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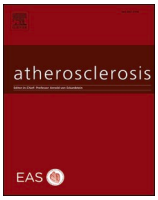

Review article

Dietary fat quality, plasma atherogenic lipoproteins, and atherosclerotic cardiovascular disease: An overview of the rationale for dietary recommendations for fat intake

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ABSTRACT

The scientific evidence supporting the current dietary recommendations for fat quality keeps accumulating; however, a paradoxical distrust has taken root among many researchers, clinicians, and in parts of the general public. One explanation for this distrust may relate to an incomplete overview of the totality of the evidence for the link between fat quality as a dietary exposure, and health outcomes such as atherosclerotic cardiovascular disease (ASCVD). Therefore, the main aim of the present narrative review was to provide a comprehensive overview of the rationale for dietary recommendations for fat intake, limiting our discussion to ASCVD as outcome. Herein, we provide a core framework – a causal model – that can help us understand the evidence that has accumulated to date, and that can help us understand new evidence that may become available in the future. The causal model for fat quality and ASCVD is comprised of three key research questions (RQs), each of which determine which scientific methods are most appropriate to use, and thereby which lines of evidence that should feed into the causal model. First, we discuss the link between low-density lipoprotein (LDL) particles and ASCVD (RQ1); we draw especially on evidence from genetic studies, randomized controlled trials (RCTs), epidemiology, and mechanistic studies. Second, we explain the link between dietary fat quality and LDL particles (RQ2); we draw especially on metabolic ward studies, controlled trials (randomized and non-randomized), and mechanistic studies. Third, we explain the link between dietary fat quality, LDL particles, and ASCVD (RQ3); we draw especially on RCTs in animals and humans, epidemiology, population-based changes, and experiments of nature.

Additionally, the distrust over dietary recommendations for fat quality may partly relate to an unclear understanding of the scientific method, especially as applied in nutrition research, including the process of developing dietary guidelines. We therefore also aimed to clarify this process. We discuss how we assess causality in nutrition research, and how we progress from scientific evidence to providing dietary recommendations.

1. Introduction

Dietary guidelines from national and international health authorities and organizations are unambiguous in their message: within an otherwise healthy dietary pattern, switching from the consumption of foods with “unhealthy” fats to the consumption of foods with “healthy” fats in the diet will reduce the risk of developing atherosclerotic cardiovascular disease (ASCVD) [1–7]. According to food-based recommendations, we should for example choose low-fat over high-fat dairy products; choose lean meat and lean meat products over high-fat variants; choose cooking oils, liquid margarine and soft margarine over hard margarine and butter; and eat fish two to three times a week, including some fatty fish

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[1–7]. This will limit the intake of saturated fatty acids (SFAs), trans fatty acids (TFAs) and cholesterol in the diet, and increase the proportion of monounsaturated fatty acids (MUFAs), omega 6 polyunsaturated fatty acids (n6-PUFAs) and n3-PUFAs, effectively preventing or improving dyslipidemia which is *a necessary and sufficient driver* of atherosclerotic development [1,2].

The scientific evidence supporting these dietary recommendations keeps accumulating. Despite this fact, a paradoxical distrust has taken root among researchers, clinicians and the general public, and the main issue involves the role of SFAs in ASCVD development [8–15]. A plethora of related factors likely contribute to this distrust, including seemingly conflicting epidemiological studies [15–18], and the strong emphasis on *other* dietary factors such as TFA, salt and sugar in prevention of chronic disease [15,19]. We as nutrition scientists should perhaps also acknowledge that we may be part of the problem. After all, it is the scientists who have developed a research system that rewards conflicting and contradictory findings more than confirmatory ones [20], who commonly claim that the dietary recommendations should be changed due to findings from their study [15,17,18], and who develop relationships with industry partners that some may consider unhealthy [20,21]. And, importantly, it is the nutrition scientists who regularly "debate" the issue of dietary fats and health [13,22,23], thereby fueling the perception that this research question is unresolved.

To combat ASCVD and the personal and economical burdens associated with its manifestation, strategies targeting high- and medium-risk subjects with innovative, state-of-the-art clinical interventions are essential [14,24,25]. Also essential, however, are the strategies that reach the *entire* population. One such public health strategy is the development of dietary recommendations that form the foundation for nutrition policy making and dietary planning [26–28]. Importantly, shifts in population dietary intake – particularly related to dietary fat quality – will cause shifts in the distribution of causal ASCVD risk factors such as plasma LDL-C [29–31]. And although this strategy enjoys broad international consensus [2], regularly appearing hot topics seem to deprecate its importance [14,32,33]. The controversy and confusion for dietary fat recommendations may thus exacerbate the social inequalities in health and cause many years of life lost [34,35].

One explanation for the persistency in the controversy regarding the dietary recommendations for fat quality may relate to *an incomplete overview* of *the totality of the evidence* for the relationship between fat quality as a dietary exposure and health outcomes such as ASCVD. Therefore, the main aim of the present narrative review is to provide a comprehensive overview of the rationale for the dietary recommendations for fat intake, except for the very long-chain omega-3 fatty acids found in fish. We will limit our discussion to ASCVD as outcome. Herein, we argue that scientific evidence solidly supports the dietary recommendations concerning fat intake. Importantly, the evidence suggests that dietary fat quality *causally* affects the risk of ASCVD via modulation of the plasma concentration of atherogenic apolipoprotein B (apoB)-containing lipoprotein particles. We will mainly use the term *LDL particles* throughout the text (commonly estimated by plasma total cholesterol or LDL cholesterol), as LDL particles usually comprise the bulk of apoB-containing lipoproteins in plasma. Secondly, the controversy over dietary recommendations for fat quality may *partly* relate to an unclear understanding of the process of developing dietary guidelines. We therefore also aim to briefly clarify this process.

2. The causal model for fat quality and ASCVD

Does dietary fat quality causally affect the risk of developing ASCVD? How should we understand the totality of the evidence for this research question (RQ)? We find it helpful to break this overarching RQ down into at least three sub-RQs that will then constitute the core framework – the causal model – by which we understand the evidence.

1. Does plasma concentration of LDL particles causally affect the risk of ASCVD?
2. Does fat quality in the diet causally affect plasma concentration of LDL particles?
3. Does fat quality in the diet affect plasma concentration of LDL particles and *thereby* the risk of ASCVD?

In the following sections, we provide a transparent overview of what we consider evidence that convincingly supports that the answer to these three RQs is *Yes*.

RQ1. *Does the plasma concentration of LDL particles causally affect the risk of ASCVD?*

The first RQ we can ask is this: does plasma LDL particle concentration causally affect the risk of ASCVD? The European Atherosclerosis Society (EAS) Consensus Panel answered this RQ in their two-part consensus statement [36,37]. In 2017, Ference et al. described the evidence describing this relationship from genetic, epidemiologic, and clinical studies [36], whereas in 2020, Borén et al. described the pathophysiological, genetic, and therapeutic insights [37]. Expectedly, the panel concluded that LDL particles *do* cause ASCVD. In the following, we give a brief summary of some main points, and refer the reader to the consensus statements for a comprehensive and detailed discussion [36,37].

1. ASCVD in animals and humans: mechanistic, pathologic and epidemiologic evidence

Most CVD is caused by *atherosclerosis*, which is a slowly progressing pathological process affecting medium- and large-sized arteries throughout life, driven by the concentration-dependent and continuous influx of apoB-containing lipoproteins particles (less than 70 nm in diameter) to the subendothelial space [36,37]. A proportion of these particles binds to proteoglycans in the extracellular matrix and becomes trapped, causing a sterile, non-resolving, low-grade inflammation, the so-called *response-to-retention* model of atherosclerosis [38]. Ever since Anitschkow's classic studies in rabbits [39], an abundance of experimental research have shown that a complex web of mechanisms contributes to the development and progression of atherosclerosis [37].

While clinical ASCVD usually manifests late in life, atherosclerosis is present already from early in life, as shown both in human autopsy studies [40–44] and studies using imaging methods [25,45–47]. Atherosclerosis is not a disease of modern societies only; CT scans of ancient mummies revealed that atherosclerosis was common in preindustrial populations spanning over 4000 years of human history, including hunter-gatherer societies [48]. However, atherosclerosis is not an inevitable process of aging either; rather, it is a pathology that progresses depending on the cumulative exposure to multiple metabolic and environmental risk factors through life [49].

Despite the multifactorial nature of ASCVD pathophysiology, dyslipidemia has a particularly central role. To experimentally induce atherosclerosis in animals, you first *must* increase the plasma concentration of LDL particles to plasma concentrations seen in humans, and then maintain this higher level over a significant amount of time [37,50], a point already noted by Anitschkow in 1913 [39]. In rodents, for example, this is commonly achieved by introducing *APOE* or *LDLR* loss-of-function mutations, or *PCSK9* gain-of-function mutations [51], all of which inhibit clearance of LDL particles via the liver, thereby humanizing the animal's cholesterol metabolism towards transporting most of its cholesterol in LDL instead of HDL particles. In contrast, animals with LDL particles below a certain threshold do not develop atherosclerosis, regardless the rest of the risk factor profile [50].

2. LDL particles and ASCVD in humans: genetics and clinical trials evidence

LDL particles are the major apoB-containing lipoproteins in circulation, and they are considered *both necessary and sufficient* for atherosclerosis development [36]. Due to the long plasma residence time for a cholesterol-rich LDL particle (over 48 h) compared to its TG-rich precursors VLDL and IDL (up to 6 h), 90 % of the lifecycle of a hepatocyte-derived apoB-containing lipoprotein is in the form an LDL particle [52].

The most compelling evidence documenting the causal link between LDL particles and ASCVD stems from classical genetic studies, Mendelian randomization (MR) studies, and clinical trials [36,37,53,54]. In short, genetic variation that increase or decrease the plasma concentration of LDL particles also increase or decrease the risk of ASCVD. Although GWAS and MR methodology have been a key tool in this understanding [55], the causal genetic link was established many decades ago [56]. Subjects with FH have hypercholesterolemia from birth, and untreated they have an approximately 20-fold increased risk of ASCVD; individuals with heterozygous FH (TC approx. 8–15 mmol/L) die on average 15 (men) to 21 (women) years earlier than the general population [57], while the homozygous variant (LDL-C approx. 12–30 mmol/L) is deadly even in early childhood, again confirming a strong dose-dependent and causal relationship between LDL particle exposure and ASCVD in humans [58].

Clinical trials have also contributed significantly to our understanding of the causal effect of LDL particles in ASCVD, of which the statin trials undoubtedly have been the most influential [59]. However, several other interventions also reduce plasma concentration of LDL particles and thereby the risk of ASCVD, including pharmacotherapy (ezetimibe, PCSK9 inhibitors, bempedoic acid, and bile acid sequestrants), ileal bypass, and diet [60,61]. Importantly, *independent* of type of intervention used to reduce plasma LDL particles, a 1 mmol/L reduction in LDL-C in a clinical trial (five years treatment) translates into an approximately 23 % reduced risk of ASCVD [59]. In comparison, a 1 mmol/L lower LDL-C associate with over 30 % lower risk in prospective cohorts (12 years follow-up), and over 50 % lower risk in MR studies (52 years follow-up) [36]. This confirms that not only the *magnitude*, but also the *duration* of exposure to LDL particles is detrimental to atherosclerosis development and ASCVD in humans, and has established that it is the cumulative exposure to LDL particles that is the fundamental driver of ASCVD [62]. Because of the evidence that has now accumulated regarding LDL particles and ASCVD in humans, prominent voices in the community have gone far in declaring LDL particles detrimental to vascular health [63,64]. For example, Toth characterized LDL particles "a waste product of metabolism and a vascular toxin" [63]. He argued that "On an evolutionary time scale, we were never meant to have the LDL-C levels we currently harbor. A truly physiologic, nonpathogenic LDL-C level is likely around 40 mg/dL (1 mmol/L)".

The successful lipid-lowering therapies to date all work by the same mechanism: ultimately, they all affect the number and activity of the LDLRs on hepatocytes, which then affect the removal of LDL particles from the plasma compartment. However, there are actors playing a role

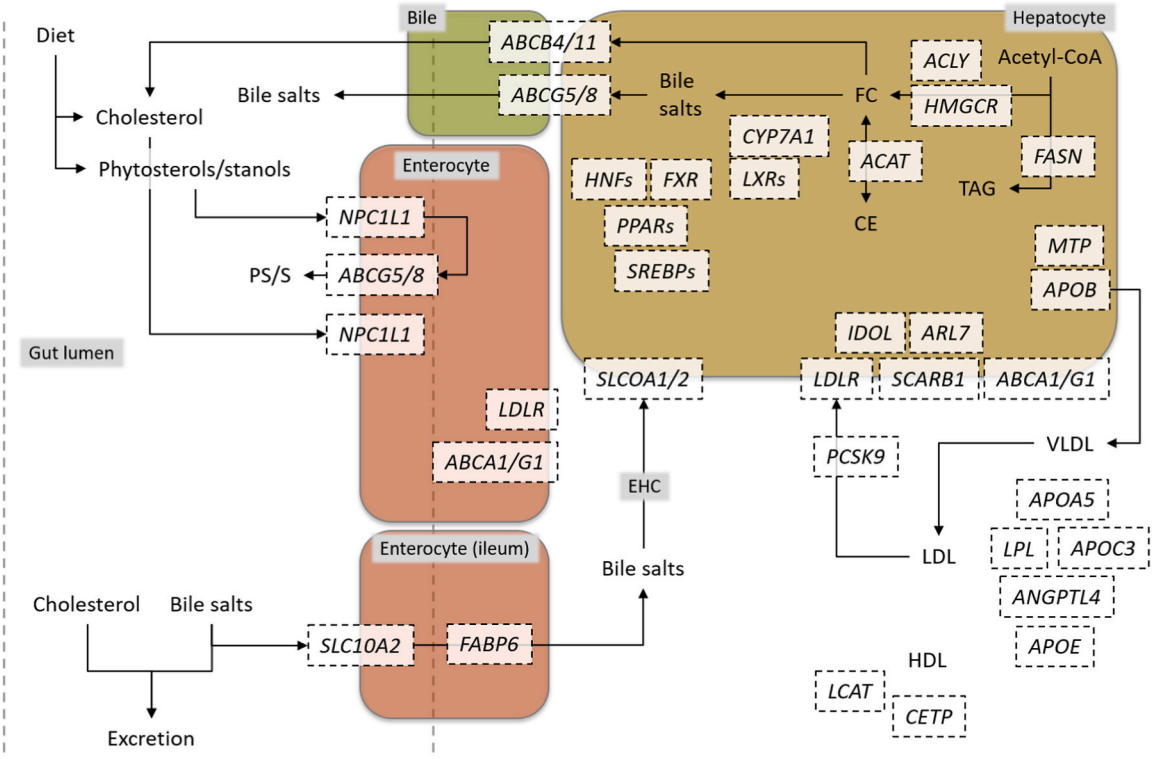

Fig. 1. Physiological determinants of variability in plasma atherogenic lipids.
ABCA1/G1, ATP binding cassette (subfamily A member 1; subfamily G member 1); ABCB4/11, ATP binding cassette (subfamily B member 4; member 11); ABCG5/8, ATP binding cassette (subfamily G member 5; member 8); ACAT, Acyl-CoA cholesterol acyltransferase; ACLY, ATP citrate lyase/synthase; ANGPTL4, Angiopoietin-like 4; APOA5, Apolipoprotein A-V; APOB, Apolipoprotein B; APOC3, Apolipoprotein C-III; APOE, Apolipoprotein E; ARL7, ADP-ribosylation factor-like 7; CE, Cholesteryl ester; CETP, Cholesteryl ester transfer protein; CYP7A1, Cholesterol 7 alpha-hydroxylase; EHC, enterohepatic circulation; FABP6, Fatty acid binding protein 6; FASN, Fatty acid synthase; FC, Free cholesterol; FXR, Farnesoid X receptor; HDL, High-density lipoprotein; HMGCR, HMG-CoA reductase; HNFs, Hepatocyte nuclear factors; IDOL, Inducible degrader of the LDLR; LCAT, Lecithin-cholesterol acyltransferase; LDL, Low-density lipoprotein; LDLR, LDL receptor; LPL, Lipoprotein lipase; LXRs, Liver X receptors; MTP, Microsomal triglyceride transfer protein; NPC1L1, Niemann-Pick C1-Like 1; PCSK9, Proprotein convertase subtilisin/kexin type 9; PPARs, peroxisome proliferator-activated receptors; PS/S, Phytosterols/stanols; SLC10A2, Ileal bile acid transporter; SLCOA1/2, Solute carrier organic anion transporter family member A 1/2; SCARB1, Scavenger receptor class B type 1; SREBPs, Sterol regulatory element-binding proteins; TAG, Triacylglycerol; VLDL, Very low-density lipoprotein.

in lipid metabolism, and we indeed know many of the main physiological determinants of the variability in plasma concentration of LDL particles, as evidenced from genetic studies and intervention trials, as well as from basic science, animal studies and cell cultures [25,37] (Fig. 1). The most important physiological determinant is the number and activity of hepatic LDLRs (supported for example by genetic variation in *LDLR*, *PCSK9* and *APOE*). Other important determinants include the effectiveness of the physical interaction between the LDL particle and the LDLR, which again affects the hepatic removal of LDL particles (supported by *LDLR* and *APOB*); cholesterol biosynthesis, which affects the need for cellular cholesterol uptake via the LDLR (supported by *HMGCR, ACLY* and *LDLR*); hepatic bile acid synthesis, which affects cholesterol excretion capacity (supported by *CYP7A1*); and enterocyte uptake and secretion of cholesterol and bile acids, which affects the hepatic cholesterol and bile acid pool (supported by *NPC1L1* and *ABCG5/G8*) [25,37] (Fig. 1). Regarding the latter, note that inactivating mutations in *NPC1L1* [65] and the NPC1L1 inhibitor ezetimibe [66] both result in lower plasma LDL-C and lower risk of ASCVD, thus confirming that cholesterol present in the GI tract can be atherogenic regardless of origin (diet or bile).

Because all LDL particles are potentially atherogenic, the *quantity* of LDL particles in circulation is the main determinant of atherosclerosis progression; however, LDL *quality* also plays a role. A multitude of physiochemical, metabolic, and functional characteristics give rise to LDL particle heterogeneity and thus variation in atherogenicity [37]. Atherogenicity is especially affected by particle-to-particle variation in the susceptibility to aggregate, oxidize and bind to proteoglycans [37]. For example, aggregation-prone LDLs have unstable apoB, and are typically sphingomyelin- and ceramide-rich particles; in contrast, aggregation-resistant LDLs have stable apoB, and are typically phosphatidylcholine- and lysophosphatidylcholine-rich particles [67]. It is likely that variation in lipid and protein composition affects the potential for modification of the LDL particle surface structure, and thus the potential for causing LDL particle instability, aggregation, and fusion [68].

RQ2. *Does fat quality in the diet causally affect the plasma concentration of LDL particles?*

Having established the importance of plasma LDL particles for ASCVD, the next RQ we can ask is this: does fat quality in the diet causally affect plasma LDL particle concentration? Indeed, metabolic ward studies (feeding trials), controlled trials, RCTs, and mechanistic studies clearly show that it does. In the following, we give an overview of some of the most relevant literature.

1. Dietary fat quality: molecular aspects and long-term dietary intake

Dietary fat quality refers to the *types* of dietary fat and their proportions in the diet (Figs. 2 and 3). Dietary fats are mainly consumed in the form of TGs, composed of three fatty acids attached to a glycerol backbone, and to a lesser degree as phospholipids and other lipids, such as cholesterol. The main *types* of fatty acids in dietary TGs are the SFAs, MUFAs, and PUFAs. Because humans cannot endogenously synthesize fatty acids of the omega-6 and omega-3 families, linoleic acid and alpha-linolenic acid comprise essential fatty acids in the human diet. The long-chained and highly unsaturated PUFAs eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, 22:6n3) are also important fatty acids. Dietary intake of cholesterol and TFAs are also considered

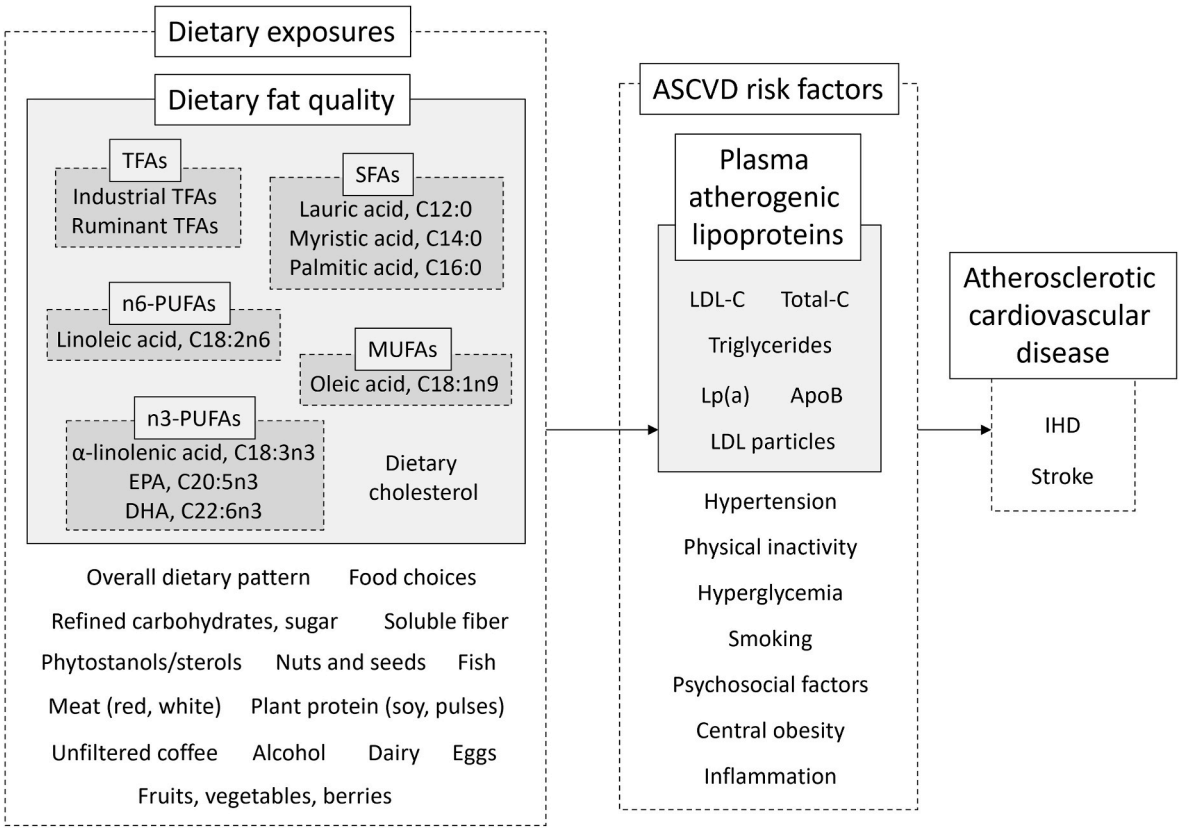

Fig. 2. The role of dietary fat quality in modifying plasma atherogenic lipids and risk of atherosclerotic cardiovascular disease (ASCVD).
The figure shows that dietary fat quality is part of a large set of dietary exposures that may affect the concentration of plasma atherogenic lipoproteins, in addition to other environmental exposures. Abbreviations: ApoB, apolipoprotein B; ASCVD, atherosclerotic cardiovascular disease; IHD, ischemic heart disease; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, trans fatty acids; Total-C, total cholesterol.

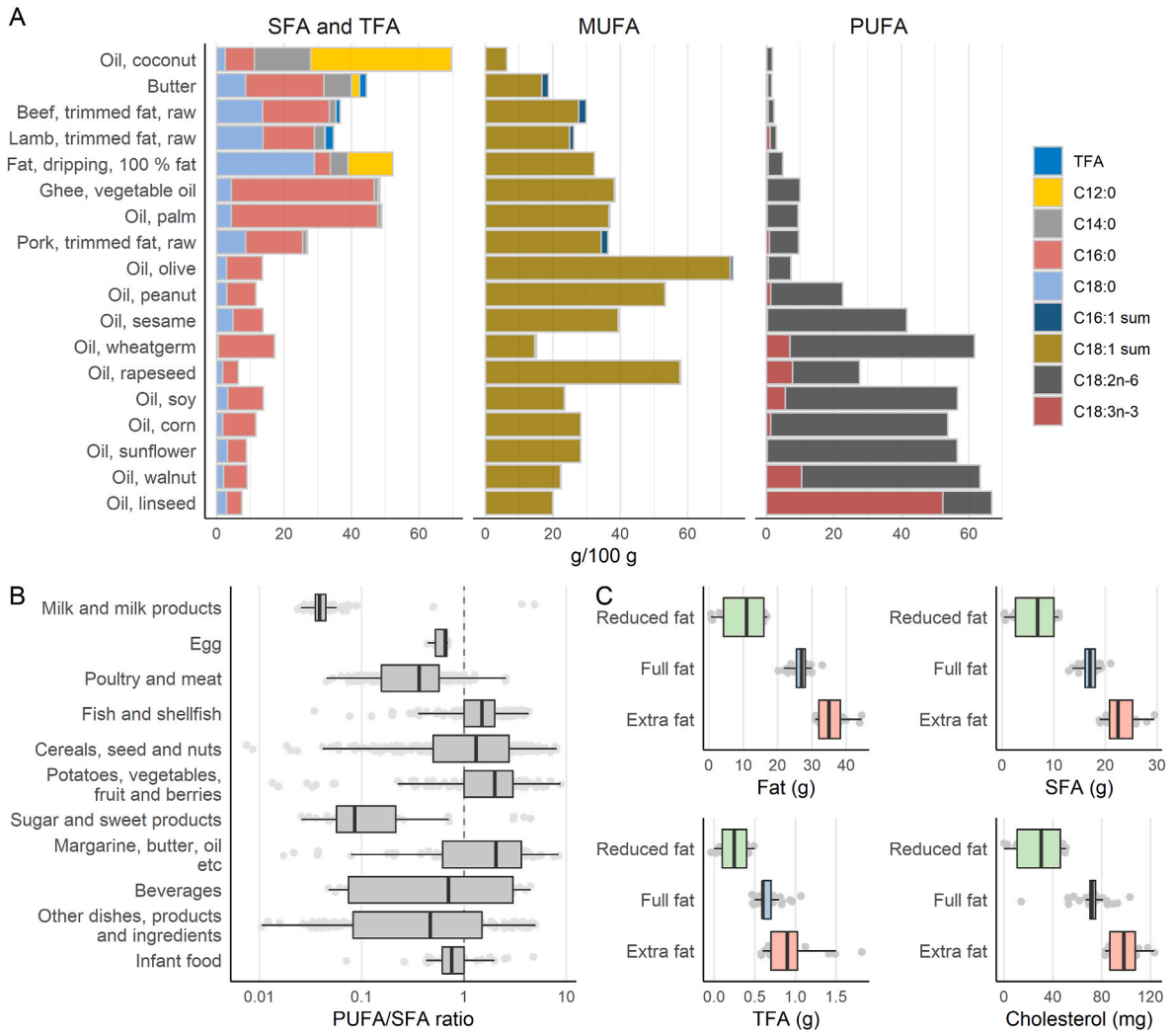

Fig. 3. Fatty acid composition in foods.
The figure displays nutrient composition data from The Norwegian Food Composition Table, publicly available at [69], with an emphasis on fats and fatty acids. Panel A shows content of saturated fatty acids (SFAs), trans fatty acids (TFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), in grams per 100 g of food, for some of the main oils and dietary fat sources (sorted by the PUFA/SFA ratio). Panel B shows the PUFA/SFA ratio (on log10 scale, for readability) across all foods in all food categories, excluding foods with either PUFA or SFA (or both) of zero g/100 g. Panel C shows fat content (total fat, SFA, TFA, and cholesterol) in three categories of cheese: extra fat cheese, full fat cheese, and reduced fat cheese. Note the large variability in the fatty acid composition of main oils and dietary fat sources (panel A), and the associated large variability in the PUFA/SFA ratio across food categories in which these fat sources are concentrated (panel B).

part of the dietary fat quality; although these are consumed in much smaller quantities than SFAs, MUFAs and PUFAs, they are often found in the same foods and thus correlate well with the intake of SFAs (Fig. 3).

Important sources of fats in foods include butter, margarines, oils and oil products, and meat and dairy products [69,70]. While MUFAs are ubiquitous in fat-containing foods, SFAs and PUFAs show a more specific distribution: SFAs are mainly found in dairy, meat, and certain tropical oils (cocoa and palm oil), and PUFAs are mainly found in vegetable oils (Fig. 3). The sources of fat typically differ in different populations, but generally, while SFA and MUFA show a wide range of intake (3–22 and 3–29 E% across populations examined in Seven Countries Study, respectively), the range in PUFA intake is typically rather narrow (3–7 E % across populations examined in Seven Countries Study) [71].

Assessment of dietary fat quality in the diet of free-living humans is a challenging task [72]. Several dietary assessment methods exist, including retrospective (24-h recall, dietary history, food frequency questionnaire [FFQ]) and prospective methods (dietary registration, duplicate portion method), each with unique strengths and limitations. In epidemiological studies, we are commonly interested in linking diet with health outcomes, and then we need to estimate the long-term *usual* intake of the dietary fat quality on the *individual* level. However, because diet is an exposure that may change over time (time-varying exposure), this requires repeated assessments of the diet with a high degree of accuracy. The same principle applies to confounding factors (time-varying confounders) and competing exposures, such as other lifestyle habits. There are also several obstacles specific to the assessment of dietary fat quality which we discuss in-depth in section RQ2 (Epidemiological studies).

Biomarkers may improve the accuracy and objectivity of the assessment of dietary fat quality, but should ideally be used in combination with traditional dietary assessment methods [73]. The exogenously produced PUFAs comprise the most relevant group of fatty acid biomarkers, although others such as odd-chain fatty acids and TFAs have specific use cases [73]. Importantly, the endogenous production of SFAs and MUFAs limits their usefulness as biomarkers of dietary intake [73]. Therefore, in practice, the most reliable fatty acid biomarkers are the essential fatty acids (C18:2n6 linoleic acid and C18:3n3 alpha-linolenic acid), as well as the marine long-chain omega-3 fatty acids (especially

20:5n3 EPA and 22:6n3 DHA [73]. Bear in mind also that different biological tissues have different turnover of fatty acids and therefore reflect different periods of dietary exposure.

2. Dietary fat quality and apoB-containing lipoproteins: controlled trials

The causal effects of dietary fat quality on plasma apoB-containing lipoproteins have been unequivocally demonstrated by hundreds of feeding trials, a type of intervention characterized by complete control of the study subject's dietary intake (close to 100 % adherence) [74–80]. From these trials, the following has been established. SFAs, TFAs and dietary cholesterol *increase* whereas PUFAs *decrease* LDL-C [76–78]. In contrast, MUFAs and the long-chained n3-PUFAs EPA and DHA are close to neutral. Importantly also, on average, the effect of SFAs is twice the effect of PUFAs [76–78], and compared to SFAs and PUFAs, the effect of dietary cholesterol is minimal [76–78]. More detailed examination has shown that only a subset of the lipid subtypes exerts effects on plasma LDL-C. Of the SFAs, myristic acid (C14:0) increases plasma LDL-C most strongly (+0.071 mM per E% increase in the diet), then palmitic acid (C16:0, +0.047 mM/E%), then lauric acid (C12:0, +0.01 mM/E%); in contrast, stearic acid (C18:0) appears neutral in isolation [79]. Industrial TFAs increase plasma LDL-C approximately as strongly as SFAs, although TFAs generated from fish oils (+0.043 mM/E%) more than vegetable oil TFAs (+0.025 mM/E%) [79,81]. Ruminant TFAs show similar effects, although with more uncertainty due to fewer studies [81]. Dietary cholesterol increases plasma LDL-C by 0.05 mM/100 mg cholesterol when modelled linearly [82], although the effect of is more complex and often considered to take a non-linear, hyperbolic shape [83,84]. Among the PUFAs, both linoleic acid (C18:2n6) and alpha-linolenic acid (C18:3n3) reduce plasma LDL-C by equal amounts (−0.017 mM/E%) [79]. In comparison to these observations, oleic acid (C18:1n9), EPA (C20:5n3) and DHA (C22:6n3) have little to no effect on plasma LDL-C in isolation [79].

The literature documenting the effect of fat quality on plasma lipids is not limited to the classic feeding trials; trials from the last decades also support the main effects [80,84–86]. In an updated review, Mensink summarized the causal effects of substituting SFAs by other fats or carbohydrates in 84 controlled trials from 1970 to 2013 (n = 2353) [85]. In essence, there was a linear effect across a wide range of intake of SFA (1.6–24.4 E%), and the effect may have been more pronounced at higher baseline lipid levels and among women (though not statistically significant). Importantly also, year of publication and type of intervention (liquid formula diet versus other types of diet) did not affect the response. Similarly, Brouwer summarized the causal effect of substituting TFAs by other fats or carbohydrates from studies from 1990 to 2014 (n = 680) [81]. Again, the effect of TFA was evident across a wide range of intake (0–10.9 E% and 0.1–3.6 E% for industrial and ruminant TFA, respectively). The variation in TC, LDL-C, TG and HDL-C is affected by many dietary and lifestyle factors other than dietary fat quality, including a Portfolio-type diet (nuts, plant protein such as soy and dietary pulses, soluble fiber, and plant sterols), filtered versus unfiltered coffee, changes in body weight, level of alcohol intake, level of physical activity, dietary intake of simple carbohydrates (sugar), and dietary intake of fatty fish (containing long-chain omega-3 fatty acids) [25,87] (Table 1). For example, the diterpenes in unfiltered coffee (cafestol, cahweol) are among the most cholesterol-increasing substances known to date [88,89]. Still, even though diterpenes, myristic acid and other dietary factors may have qualitatively important effects on plasma LDL-C, *palmitic acid* is likely among the most *quantitatively* important dietary component determining the variation in LDL-C. This is because dietary intake of palmitic acid is both *high* and *variable* within and across populations.

Dietary fat quality also affects the LDL quality by remodeling of the LDL lipidome. In a three-week RCT, subjects consumed 1000 kcal per day extra of either unsaturated fat, saturated fat, or simple sugars [90]. LDL aggregation increased in the saturated fat group only, and this effect was linked to increased sphingolipid and saturated triacylglycerols in LDLs and plasma, as well as a reduction of clusterin on LDL particles. Interestingly also, proteoglycan binding of plasma lipoproteins decreased in the unsaturated fat group [90]. Moreover, in a six-months RCT, subjects consumed either a plant stanol ester-enriched or plain spread [91]. Plant stanols decreased the LDL aggregation susceptibility, especially among normal weight subjects, and this effect was linked to a decreased proportion of LDL-sphingomyelins and increased proportion of LDL-triacylglycerols. Also, LDL binding to proteoglycans was decreased in the plant stanol ester group, and this was linked to the decreased serum LDL-C [91].

Despite the evidence presented above, critics have repeatedly questioned the effects of dietary fat quality on plasma apoB-containing lipoproteins. A common criticism concerns the effect of *foods* compared to *nutrients*, arguing that because we do not eat fatty acids in isolation, different foods may differ in their effect on plasma apoB-containing lipoproteins. This is to some extent a valid criticism, although there are important nuances. It is true that we often extrapolate from studies on isolated nutrients to provide recommendation on foods. But absence of evidence is not evidence of absence of effect; even if we lack evidence of the health effect of a specific food item, this does not equate to that food having no health effect at all. We can still make useful recommendations for any given food compared to another based on the nutrient content and other factors, such as the context and overall dietary pattern in which that food is usually consumed.

Cheese is often claimed to have a neutral effect on plasma apoB-containing lipoproteins [15], but the data suggest that this depends on

Table 1
Toolbox of lifestyle interventions to improve dyslipidemia.

Lifestyle intervention	TC- and LDL-C-reducing effect	TG-reducing effect	HDL-C-increasing effect[a]
Reduce dietary SFA	+++	+	
Reduce dietary TFA	+++		+++
Avoid unfiltered coffee	+++	+++	
Reduce excessive body weight	++	+++	++
Reduce dietary cholesterol	+		
Reduce alcohol intake		+++	
Increase MUFAs and PUFAs in exchange for SFAs	+++	+	
Increase dietary fiber	++		+/−
Increase habitual physical activity	+	++	+++
Increase MUFAs and PUFAs in exchange for sugars		++	++/+
Increase intake of fatty fish or omega-3		++	
Use foods with phytosterols/phytostanols	++		

Table adapted from Refs. [25,89].
HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TC, total cholesterol; TFA, *trans*-fatty acids; TG, triglycerides.
[a] HDL-C is not a causal risk factor for ASCVD; on population level, it can generally be interpreted as a marker of long-term exposure to plasma TGs.

the comparator food. High-fat cheese may lower LDL-C slightly compared to butter [92], maybe because of variation in fatty acid composition and calcium content, and different food matrices. Some studies are more surprising at first glance. Results from Raziani et al. for example, suggested that low-fat cheese had no favorable effect on plasma LDL-C compared to high-fat cheese, despite differing in total fat content only [93]. At closer inspection, the observed change in plasma LDL-C in that study was almost exactly as estimated by the predictive equations [79], but the study was underpowered to detect a statistically significant difference between the groups [94]. Furthermore, the impact of the fat quality in dairy was elegantly demonstrated by the RESET study [95]; in a 12-week controlled crossover trial, use of MUFA-enriched dairy products attenuated the increase in LDL-C compared to standard dairy products (0.03 mmol/L vs. 0.19 mmol/L increase). The fatty acids in dairy thus seem to behave similar to fatty acids found in other foods.

Another common pushback relates to inter- and intraindividual variation in response to dietary fats. Critics would argue that some people respond with changes in plasma LDL-C while others do not. While partially true, this claim also needs some nuance. First, it is important to distinguish between *random* and *non-random* variation in response. Random variation can for example be statistical errors, for example analytical errors such as sampling and measurement errors, and variation resulting from aspects of the research methodology. Standard parallel arm intervention studies can be susceptible to *regression toward the mean*; this is an artefact where a subject with an extreme value for a measurement at baseline most likely has a value closer to the mean for that measurement at the end-of-study. Unaware of regression toward the mean, one might incorrectly interpret such variation in repeated measurements as a true change in the measurement. Non-random variation, on the other hand, arise from various types of biases, for example selection bias or confounding bias, or from a true biological effect. While the impact of random variation usually can be handled by increased power, non-random variation caused by biases needs a thorough methodological setup – a rigorously planned and executed research study. In fact, to determine true, biological inter- and intraindividual variation in response, the response should be shown not only once, but *many* times, for each study subject; it should be highly *replicable*. And those studies are infrequent [77].

True biological variation in LDL-C response to changes in dietary fat quality is not an either-or phenomenon – it is a *continuum* of responses [96]. In general, hyper- and hyporesponsiveness takes the form of a normal distribution, as is standard for all random variables that are determined by many independent processes; relatively few people have a strong or weak response, while most people have a response close to the average [96]. And controlled trials demonstrate that the average response is *highly predictable* [79]. However, *true biological variation* in response likely results from numerous factors, such as baseline diet and plasma LDL-C, sex, age, certain health parameters such as obesity, and genetic variation. The complete role of genetics is far from characterized, although some of the key players seem to be *APOE* polymorphisms and whole-body cholesterol homeostasis, including regulation of bile acid production and cholesterol synthesis [96]. In addition, interindividual variation in response to dietary cholesterol accounts for a significant proportion of the interindividual variation in response to changes in fat quality [96]. Absorption of dietary cholesterol varies considerably, in part due to genetic variation in cholesterol absorption and biosynthesis, and the presence of cholesterol absorption inhibitors in the GI tract, such as plant sterols and stanols.

Another important issue that has been addressed is the safety of modification of plasma cholesterol. Cholesterol is indeed vital to every human cell, and many cells do acquire some cholesterol via uptake. However, all cells can synthesize the cholesterol they need, and cellular cholesterol deficiency occurs only when its synthesis is defective [97–99]. For example, individuals with heterozygous FH exhibit a 50 % reduction, while those with homozygous FH show a complete absence of LDLR-mediated cellular LDL uptake, but clinical symptoms are not consistent with cholesterol deficiency. In contrast, cholesterol synthesis defects such as Smith-Lemli-Opitz syndrome is detrimental already in fetal life. Because every cell can synthesize cholesterol, no cell has an absolute requirement for cholesterol *uptake* [98–100]. Plasma LDL-C levels can therefore be very low with few adverse effects. Even extremely low LDL-C caused by rare genetic disorders like abetalipoproteinemia is not associated with signs of cholesterol deficiency; instead, they are characterized by fat accumulation in hepatocytes and enterocytes due to absent chylomicron and VLDL synthesis, and fat-soluble vitamin deficiency [101]. Large RCTs have repeatedly verified the safety of lipid-lowering therapy [59]; for example, a recent observational analysis of the FOURIER trial showed that it was both effective and safe to reduce the LDL-C down to less than 20 mg/dL (0.52 mmol/L) using a PCSK9 inhibitor on top of statin therapy [102]. Accumulated clinical data thus support evidence from naturally occurring genetic variants [103].

The issue of safety is especially relevant for the most vulnerable groups in society; because most lipid-modification trials have been conducted in adults, extrapolating to children, for example, *could* pose a risk. However, the STRIP and DISC studies showed that dietary modification effectively lowers LDL-C and other risk factors for ASCVD in children and adolescents, while still allowing optimal growth and development [104–106]. It is equally safe to modify plasma lipids by statin therapy in children with FH [59,107,108].

3. Modification of plasma concentration of LDL particles by dietary fat quality: mechanistic evidence

What, then, causes dietary fat quality to affect the plasma concentration of LDL particles? Mechanisms linking fat quality and plasma lipids should be evaluated considering known physiological systems, as discussed previously (Fig. 1). However, a complicating factor is that experimental animal models vary in their lipid and lipoprotein metabolism. In fact, inter-species variation in lipid metabolism account for inter-species variation in *response* to dietary exposures [39,109–111]. For example, rats and dogs convert cholesterol to bile acids more effectively than rabbits, which explains why Anitschkow's initial findings in rabbits were irreproducible in rats and dogs, rendering these experimental animals unsuited to study the molecular effects of dietary fat quality on plasma lipids and ASCVD [39]. Mice are also resistant to developing atherosclerosis, and one common way to humanize the lipoprotein metabolism in mice include introducing loss-of-function mutations in the *APOE* gene, which, importantly, renders the animals much more sensitive to high-fat diets [109,111]. Other experimental animals, such as pigs, rabbits, and non-human primates, are characterized by a more human-like lipoprotein metabolism and are therefore naturally better suited to study the molecular effects of dietary exposures on plasma lipids [109,111].

Therefore, although still *partly* an open question, we do know *some* of the physiological mechanisms that mediate the effect of dietary fat quality on plasma concentration of LDL particles, discussed next.

An important mechanism by which dietary fat quality affects plasma LDL particles is by the regulation of the LDLR in liver [112]. Specifically, improved fat quality (for example exchanging dietary SFA by PUFA) will increase the expression of the LDLR which in turn improves the hepatic removal of LDL particles, and thereby the LDLR-mediated reverse cholesterol transport from plasma and peripheral tissues via liver to the gut for excretion with feces [113–118]. The effect on LDLR expression may result from lipids affecting the cellular cholesterol homeostasis via the cholesterol sensing SREBP2 system [56,112]. Notably, following a series of *in vitro* studies, Dietschy proposed the following model: chylomicron-derived fatty acids with chain length of 12–16 carbons were poor substrates for the ACAT reaction compared to for example oleic acid (C18:1n9), resulting in less free cholesterol being esterified and directed to lipid droplets for storage, and instead entering the

membrane pools as free cholesterol, including the regulatory pool of the ER [100]. Elevated free cholesterol in the regulatory pool would then suppress SREBP2-mediated expression of LDLR, and result in elevated plasma LDL-C.

Regardless of specific molecular mechanisms involved, several aspects of the dietary fat quality affect the regulation of hepatic LDLR expression, including the SFAs, TFAs, n6-PUFAs, dietary cholesterol, and the diterpenes in unfiltered coffee (cafestol, cahweol). Other dietary elements act via regulation of intestinal cholesterol uptake, such as dietary cholesterol, phytostanols/sterols, dietary fiber (beta-glucans), and calcium. Additionally, dietary fiber (beta-glucans) regulates bile acid and cholesterol excretion to the gut [112,119].

Finally, several lifestyle factors affect plasma concentration of TGs and thereby plasma concentration of apoB-containing lipoproteins [25,53] (Table 1). Accumulation of ectopic fat in the liver will increase the secretion of TG-rich VLDL particles, and this is exacerbated by adiposity, physical inactivity, and intake of dietary factors such as SFAs, alcohol and simple carbohydrates (sugar), and improved by dietary intake of n6-and n3-PUFAs. Variation in the LPL-mediated lipolysis of TGs in circulating lipoprotein particles also affects plasma TGs, which can be worsened by physical inactivity.

RQ3. *Does fat quality in the diet causally affect the plasma concentration of LDL particles and thereby the risk of ASCVD?*

In short, the preceding two sections covering RQ1 and RQ2 concluded with the following.

1. That the LDL particle concentration causally affects ASCVD, largely because:
a. Atherosclerosis is the main cause of most CVD,
b. LDL particles are both a necessary and sufficient driver of atherosclerosis, and
c. Modification of the plasma concentration of LDL particles will affect atherosclerosis mainly by two characteristics: the *magnitude* and *duration* of the modification (absolute change from baseline and number of years maintained), and
2. That fat quality is the *main* environmental determinant of plasma LDL particle concentration, especially the variation in SFA intake.

Under these assumptions, we can finally ask the RQ that we set out to answer: does fat quality in the diet causally affect the plasma concentration of LDL particles and thereby the risk of ASCVD? Indeed, several lines of evidence suggest that the *observed* effect of dietary fat quality on ASCVD is close to the predicted effect. We can answer this third RQ with controlled trials in humans and animals, epidemiological studies, and ecological studies.

1. Controlled trials in humans and animals

Researchers have examined RQ3 in numerous controlled trials; however, surprisingly few are of solid enough quality to be included in the highest-quality evidence summaries [120–122]. While Hooper et al. and Mozaffarian et al. included 15 trials (16 comparisons) and eight trials in their analyses, respectively [120,121], Sacks et al. described only *four* "core" trials (The Wadsworth Hospital and Veterans Administration Center in Los Angeles, The Oslo Diet-Heart Study, The British Medical Research Council, and The Finnish Mental Hospital Study) [123–126], and an additional six "noncore" trials (STARS, DART, Houtsmuller et al., Rose et al., The Minnesota Coronary Survey, and The Sydney Heart Study) [127–132]. Criteria for inclusion among the core trials were overall quality of the study design, execution of the trial, and the adherence to the dietary protocol. Specifically, the studies had to be a fair comparison of exposure to dietary SFA versus PUFA, be unconfounded by TFAs, have control of the dietary intake, have a duration of at least two years, verified adherence by objective biomarkers (change in plasma LDL-C or biomarkers of fatty acid intake), and assessed ASCVD outcomes by validated methods. The noncore trials, in contrast, were studies that failed to meet at least one of these criteria, thereby diluting the effect. In the *strictest* analyses including only the core trials, replacing SFA by similar amounts of PUFA reduced plasma cholesterol by 13–16 % from baseline, and reduced the risk of ASCVD with approx. 30 %, over the course of four to eight years [122].

Why were Sacks et al.'s criteria so crucial? Because they effectively focused on the controlled trials which had the greatest control over the exposure and comparator (dietary fat quality), the mediator (plasma LDL-C) and the outcome (ASCVD), plus all other relevant confounders and competing exposures (such as TFA). In addition, they focused on trials that lowered LDL-C a significant amount from baseline (magnitude) and maintained that change over a significant period (duration), both essential requirements to evaluate RQ3 in the causal framework. Of note, the reduction in plasma cholesterol was consistent with the change in dietary fat quality, as predicted by Keys' equation, and the reduced risk of ASCVD was consistent with the change in plasma cholesterol, as predicted by statin trials [122].

Moreover, because atherosclerosis takes time to develop, duration of intervention is an important, independent variable [120–122]. Sacks et al. chose a duration of at least two years, a sensible criterion considering that the turnover of tissue fatty acids occur slowly, and that there is a one-to two-year lag on the ASCVD benefit of statin therapy.

Some dietary interventions in humans have assessed atherosclerosis by imaging. For example, classical human trials showed reduced atherosclerosis when SFA was lowered in the diet, as measured by coronary angiography [127,133]. More recently, dietary fat modification from infancy to young adulthood had favorable effects on both lipid profile and insulin sensitivity [134] as well as surrogate measures of atherosclerosis, such as IMT and arterial distensibility of the abdominal aorta and carotid artery, measured by ultrasonography [135].

Compared with experiments in humans, experiments in animals are much more prevalent, and they also provide a far richer detail of the link between dietary fat quality, LDL particles, and atherosclerosis [109,111]. Feeding diets with different fat quality to non-human primates modify their plasma LDL particle levels and either *induce* or *prevent* the development of atherosclerosis [122,136,137]. This is important and convincing evidence, since such experiments cannot be performed in human subjects, and monkeys represent humans' closest living relatives.

2. Epidemiological studies

RQ3 has also been examined in numerous epidemiological studies, mainly prospective cohort studies and nested case-control studies. However, as for the controlled trials, the quality of the studies is variable, and only a subset is included in the highest-quality evidence summaries [5–7,122,138,139]. Among the most common limitations in the epidemiological studies examining RQ3 is inaccurate assessment of the dietary exposure and its comparator. For an *honest* comparison between different levels of SFA intake on the risk of ASCVD using observational study design, for example, it is essential that the dietary fat quality is assessed to a high degree of accuracy, and that the substitute nutrient is specified.

Then what do observational studies that *do* provide honest comparisons tell us? They tell us that each 5 E% lower intake of SFA and concomitantly 5 E% higher intake of PUFA associates with 25 % lower risk of ASCVD over the course of 24–30 years; likewise, exchanging 5 E% SFA for similar amounts of MUFA or whole-grain carbohydrates associates with 15 and 9 % lower risk of ASCVD, respectively, over the course of 24–30 years [122,140]. These effects can be interpreted as substitution effects [72]. In contrast, substituting 5 E% of SFA by refined-grain carbohydrates and added sugars both associates with 1 % higher risk of ASCVD, while a substitution of 2 E% of SFA by TFA associates with 5 % higher risk of ASCVD [122,140]. As for the clinical trials, note the critical importance of considering the duration of follow-up; in a similar pooled analysis of eleven cohort studies with only

4–10 years of follow-up, substituting 5 E% of SFA by an equivalent amount of energy from PUFA was associated with mere 13 % lower risk of ASCVD [141].

In SRs developed for the 2020 DGA, the effect of dietary cholesterol on blood lipids or ASCVD was considered inconclusive because of a small number of studies and inconsistent results [4]. Reasons include a relatively mild effect of dietary cholesterol on plasma lipids, large inter-individual response, low average dietary intake, and low variation in intake. However, high-quality prospective cohorts still conclude that higher intake of dietary cholesterol increases the risk of ASCVD, especially using substitution analyses [142,143]. Eggs are a major source of cholesterol in the diet, and thus of the variation in cholesterol intake. In a recent study, researchers found a stepwise increased risk of ASCVD up to ≥10 eggs per week, but only among people with high genetic risk [144].

Observational studies using *objective biomarkers* support these data: higher tissue levels of PUFAs – both omega 6 and omega 3 fatty acids – associate with lower risk of ASCVD [145–147].

These analyses also suggest that there are many ways to improve the fat quality of the diet. Unsaturated fats are often emphasized as the substitute nutrients. However, because dietary PUFA intake is recommended not to exceed approximately 10 E% due to potential adverse effects, a significant amount of SFA could also be replaced by food sources containing MUFAs, complex carbohydrates and plant-based protein, such as non-tropical vegetable oils, liquid and soft margarines, fatty fish, nuts and seeds, avocado and olives, mayonnaise- and oil-based spreads, pesto and dressings, whole grains, fruits, vegetables, berries, legumes (peas, beans, lentils), and potatoes.

Observational studies are undoubtedly among the types of studies that generate the most confusion. Controversial and misleading findings often arise when the observational study design is *inaccurate* and therefore *inappropriate* to answer a given research question. To address a research question such as the effect of fat quality on risk of ASCVD, we *must* consider existing knowledge both in the design and analysis of an observational study. This could be formalized in a PICOTSS statement [27] or a target trial protocol [148]. The aim of these exercises is to gain control of all aspects of the study design and analysis that might affect the effect of the exposure (dietary fat quality) on the outcome (ASCVD). High-quality observational studies should reduce various sources of bias and thereby *emulate or imitate a clinical trial* [122,148]. And indeed, results from well-conducted observational studies often align nicely with results from well-conducted clinical trials [148,149].

Let us revisit the claim we made initially in this section, and ask: *why* is inaccurate assessment of dietary fat quality so common in observational studies? Usually because of measurement error [19]. In addition, the intra-individual (day-to-day) variation in SFA intake is large compared to the inter-individual variation [150–153], and it has been estimated that you need approximately 8–20 individual 24HRs to *reliably* (±20 %) estimate mean SFA intake in a sufficiently large proportion of a sample [153,154]. As few cohort studies have included repeated intake assessments during follow-up, the exposure status may be misclassified, and the effect of the cumulative exposure is thus not considered.

Residual confounding is often also present, as dietary SFA intake correlates with both unhealthy and healthy behaviors depending on the context, especially regarding socio-economic status and food environment, and hence food sources containing SFA [18]. One way to meet this issue is to compare energy from SFA with other sources of energy. Substitution models are indeed more realistic considering that free-living humans maintain a relatively stable weight over time. Also, a substitution of SFA with PUFA, specifically, will amplify the known association between dietary fat quality and plasma concentration of LDL-C, which is predicted based on controlled trials, but may be absent in observational studies. One reason for this discrepancy between clinical trials and observational studies is that within homogenous populations, there is usually a high variation in plasma LDL-C but a low variation in dietary intake of SFA [19].

This leads us to another issue: over-adjusting for mediators [19]. Because LDL-C is the mediator on the causal pathway that links dietary fat quality and ASCVD, LDL-C *should* be predictably different across strata of dietary intakes. However, incorrectly adjusting for this mediator – or correlated measures – will result in null findings [16].

Ancel Keys acknowledged many of these limitations over half a century ago [155]. Because of the inherent difficulty in adequately assessing SFA intake on an individual level, our expectation is actually that there is *no* association between estimated SFA intake and plasma LDL-C or ASCVD in an epidemiological study [155]. However, as Keys also noted, we may still get a good estimate of the population average SFA intake, and can thereby compare across heterogeneous populations when there is a sufficiently wide range in dietary intake of SFA and plasma LDL-C [155].

3. Population-based changes: ecological studies

Well-conducted controlled trials try to examine the efficacy related to RQ3 under ideal conditions, for a short time period, and in a selected sample of the population. Well-conducted observational studies try to emulate the same, but for a longer time period, and in a larger sample of the population. However, when suitable data exists, RQ3 can even be examined and monitored over time in *entire* populations. Although ecological studies have a dubious reputation, they can be quite informative when we consider other sources of evidence on the same topic.

For example, what happens if we shift the population distribution of LDL-C to the left or right by dietary means? What do ecological studies tell us? Based on prior knowledge, we would expect that shifts in the dietary fat quality for an entire population would cause shifts in the population distribution of plasma LDL-C, which again would cause shifts in the population burden of ASCVD [29–31]. And this is indeed what we find when we examine evidence from populations experiencing planned policy change or unplanned experiments of nature.

For decades, policy change has been a core strategy to improve population health, one that enjoys broad international consensus [2]. In the 1970s, both Norway and Finland were world-leading in burden of ASCVD. Since then, both countries implemented measures to reduce the ASCVD incidence, with great success: within few decades, the risk of ASCVD fell to similar level as the Mediterranean countries. Most of the impressive improvement in ASCVD risk in Norway [156,157] and Finland [158–161] has been attributed to a decrease in LDL-C, and most of the decrease in LDL-C has been attributed to changes in diet, especially improved dietary fat quality (lower dietary intake of SFA and TFA), and a switch from unfiltered to filtered coffee [162]. While Finland always had a low dietary TFA intake, a reduction in dietary TFA intake partially explained the reduction in LDL-C in Norway [19]. Note also that changes in dietary PUFA intake probably had relatively little influence, at least in Norway, as estimated PUFA intake remained stable at 5–8 E% [19]. In other words, since the 1970s, Norway and Finland have effectively shifted the population distribution of LDL-C to the left – even before the statin era – by improving the dietary fat quality for the entire population, thereby reducing the population burden of ASCVD.

Population-based changes in LDL-C have been observed in many countries around the world from the 1980s onwards [163,164]. And while improved medical treatment is often cited as a major contributor to these changes, we believe that population dietary fat quality may also have had major impact. Statins, for example, became relevant following the 4S trial in 1994 and was being widely prescribed no earlier than the 2000s, long after the incidence of ASCVD had fallen dramatically. Interestingly also, some low-risk countries have experienced a shift towards higher-risk, such as China [165,166], Syria [167], and Tunisia [168], possibly resulting from changes in dietary intake and level of ASCVD risk factors rather than worsening of the medical treatment available to the population.

Experiments of nature are also informative. Both World Wars, for

example, caused dramatic changes in food supply for many countries, which associated with changes in the incidence of ASCVD. Norway, for example, experienced reduced incidence of ASCVD during World War II, which was associated with reduced food availability, especially foods of animal origin [169,170]. Notably, among the many factors that changed simultaneously, the war caused a drastic reduction in dietary intake of SFA from meat and dairy that would be predicted to reduce the population plasma LDL-C and risk of ASCVD. After the war ended, the population dietary intake returned to pre-war levels, while also being more influenced by American food culture. Consequently, the incidence of ASCVD increased again until the 1970s, in which public health measures were implemented.

Another informative data source is the Japanese migration studies. Japanese migrants moving from *low*-risk Japan to *medium*-risk Hawaii or *high*-risk San Francisco experienced concurrent elevations in plasma LDL-C and an associated increased risk for ASCVD, all of which was related to variation in population dietary intake – in particular dietary fat quality [171–173]. Similar results were found for populations living traditional hunter-gatherer lifestyles. In the indigenous Tsimane Indians, the lifetime exposure to ASCVD risk factors was low and ASCVD was virtually absent; however, the transition to an industrialized society was paralleled by an increase in both ASCVD risk factors and ASCVD as measured by CAC score [174]. Much of this change was linked to diet, including dietary fat quality.

3. Developing dietary guidelines

The controversy regarding the dietary recommendations for fat quality may also relate to an unclear understanding of the process of developing dietary guidelines. Therefore, we include a discussion of how nutrition research produces evidence and knowledge which can be used for recommendations.

3.1. The scientific method

The principles of the scientific method form the foundation for nutrition research. Science has been extremely successful in advancing mankind; one reason for this success is the willingness to *admit ignorance*, and that any belief at some point *may be refuted*, which contrasts with the pre-modern religious traditions that claimed omniscience and infallibility [175]. In fact, the scientific method *itself* has not always been the same; instead, it has been subject to changes as scientific reasoning and the philosophy of science has evolved [176]. Formulated in the simplified *hypothetico-deductive framework*, the scientific method contains five mains steps [177].

1. We observe the state of the world (observation).
2. We formulate a hypothesis (induction).
3. We formulate testable predictions based on the hypothesis (deduction).
4. We test our prediction by collecting new data (experiment/observation).
5. We assess the outcome of the test and evaluate the hypothesis (verification/falsification).

Probabilistic or *Bayesian* thinking is similar in that you should *update your priors* (background knowledge and assumptions) *when new evidence emerges* [178]. Because of these characteristics, building a causal framework using the scientific method enables probabilistic decision making under complexity and uncertainty, for example when encountering imperfect data as in nutrition research.

3.2. Evidence integration to inform decision making

We assess the relationships between dietary exposures and health outcomes using evidence integration methods [179], for example as described by WCRF (World Cancer Research Fund) [180], GRADE (Grading of Recommendations, Assessment, Development and Evaluations) [181], OHAT (Office of Health Assessment and Translation) [182], HEALM (Hierarchies of Evidence Applied to Lifestyle Medicine) [179], and NutriGrade [183], which represent modernized versions of the classic Bradford-Hill considerations for causal relationships [184]. A key part of evidence integration is the use of systematic reviews (SRs) [27,28,185]. Importantly, SRs provide a grading of the *confidence* in the body of evidence, which is then translated into *level of evidence* for health effects [182]. For example, WCRF uses the following confidence gradings (with corresponding level of evidence in parenthesis): convincing (strong evidence), probable (strong evidence), limited-suggestive (weak evidence), limited-no conclusion (weak evidence), and substantial effect unlikely (strong evidence). To inform dietary guidelines, we usually require that there is strong evidence for a health effect [27].

Despite being available for about two decades only, SR methodology is today considered critical in reliable assessment of exposure-outcome relationships, and only the *highest-quality* SRs are part of the knowledge base for development of dietary guidelines and nutrition recommendations [27,28,185].

However, regardless of the rigor, evidence integration cannot once and for all *prove* that a dietary exposure has an effect on a health outcome, as the totality of the evidence may change in the future. Therefore, the best we can do is to transparently communicate the evidence, including all its uncertainties, and provide a *best explanation* [180,186]. As described, our degree of confidence in any such explanation is determined by grading systems, and explanations that we feel *sufficiently confident in* (that is, *strong* evidence) are used to inform dietary guidelines. In other words, *best explanation* informs *best judgement* [180,186,187]. Dietary guidelines are therefore a result of probabilistic decision-making under uncertainty, and that may change with time.

4. Conclusions

We conclude by reiterating our main message: within an otherwise healthy dietary pattern, switching from the consumption of foods containing unhealthy fats (SFAs, TFAs and cholesterol) to the consumption of foods containing healthy fats (MUFAs, n6-PUFAs and n3-PUFAs) will prevent or improve dyslipidemia, reduce the cholesterol burden and rate of atherosclerosis progression, and reduce the risk of clinical ASCVD [1–7] (Fig. 4). In practical terms, we should for example choose low-fat over high-fat dairy products; choose lean meat and lean meat products over high-fat variants; choose cooking oils, liquid margarine and soft margarine over hard margarine and butter; and eat fish two to three times a week, including some fatty fish [1–7]. These food-based recommendations are reasonable, and they leave plenty of room for variation in the diet.

The dietary recommendations for fat intake are now well established. In fact, we believe that there is a low probability that they will *fundamentally* change in the future, considering the robustness of the totality of the evidence. Goldstein and Brown were quite clear in their message regarding statin therapy: “the main questions that remain about LDL-C and ASCVD are: 1) who to treat, 2) when to treat, and 3) how long to treat” [56]. Given the relevance of the dietary fat quality in this context, we are inclined to suggest that *everyone* can safely and effectively adopt a healthy fat quality within an otherwise healthy dietary pattern, they can adopt it *from weaning*, and they can maintain it *throughout life*.

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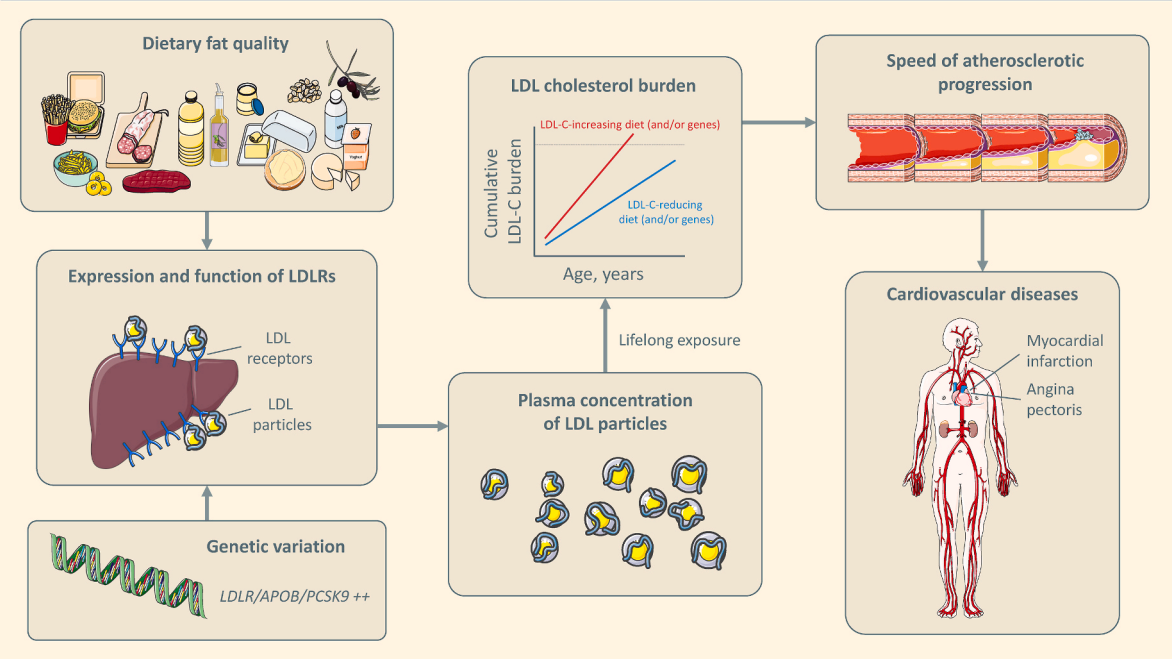

Fig. 4. Graphical abstract.
Dietary fat quality – especially the variation in dietary intake of saturated fatty acid (SFA) – is the main environmental determinant of plasma concentration of LDL particles (usually estimated by plasma LDL cholesterol [LDL-C]). Most of the effect of dietary fat quality on LDL particle concentration is via regulation of the expression and function of the LDL receptor (LDLR) in the liver (SFAs, monounsaturated fatty acids [MUFAs], trans-fatty acids [TFAs], and dietary cholesterol); however, some of the effect is via non-LDLR-related mechanisms, such as via activation of Liver X Receptor (n-6 polyunsaturated fatty acids [PUFAs], not shown in the figure). Genetic variation in some genes, for example LDLR, APOB and PCSK9, also affects the expression and function of LDLRs, and thus the plasma concentration of LDL particles. LDL particle concentration affects atherosclerotic cardiovascular disease (ASCVD) largely because 1) atherosclerosis is the main cause of most CVD; 2) LDL particles are both a necessary and sufficient driver of atherosclerosis; and 3) the effect of the plasma concentration of LDL particles on atherosclerosis is determined mainly by two characteristics: the absolute concentration and number of years maintained. Therefore, regardless of the underlying cause (dietary fat quality or genetic variation), variation in lifelong exposure to LDL particles give rise to variation in cumulative exposure (cumulative LDL-C burden), and thus variation in speed of atherosclerotic progression and incident atherosclerotic cardiovascular disease.

CRediT authorship contribution statement

Jacob J. Christensen: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Erik Kristoffer Arnesen:** Investigation, Validation, Writing – review & editing. **Amanda Rundblad:** Validation, Writing – review & editing. **Vibeke H. Telle-Hansen:** Validation, Writing – review & editing. **Ingunn Narverud:** Validation, Writing – review & editing. **Rune Blomhoff:** Supervision, Validation, Writing – review & editing. **Martin P. Bogsrud:** Validation, Writing – review & editing. **Kjetil Retterstøl:** Investigation, Validation, Writing – review & editing. **Stine M. Ulven:** Validation, Writing – review & editing. **Kirsten B. Holven:** Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

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