association of apoE and/or apoC3 containing HDL subspecies with CHD. Figure summarizing data from Morton, Koch *et al.* JCI Insight. 2018. apoC3, apolipoprotein C3; apoE, apolipoprotein E; CHD, coronary heart disease; HDL, high-density lipoprotein.

metabolism (Fig. 2, right panel), [39]. The study participants were 6 men and 3 women, overweight or obese and with mild dyslipidemia, having mean body mass index of 29 kg/m², HDL-cholesterol levels of 41 mg/dL, and triglyceride levels of 151 mg/dL. They received diets either high in carbohydrate or monounsaturated fat, each for 4 weeks, in a crossover design.

HDL that contains apoE comprised 5–7% of the plasma total apoA1. On both diets, HDL that contains apoE had greater secretion rates than HDL that lacks apoE, faster conversion to larger sizes, and 10 times faster clearance from the circulation [39].

The high unsaturated fat diet had substantial effects on HDL that contains apoE, increasing the secretion rate of its main protein, apoA1, by 150%,

and increasing the FCR of apoA1 of the larger HDL sizes, $\alpha 1$ and $\alpha 2$, by approximately 150% (Fig. 4a, solid blue arrows) [39]. Compared to the relatively small plasma pool size of the HDL that contains apoE, 5%, a relatively larger amount of apoA1 secretion, 16%, was in HDL that contains apoE. The high unsaturated fat diet also tended to increase size expansion flux of small, discoidal to large spherical HDL and size contraction of small spherical to discoidal HDL ($\alpha 3$ to pre β) (Fig. 4a, blue arrows). In summary, a high unsaturated fat diet compared to a high carbohydrate diet increased markedly several metabolic pathways involving HDL that contains apoE that are crucial to reverse cholesterol transport. These are secretion, size expansion, remodeling of medium size ($\alpha 3$) HDL to generate small discoidal HDL, and clearance of large-size HDL from the circulation. We consider this kinetic behavior in response to unsaturated fat to be potentially atheroprotective because it

reflects faster transport of cholesterol through the HDL system.

Dietary unsaturated fat also affected the metabolism of apoE itself on HDL (Fig. 4b) [40^{***}]. Dietary unsaturated fat reduced the secretion of apoE on HDL, reduced the irreversible removal and clearance of apoE from HDL, and tended to reduce the movement of apoE from small to large HDL (Fig. 4b, red arrows). The upshot is that unsaturated fat enhances the retention of apoE on HDL as it circulates, allowing the apoE to continue to stimulate reverse cholesterol transport.

Interactive role of apolipoprotein C3 and apolipoprotein E on high-density lipoprotein metabolism

ApoC3 is present in about 10% of HDL, as measured by plasma total apoA1 concentration. As discussed before, HDL that contains apoC3 is correlated with

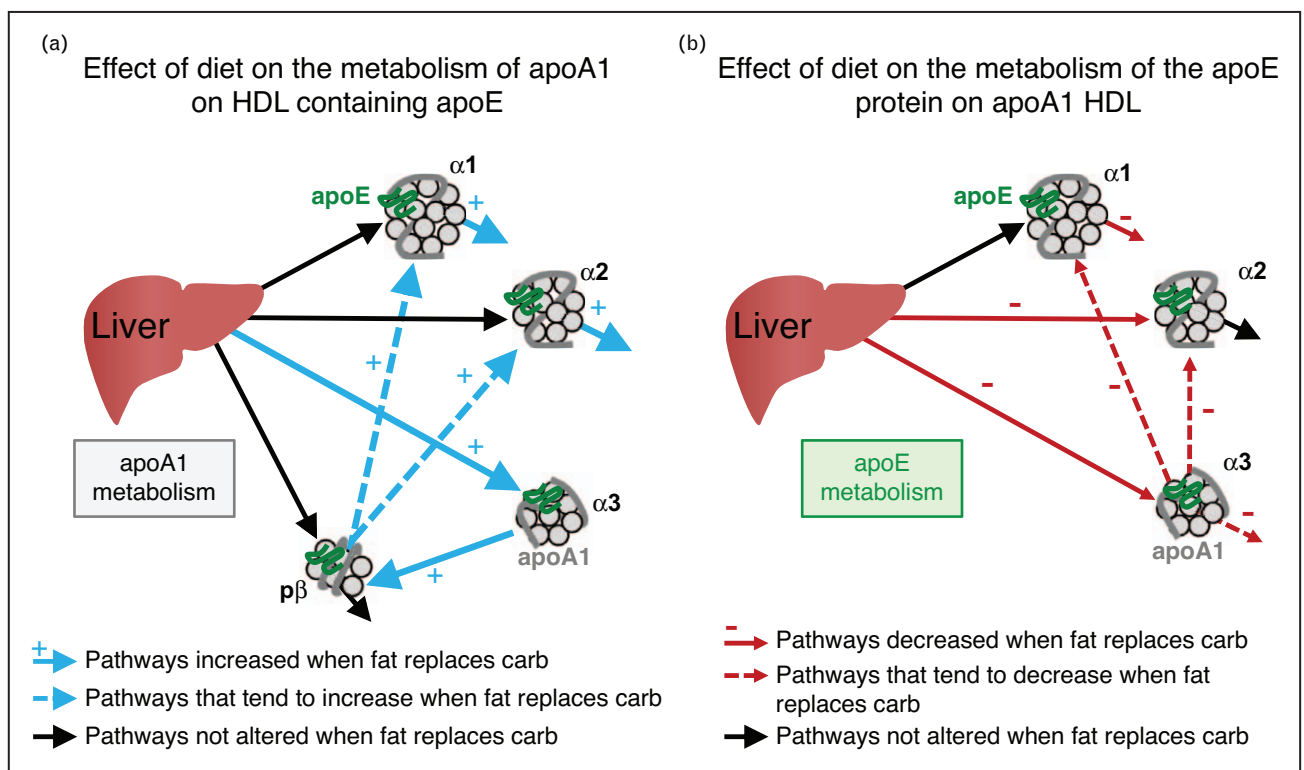


FIGURE 4. Effects of dietary unsaturated fat and carbohydrate on the metabolism of apoA1 in HDL that contains apoE (a), and of the apoE protein on HDL (b). Unsaturated fat, when replacing carbohydrate, increases apoA1 flux from the liver to medium $\alpha 3$ particles containing apoE and from $\alpha 3$ to pre β (solid blue arrows from the liver to $\alpha 3$ and from $\alpha 3$ to pre β). ApoE also increases the catabolic rate of large $\alpha 1$ and $\alpha 2$ particles (arrows out of $\alpha 1$ and $\alpha 2$) and tends to increase the size expansion of small pre β HDL to larger $\alpha 1$ and $\alpha 2$ (dashed blue arrows from prebeta to $\alpha 1$ and $\alpha 2$). On the other hand, unsaturated fat, when replacing carbohydrate, decreases the flux of apoE protein itself from the liver to $\alpha 2$ and $\alpha 3$ (red arrows from liver into $\alpha 2$ and into $\alpha 3$) and decreases the catabolic rate of apoE on $\alpha 1$ and $\alpha 3$ HDL (red arrows out of $\alpha 1$ and out of $\alpha 3$). Figure summarizing data from Morton *et al.* JCI Insight. 2019 (a) and Andraski *et al.* Arterioscler Thromb Vasc Biol. 2019 (b). HDL, high-density lipoprotein.

CVD risk factors, and higher risk of CHD. Does HDL that contains apoC3 compared to HDL lacking apoC3 affect HDL metabolism? In the metabolic protocol described before, HDL that contains apoC3 was prepared from the total HDL apoA1 by anti-apoC3 immunoaffinity chromatography [27]. There was no apparent effect on secretion, interconversion or metabolism, compared to HDL that lacks apoC3. However, HDL subspecies that contain both apoC3 and apoE had metabolism similar to HDL that contain apoC3 rather than HDL that contain apoE (Fig. 5a) [23]. In other words, apoC3 abrogated the apoE-mediated metabolic pathways that define reverse cholesterol transport. This demonstrates a direct or indirect interaction between apoC3 and apoE on HDL affecting metabolism and risk of CHD. The findings also suggest a mechanism by which apoC3 on HDL impairs HDL function in reverse cholesterol transport, and increases atherogenesis and risk of CHD. The effect of diet on the apoE and apoC3 interaction is not known, however we hypothesize that since dietary unsaturated fat accelerates HDL metabolism via the HDL apoE system, apoC3 would block such beneficial effects.

Dietary unsaturated fat and high-density lipoprotein apolipoprotein C3

Dietary fat reduces secretion of apoC3 on HDL but does not affect clearance of apoC3 from HDL (Fig. 5b) [40^{***}]. The result is a reduction of the apoC3 concentration of HDL. This may represent another potentially beneficial action of unsaturated fat on HDL subspecies and metabolism.

DIET, THE HIGH-DENSITY LIPOPROTEIN PROTEOME AND ITS METABOLISM

The proteome of HDL spans a large number of proteins involved in many types of actions [41–44]. The proteins are organized into subspecies. Davidson and colleagues make the point that protein-defined subspecies are distinct from subtypes or subfractions based on size or density, and are a better probe to discover and characterize specific HDL functions conferred by the defining protein [45^{***}], and their relationship to diseases [46]. We and our colleagues aimed to study metabolism of several proteins in apoA1 HDL and the dietary effects [40^{***}]. Twelve participants participated in the dietary protocol

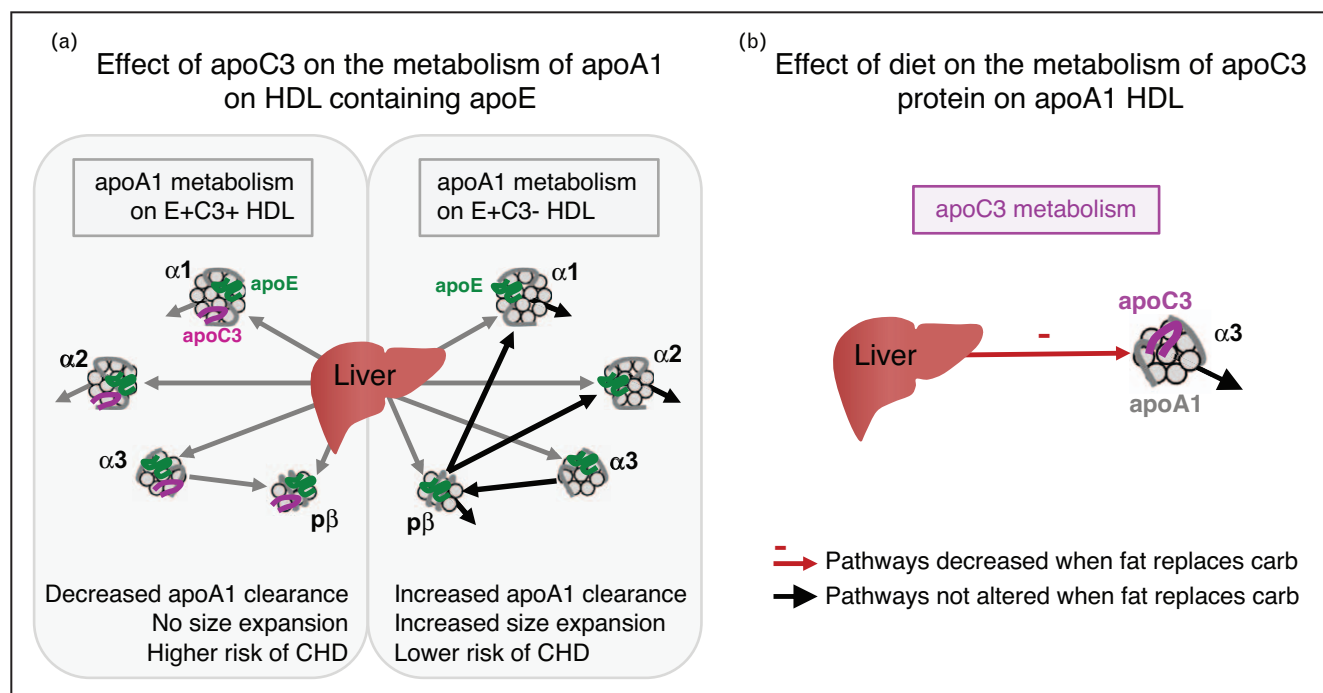


FIGURE 5. Effects of apoC3 on the metabolism of apoA1 on apoE-containing HDL (Panel a) and the effect of diet on apoC3 on HDL (Panel b). (a) ApoC3 on HDL that contains apoE mitigates the beneficial metabolic effects of apoE. ApoC3 decreases apoA1 clearance rates, decreases size expansion, and increases the risk of coronary heart disease (CHD). Data summarized from Morton, Koch. JCI Insight. 2018. (b) Dietary unsaturated fat, when replacing carbohydrate, decreases apoC3 synthesis but does not alter its clearance rate. Figure summarizing data from Andraski *et al.* Arterioscler Thromb Vasc Biol. 2019. apoC3, apolipoprotein C3; apoE, apolipoprotein E; CHD, coronary heart disease; HDL, high-density lipoprotein.

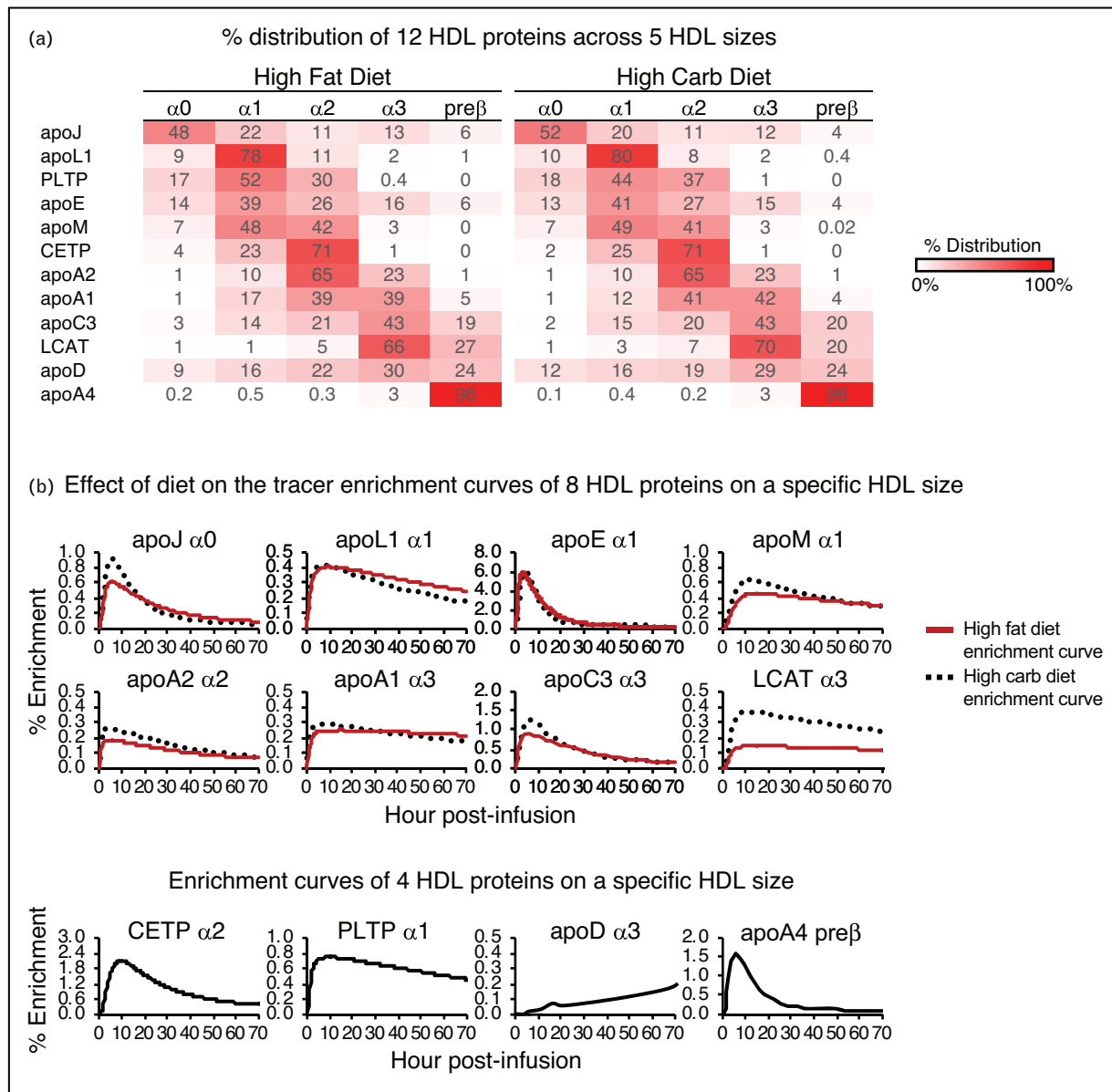


FIGURE 6. Effects of dietary unsaturated fat and carbohydrate on the HDL proteome and the tracer enrichment curves of several HDL proteins. (a) Average ($n = 12$ participants) percentage distribution of 12 HDL proteins across 5 HDL sizes on a high unsaturated fat and a high carbohydrate diet. Each protein has a distinct distribution across HDL size, and diet does not alter this distribution. (b) Representative enrichment curves from the 12 HDL proteins that have been monitored by parallel reaction monitoring mass spectrometry. Eight of these proteins (top panel) were analyzed on a high fat and high carbohydrate diet. The effect of diet on the remaining 4 proteins (bottom panel) was not studied. Enrichment curves are shown for the size fraction in which each protein is most abundant. Figure summarizing data from Andraski *et al.* *Arterioscler Thromb Vasc Biol.* 2019 (a, b top panel); Singh *et al.* *J Lipid Res.* 2016, and Singh *et al.* *JCI Insight.* 2021 (b, bottom panel). HDL, high-density lipoprotein.

and tracer kinetics procedure described before. ApoA1 HDL was prepared from plasma by immunoaffinity chromatography, separated into 5 HDL sizes by gradient gel electrophoresis, and prepared for analysis by LC-MS/MS. The HDL proteome in each HDL size on each diet was determined by data-dependent acquisition (Fig. 6a). Additionally, the metabolism of 8 HDL proteins across the 5 HDL sizes was studied on

each diet by parallel reaction monitoring (PRM) mass spectrometry. Analysis by PRM allows the tracer enrichment quantification not only of apoA1, but of multiple HDL proteins simultaneously [44]. The proteins monitored were apoA1, apoA2, apoC3, apoE, apoJ, apoL1, apoM, and lecithin-cholesterol acyltransferase (LCAT) [40²²]. In addition to these proteins, PRM has also been used to characterize

the metabolism of PLTP, CETP, apoD, and apoA4, but the effect of diet on these proteins has yet to be determined (Fig. 6b) [44,47].

The results showed that each protein has a unique distribution in the HDL size spectrum (Fig. 6a), and unique metabolism (Fig. 6b) [40,44,47]. Diet affected the metabolism of all these HDL proteins except LCAT (Fig. 7) [40]. Unsaturated fat when replacing carbohydrate, decreased the FCR of apoA1 and apoA2 on medium-size α_3 HDL; apoE on α_3 and α_1 HDL; and apoM on α_2 HDL (Figs. 4 and 7). Additionally, unsaturated fat decreased the secretion of apoC3 on α_3 HDL and apoJ and apoL1 on the largest

HDL (Figs. 5 and 7) [40]. Viewed in the opposite way, dietary carbohydrate increased the FCR of apoA1 and apoA2, as a mechanism for reducing HDL concentration, supporting some although not all previous studies (Table 1).

Interestingly, carbohydrate increased the catabolism of proteins mainly in the medium α_3 HDL size. This effect may be due to an increase in apoC3 secretion in this size fraction (Fig. 5b), indicating that apoC3 may destabilize the HDL particle and disrupt the binding of other proteins to HDL. Carbohydrate may also decrease the stability and protein binding affinity by altering the lipid composition of α_3 HDL

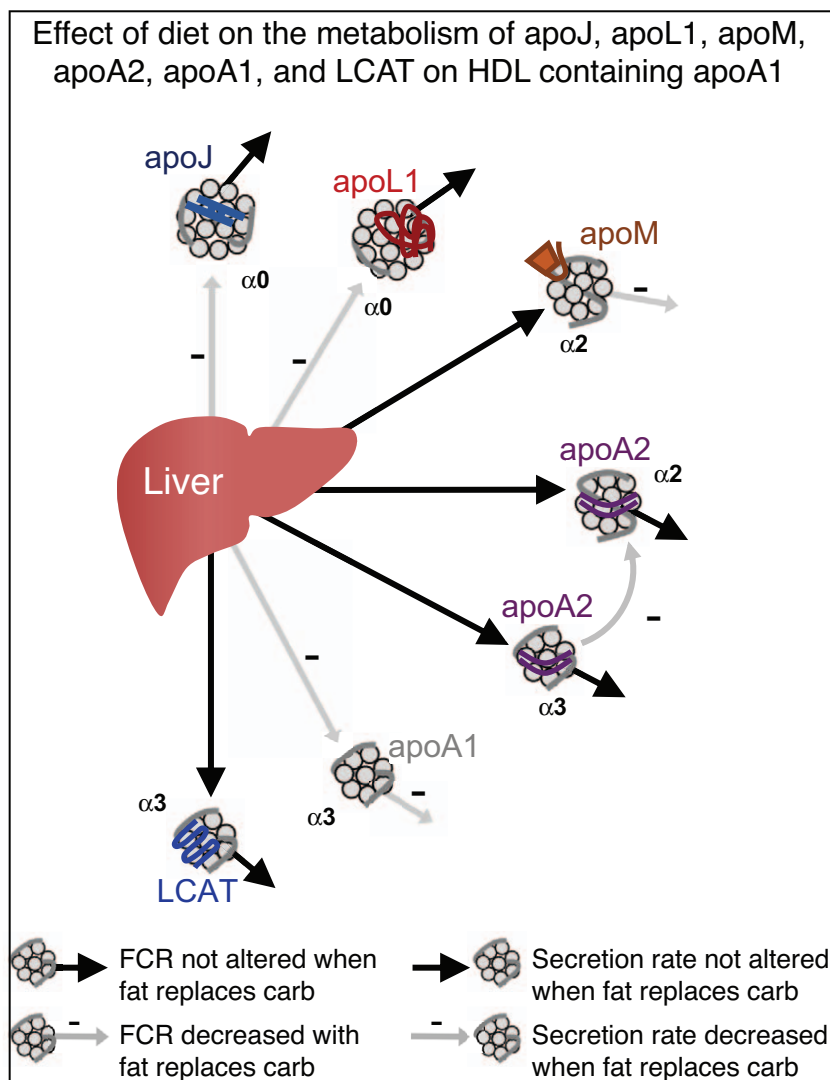


FIGURE 7. Unsaturated fat, when replacing carbohydrate, decreases the secretion rate and catabolic rate of several proteins on specific HDL sizes. Fat, when replacing carbohydrate, decreases the secretion of apoJ and apoL1 on large α_0 and of apoA1 on α_3 HDL. Fat also decreases the catabolic rates of apoM on α_2 and apoA1 on α_3 HDL. For apoA2, fat decreases the secretion of apoA2 on α_2 by decreasing its rate of conversion from α_3 . Only the metabolism of LCAT is not altered by diet. Small, grey arrows indicate pathways that are decreased when fat replaced carbohydrate. Large, black arrows indicate pathways that were not altered when fat replaced carbohydrate. Figure summarizing data from Andraski *et al.* *Arterioscler Thromb Vasc Biol.* 2019. HDL, high-density lipoprotein.

[48,49]. Finally, diet did not affect the size distribution of any of these HDL proteins (Fig. 6a), or alter the composition and size distribution of the full set of 145 HDL-associated proteins identified [40^{***}].

These findings also greatly expanded our view of the metabolic structure of the HDL particle system. Not only are apoA1-HDL particles across the HDL sizes primarily secreted and remain in a given size through their time in circulation (Fig. 2, new model), but the additional proteins studied follow the same pattern, although each protein has a distinct size distribution and metabolism, likely indicating a unique function (Figs. 4, 5 and 7).

CONCLUSION

Dietary unsaturated fat is known for its LDL-cholesterol lowering, compared to either saturated fat or carbohydrate, and this is an important reason why unsaturated fat decreases risk of CHD. The mechanisms and clinical meaning of dietary unsaturated fat raising of HDL-cholesterol are now at least partially explicated. Studies investigating the effect of unsaturated fat, when replacing carbohydrate, on total plasma apoA1 HDL metabolism have yielded mixed results as to underlying mechanisms. However, studies of HDL subspecies are starting to uncover hitherto unknown mechanisms that link HDL subspecies to dyslipidemia and atherogenicity. HDL that contains apoC3 or apoE are minor subspecies that have large opposing effects on metabolism, offering an explanation for HDL-related risk of CHD. Dietary unsaturated fat enhances the atheroprotective role of apoE in reverse cholesterol transport; and diminishes the detrimental HDL that contains apoC3. Additionally, advancements in mass spectrometry technology have increased our ability to monitor the metabolism of apoA1 as well as several additional proteins on HDL, further enhancing our knowledge of how proteins, such as apoE and apoC3, as well as others, may be orchestrating HDL function. Together, studying the metabolism of HDL subspecies and the proteins that reside on these particles may provide a methodology to gain a better mechanistic understanding of interventions that affect HDL, and a means to predict effects on diseases.

Acknowledgements

None.

Financial support and sponsorship

This work was supported by research grants from the National Institutes of Health [R01HL095964 (F.M.S.); R01HL123917 (F.M.S.)].

Conflicts of interest

F.M.S.: 1) US Federal Trade Commission: Expert witness. 2) Pfizer: consultant, expert witness. 3) Patent: Inventor on US patent awarded to Harvard University for HDL proteins as diagnostic and therapeutic targets. 4) Consultant for Inventiva, Dalcour, and Baim Institute. A.B.A.: none

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