

## Does variation in serum LDL-cholesterol response to dietary fatty acids help explain the controversy over fat quality and cardiovascular disease risk?

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- 1 Does variation in serum LDL-cholesterol response to dietary fatty acids help explain the
- 2 controversy over fat quality and cardiovascular disease risk?
- 3
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### 17 Declarations of interests.

- 18 JAL is Deputy Chair of the UK Government's Scientific Advisory Committee on Nutrition (SACN)
- and was a member of SACN's Working Group on 'Saturated Fats and Health'. JAL Chairs and
- 20 RPM is Deputy Chair of the International Life Sciences Institute (ILSI) committee on 'Individual
- 21 Saturated fatty acids and Cardiovascular Risk'.
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### 27 1. Abstract

Background and Aims: Controversy over fat quality and cardiovascular disease risk 28 29 stems from a series of meta-analyses of prospective cohort and randomised 30 intervention trials, which found little evidence for a significant relationship between the intake of saturated fat and disease endpoints. Possible explanations for these null 31 findings include difficulties inherent in estimating true food intake, the confounding 32 effects of macronutrient replacement and food composition, and marked inter-33 individual variation in the response of serum LDL-cholesterol. The aim of this narrative 34 35 review was to present evidence for the existence and origins of variation in serum LDL-36 cholesterol response to the replacement of dietary saturated fat, and its potential to 37 explain the controversy over the latter. Methods/Results: The review provides evidence to suggest that variation in LDL-responsiveness may harbour significant 38 potential to confound the relationship between saturated fat and atherosclerotic 39 40 cardiovascular disease risk, thus undermining the effectiveness of the dietary guideline 41 to replace saturated fat with unsaturated fat. Conclusions: the identification and application of a simple biomarker of this phenomenon, would make it possible to tailor 42 dietary guidelines to LDL responsive individuals, who stand to gain a greater benefit to 43 their cardiovascular health. 44

### 47 2. Introduction

### 48 Serum low density lipoprotein, saturated fat; consensus amidst controversy

49 Since the discovery of low-density lipoprotein (LDL) in 1955, knowledge of its now established 50 roles as a causal risk factor in the pathogenesis of atherosclerotic cardiovascular disease (ASCVD) and target of cholesterol-lowering therapy, has made an incalculable contribution to 51 52 the reduction in morbidity and mortality from this disease worldwide. This remarkable progress in medical science has occurred against a backdrop of controversy and scepticism 53 over the strength of evidence to support the link between raised LDL cholesterol and ASCVD 54 [1], and more recently, dietary recommendations to lower serum LDL by reducing intake of 55 56 saturated fatty acids (SFA) [2,3]. In 2017 and 2020 [4, 5], consensus panels for the European 57 Atherosclerosis Society (EAS) concluded that LDL is a causal risk factor for the development of ASCVD. Simultaneously, independent expert scientific nutrition advisory committees 58 59 confirmed the validity of dietary guidelines to reduce SFA, by their replacement with 60 unsaturated fatty acids [6, 7], particularly polyunsaturated fatty acids (PUFA), in part, on the strength of the effect of this dietary change in lowering serum LDL-cholesterol (LDL-C). While 61 debate over the validity of this recommendation was positive in reinforcing its relevance to 62 human health, it also exposed weaknesses in the evidence for the impact of SFA on ASCVD, 63 64 and urgent need for a better understanding of the complex relationship between SFA and 65 serum LDL. The latter included gaining further insight into the effects of the specific macronutrient which replaces SFA in the diet, SFA in whole foods and dietary patterns [8], and 66 impact of inter-individual variation in the response of serum LDL to the reduction of SFA. The 67 following narrative review examines the evidence for the origins of this variation in serum LDL-68 C, and its potential contribution to the controversy over fat quality and ASCVD. Emphasis has 69 been placed on metabolic rather genetic determinants of this phenomenon, in areas where 70 71 the evidence is sufficiently robust to be appraised. The roles of obesity and related conditions 72 of insulin resistance in different genders and ethnic groups, while important, especially to the cardiometabolic origins of variance in LDL, were considered to lie beyond the scope of the 73 74 review.

#### 77 2. LDL cholesterol, apo B, and models of cholesterol homeostasis

78 The concentration of serum LDL is most commonly represented by its cholesterol content 79 ('LDL-C') but can also be expressed in terms of its total lipid and protein mass, or 80 concentration of its main structural protein, apoprotein B (apo B-100). Since each LDL particle carries a single polypeptide chain of apo B-100, this protein conveys information about the 81 82 number of LDL particles. While both total serum cholesterol and apo B are informative with respect to the association between LDL and cardiovascular risk, the most recent guidelines 83 from the European Cardiovascular Society and EAS, report that serum apo B provides the most 84 85 accurate marker of ASCVD, by providing a measure of the total number of atherogenic 86 lipoproteins in serum [9]. Moreover, serum apo B can be measured directly, inexpensively, 87 and with greater accuracy and precision than LDL-C, which is mostly calculated indirectly from 88 the Friedewald equation [10]. While these advantages confer greater all-round clinical utility 89 upon serum apo B [11, 12], LDL-C has remained the primary target for lipid-lowering drug therapy, in part, because of its relatively greater prominence in the mechanism to explain the 90 regulation of serum LDL and whole-body cholesterol homeostasis [13]. The lowering of serum 91 92 LDL-C is also the main target for the dietary management of ASCVD risk, by approaches such as the Portfolio Diet [14], though subtle differences exist between this approach and the 93 94 dietary management of elevated serum apo B [15].

95 The widely accepted view of serum LDL is that it provides cells with an available source of cholesterol, the uptake of which requires less energy than cholesterol biosynthesis. This view 96 97 is supported by a model of cholesterol homeostasis, whereby the cellular uptake of LDL is regulated by the expression and activity of cell surface LDL-receptors, the gene-transcription 98 99 of which is regulated by the amount of intra-cellular free cholesterol [13]. The size of the intracellular pool of free cholesterol is governed by the rate of cholesterol biosynthesis, export of 100 cholesterol from the liver as bile acids and free cholesterol in bile, reabsorption of these bile 101 102 acids and cholesterol in the gut, and uptake of serum LDL by LDL-receptors. Cholesterol biosynthesis co-ordinates with these other processes in a reciprocal fashion, to maintain a 103 104 mass of intra-cellular cholesterol that is appropriate for the requirement of cells, and, at the 105 same time, regulates and concentration of serum LDL [13]. However, because this traditional 106 model was largely developed in fibroblasts in vitro, it does not reflect the complexity of

cholesterol homeostasis in vivo [16]. In a mutually inclusive update of this conventional model, 107 108 it has been proposed that cholesterol entering the liver in LDL, HDL or chylomicrons has 109 different fates. In this updated model, LDL-derived cholesterol is largely shunted into the 110 production of VLDL, without influencing the regulatory pool of intra-cellular cholesterol or expression of LDL-receptors, and HDL-derived cholesterol is incorporated into the production 111 of bile acids. Most critically, it is the uptake of cholesterol into the liver in chylomicrons, and 112 presumably their remnants, that enters the regulatory pool of intra-cellular cholesterol, and 113 therefore is chiefly responsible for suppressing the activity of LDL-receptors [16]. This latter 114 115 pathway has major implications for the metabolic coupling of serum LDL and triacylglycerol-116 rich lipoproteins and their remnants, and atherogenic roles of these lipoproteins in ASCVD.

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# Influence of dietary fatty acids on serum LDL-C; fundamental importance of macronutrient replacement

Arguably the strongest evidence to support dietary SFA as a modulator of total serum 120 cholesterol, comes from tightly controlled, metabolic ward studies in the early 1950s, in which 121 122 total serum cholesterol was manipulated by altering the relative proportions of dietary SFA and unsaturated fatty acids, from animal and plant sources, within milk shakes [17-19]. These 123 124 findings were later supported by the outcome of epidemiological studies of Ancel Keys [20, 21], which laid the foundation for the 'diet-heart hypothesis' and earliest guideline to reduce 125 126 intake of total fat and SFA to prevent heart attacks in the USA in 1961 [22]. Further evidence for the efficacy for this hypothesis would follow from randomised intervention trials (RCT) [23-127 128 25], and the most comprehensive meta-analysis to date of RCTs, that showed a 27% reduction in cardiovascular events in response to the replacement of SFA with polyunsaturated fat [26]. 129 130 A fundamental principle that distinguishes the relatively subtle physiological effects of diet from the pharmacological effects of drugs, is the obligation to replace a removed 131 132 macronutrient with a substitute macronutrient to render the diet viable. In the case of SFA, 133 the substitute macronutrients of choice are either unsaturated fatty acids (PUFA or MUFA),

134 carbohydrates or proteins. The replacement of SFA with unsaturated fats or carbohydrates

have been shown to reduce serum LDL-C, in a dose-response fashion, with contributions to

these effects coming from both the removal of SFA, and the type and quality of substitute

macronutrient [27]. Isocaloric replacement of 1% energy from dietary SFA with PUFA, chiefly 137 in the form of linoleic acid, has been shown to be more effective in lowering serum LDL-C 138 139 (mean change -0.055, 95% CI -0.061 to -0.050 mmol/L P <0.001) than the equivalent 140 replacement of SFA with either MUFA (mean change -0.042 mmol/L, 95% CI -0.047 to 0.037 mmol/L, P <0.001) or carbohydrate (mean change -0.033, 95% CI -0.039 to -0.027 mmol/L, P 141 <0.001) [28]. Nevertheless, increased demand for low fat diets and food products has 142 143 invariably favoured the replacement of SFA with carbohydrate in preference to unsaturated fat in the USA and UK. The latter dietary exchange is estimated to be associated with an 144 145 unfavourable increase in serum triacylglycerol (mean change 0.011, 95% CI 0.007 to 0.014 146 mmol/L, P<0.001) [28], and raises the significance of carbohydrate quality, specifically in 147 relation to the opposing effects of dietary fibre and free sugars on serum triacylglycerol and 148 other cardiometabolic risk factors.

Other relevant dietary sources of variation in serum LDL-C, include the effects of specific dietary fatty acids of variable chain length and capacity to raise and lower serum LDL-C [29], and other constituents in whole foods (e.g. minerals, food matrix), meals, and dietary patterns [30], which can alter the bioavailability and exposure to dietary SFA.

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### 154 4. Evidence for variation in serum LDL-C in response to dietary cholesterol and SFA

Serum LDL-cholesterol varies within (intra) and between (inter) individuals in response to intrinsic factors (e.g. polymorphism and expression of genes, hormones) and extrinsic factors (e.g. diet, behaviour), and interactions between the two. Estimates for the proportion of interindividual variation in serum LDL-C that can be ascribed to genetic heritability in and between populations, though wide ranging (20-90%) [31], still accommodates a significant contribution from environmental factors, including diet and nutrient-gene interactions.

161 The first reports of hyper and hypo-responsiveness of serum LDL to diet were in response 162 to variable amounts of dietary cholesterol from eggs [32, 33]. This variation was not an acute 163 artefact of the experimental design or due to variation in dietary compliance, but a 164 reproducible phenomenon that would manifest in response to a second exposure to the same 165 diet [34, 35]. It was established that dietary cholesterol and SFA exert additive, and even 166 synergistic effects on serum LDL-C, but also that dietary cholesterol could exert its effects on 167 LDL in the absence of SFA. Hyper and hypo-responsiveness in serum LDL-C was described as differing degrees of change at either end of a continuous spectrum of responses to dietary
cholesterol, rather than two discrete distributions or phenotypes [36]. In retrospect, the latter
would be unlikely in view of the multiple genes and metabolic variables contributing to interindividual variation.

The most well documented example of inter-individual variation in serum LDL-C in 172 response to a reduced intake of SFA in men and women, comes from the effects of the US 173 174 National Cholesterol Education Programme's (NCEP) Step 2 diet [37]. Low in total fat (18-29% energy) and SFA (4-7% energy), the Step 2 diet has been shown to produce dramatic 175 176 reductions in serum LDL-C and significant variation between individuals. Exposure to this diet 177 from between 4.5-24 weeks was reported to produce changes in serum LDL-C ranging from +3 178 to -55% and +13 to -39% in men and women, respectively. In this case, 48% of this variation 179 could be accounted for by baseline LDL-C concentration and age in men, and 13% to age in 180 women (Figure 1A). After taking into consideration variation in dietary compliance, and 181 controlling for this and other extrinsic factors, significant variation was attributed to apo E 182 genotype. Significant variation in serum LDL-C has also been observed in response to an increased intake of SFA in two randomised controlled intervention studies; 'Dletary fat & 183 184 VAScular function' 'DIVAS' study, Figure 1(B), and 'Reading, Imperial, Surrey, Cambridge & Kings' ('RISCK') study' [38, 39]. Rigorous control of confounding, extrinsic factors and dietary 185 186 compliance in these studies, provided further evidence to suggest that the variation in serum LDL-C originated from intrinsic biological differences in the metabolic handling and impact of 187 dietary SFA on cholesterol homeostasis between individuals. 188

189

190 5. Origins of variation in serum LDL-C in response to diet and SFA

### 191 **5.1** Confounding influences of inter and intra-variation in serum LDL-C

192 Dietary guidelines to reduce disease risk are primarily designed for human populations that show inherent variability in risk susceptibility, dietary compliance, and response to 193 dietary recommendations. When variation in an outcome measure (serum LDL-C) in response 194 195 to an intervention (replacement of dietary SFA) is greater between individuals than the average response of the study population, this will reduce the ability of that study to 196 demonstrate a significant effect of the intervention on that outcome measure. It is evident in 197 198 each of the studies shown in Figures 1 (A) & (B) that the magnitude of inter-individual 199 variation in response to SFA intake is greater than the mean response, which will effectively

200 reduce the significance of the dietary intervention [40]. Similarly, the amount of error and 201 ability to demonstrate a significant association between two variables depends on the ratio of 202 the intra to inter-variability in these variables. If intra-variation is greater than the inter-203 individual variation, this will attenuate the strength of association between the two variables [41]. While this has been reported to apply to the association between serum LDL-C and 204 dietary SFA, this is not supported by observations of inter and intra-variation in LDL-C in 205 206 response to diet. A comparison of inter and intra-individual variation in total serum 207 cholesterol in 58 men, on six different dietary regimens for between 3-10 weeks, showed that 208 inter-individual variation (between men) was nearly two-fold greater than variation within 209 these men [42] (Figure 2). Irrespective of this difference, it is likely that both inter- and intra-210 variation will attenuate the strength of associations between LDL-C, SFA and CVD, and reduce 211 the strength of the statistical evidence on which dietary recommendations are based, even 212 within dietary compliant cohorts. Identification of this variation in LDL response to SFA, 213 together with an increased understanding of the metabolic origins of these traits, would 214 provide the opportunity to tailor dietary recommendations to serum LDL-C-responsiveness, to enhance the effects of this dietary change in a more personalised dietary approach. 215

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### 217 5.2 Mechanistic insights from the effects of dietary cholesterol in metabolic studies

218 The human liver and gut work in concert to regulate the rates of endogenous cholesterol 219 synthesis and absorption, through a reciprocal mechanism that suppresses cholesterol 220 synthesis in the liver in response to increased cholesterol absorption in the gut, and vice versa. 221 This mechanism is largely driven by the inter-connecting entero-hepatic circulation that 222 produces and reabsorbs bile acids (and biliary cholesterol) to facilitate the absorption of dietary fat and cholesterol [43]. As discussed previously, the reciprocal relationship between 223 224 the absorption of dietary cholesterol, and biosynthesis of cholesterol, chiefly in the liver, 225 effectively controls the amount of free cholesterol (FC) within cells, which ultimately regulates 226 the concentration of serum LDL-C by adjusting the uptake of LDL into cells via membrane LDL 227 receptors. Expression of LDL-receptors is governed by a mechanism of inhibition feedback 228 that modulates the transcription of the LDL-receptor gene by 'sensing' the level of intra-229 cellular free cholesterol. This mechanism also forms the basis of our understanding of the 230 differential effects of dietary fatty acids on serum LDL-C, mediated through differences in the

esterification of intra-cellular cholesterol, as described in the pioneering work of John Dietschy[44, 45].

233 While it is often assumed that the 'push-pull' reciprocity between cholesterol biosynthesis 234 and absorption is finely attuned, there exists the possibility for inter-individual variability in the magnitude to which these variables can respond to each other and become misaligned. 235 Imbalance in these processes would manifest as distinct metabolic phenotypes or 236 237 'metabotypes' characterised by either higher cholesterol synthesis (low absorption) or higher absorption (low synthesis). Evidence from metabolic studies for the existence of such 238 239 metabotypes, who are respectively less and more sensitive to dietary cholesterol, may 240 underlie the phenomenon of hypo and hyper-responsiveness of serum LDL-C to dietary 241 cholesterol, which may, in part, be an inherited trait [46]. The relatively greater efficacy of 242 LDL-lowering drugs that either inhibit cholesterol synthesis or block absorption in the gut (e.g. 243 statins and ezetimibe) in synthesisers or absorbers of cholesterol, respectively, provides 244 further evidence for the existence of these discrete metabotypes [47, 48]. Factors governing 245 the absorption and synthesis of cholesterol are summarised in Figure 3.

246

### **5.3 Key role of bile acids in the absorption of dietary SFA and cholesterol**

The additive and even synergistic effects of dietary SFA and dietary cholesterol on serum 248 249 LDL-C, reflect the fact that these dietary lipids share common determinants of cholesterol 250 homeostasis. While congruence in the response of serum LDL-C response to these dietary components may be helpful in explaining the origins of variation in serum LDL-C to dietary 251 252 SFA [49], dietary fatty acids and cholesterol are absorbed by different mechanisms. The bulk 253 of dietary SFA (98%) is absorbed in the upper jejunum, whereas about 50% of cholesterol in the gut lumen is absorbed throughout the small intestine, via a series of regulatory transport 254 255 proteins. However, since the absorption of both dietary lipids depends on the production and 256 resorption of bile salts in the entero-hepatic circulation, the metabolism of bile acids provides 257 a credible link between dietary SFA, cholesterol synthesis and absorption [47], which could 258 help to explain variation in LDL-C response to SFA.

Bile acids are the products of metabolic events occurring primarily between the liver and gut microbiota. Primary bile acids are synthesised in the liver from cholesterol and conjugated with either taurine or glycine to form bile salts, which are stored in the gall bladder and

secreted into the bile. This conjugation step enhances bi-polarity, which increases the 262 263 capacity of bile acids to emulsify dietary fat for absorption. Conversely, bacterial bile salt 264 hydrolases (BSH) deconjugate primary bile salts in the gut, reducing their efficiency to 265 emulsify dietary fat [50, 51]. The circulating bile acid pool contains more than 30 known bile acids, the diversity of which is largely driven by the gut microbiota. In addition to facilitating 266 fat absorption, bile acid production drives the flow of bile. Bile acids also act as key cell 267 signalling molecules, which serve as ligands for nuclear receptors that regulate the 268 transcription of genes involved in lipid metabolism [52-54]. The gut microbiota shares a bi-269 270 directional relationship with dietary fat, by influencing the absorption of fat through bile salts, 271 and, in turn, being modified by dietary fat. The BSH activity of certain bile acid-deconjugating 272 lactobacilli and bifidobacteria may be especially relevant in the former respect, by reducing 273 the absorption of dietary fat and lowering serum LDL-C, as shown in human intervention 274 studies with probiotics [55]. The microbiota may also influence the effects of dietary SFA on 275 serum LDL-C through the production of short chain fatty acids (SCFA) [56]. Acetate and 276 propionate have been shown to stimulate and inhibit cholesterol biosynthesis, respectively. 277 Propionate may also inhibit the uptake of acetate into hepatocytes, thus producing down-278 stream effects on cholesterol metabolism. In this respect, a high SFA diet has been reported 279 to increase the excretion of SCFA, which attenuated the significant reduction in serum LDL-C 280 when switching to a low SFA diet [56].

281

### 282 **5.4 Relevance of LDL particle size distribution and subclass phenotype**

In keeping with the other main classes of serum lipoproteins, LDL shows structural and
metabolic heterogeneity and exists as a variable number of discrete LDL subclasses [57]. When
characterised and quantified by their hydrated density, particle size, and unique magnetic
signatures, LDL subfractions express a gradient of increasing atherogenic potential on moving
from large, buoyant LDL, to small, dense LDL [5, 58].

Dietary SFA have been reported to act primarily on larger LDL particles [59, 60], and since larger LDL is associated with lower ASCVD risk, this idea has been invoked to explain the lack of evidence for a direct link between SFA and ASCVD. A potential flaw in this idea lies in the fact that if larger LDL were unrelated to CVD risk, this would tend to negate the positive risk

association between serum LDL-C and ASCVD in populations, since for most people without a 292 293 predominance of small, dense LDL, the bulk of LDL mass will reside in 'larger' cholesterol-rich 294 subfractions. Mechanistically, there is evidence to suggest that larger LDL express a higher 295 affinity for LDL-receptors than smaller, dense LDL [61]. As such, the effect of adding or replacing dietary SFA on LDL-receptor activity should be to selectively increase or decrease 296 larger LDL, respectively. However, this may not be the case if the uptake of cholesterol from 297 298 LDL has a minimal effect in regulating intra-free cholesterol and production of LDL receptors in vivo. It could also be off-set by the nature of substitute macronutrient, with refined 299 300 carbohydrate producing the opposite effect to SFA on large LDL [59]. Understanding how LDL 301 particle size influences the effect of SFA replacement on serum LDL-C, and LDL particle 302 number (LDL-apo B), has been difficult to establish, and may depend on the initial distribution 303 of LDL particle size (LDL subclass phenotype), dietary exchanges, and threshold effects of SFA 304 intake [62].

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### 306 **5.5 Genetic polymorphism in apoprotein E**

307 Numerous common single nucleotide polymorphisms have been reported to influence the 308 response of serum LDL-C to dietary fats, the address of which lies beyond the scope of this 309 review [63-71]. Of all common genetic traits studied to date, two missense single nucleotide 310 polymorphisms in the apoprotein E gene (rs429358 and rs7412 at codons 112 and 158, 311 respectively) are by far the most well documented in relation to variance in serum LDL-C and diet. These polymorphisms produce different isoforms of apoprotein E with variable capacity 312 to function as ligands for the binding of triacylglycerol-rich lipoproteins and their remnants, 313 and HDL, to cell surface receptors, including LDL receptors. They are reported to account for 314 315 up to 8-10% of variance in serum LDL-C in populations [72], primarily, by influencing the regulatory pool of intra-cellular free cholesterol and activity of LDL-receptors, as described 316 317 previously. Apo E polymorphism has also been linked to variation in serum LDL-C response to changes in dietary SFA and cholesterol [73, 74]. Most notably, carriers of the  $\varepsilon$ 4 allele (apo E4 318 319 isoform) tend to have elevated serum LDL-C (5-10%) and are consistently more responsive to 320 changes in SFA, primarily because of the common pathways by which dietary SFA, and to a lesser extent, dietary cholesterol elevate serum LDL-C by modulating intra-cellular cholesterol 321 322 and the expression of LDL receptors. Carriage of the apo E4 variant has also been shown to be

more effective in lowering serum LDL-C and apo B than wild type (E3/E3), when SFA is

replaced with low glycaemic index carbohydrates [75].

325

### 326 6. Future perspectives and conclusions

327 The cardiovascular risk that can be attributed to elevated serum LDL-C in a population is a 328 function of the absolute risk (mortality associated with the concentration of raised LDL-C over 329 a prospective follow-up period), and number of people with that level of serum LDL-C. Moderately elevated serum cholesterol is extremely common in populations, but carries a 330 relatively low absolute risk in comparison to some other risk factors, such as blood pressure, 331 making both total serum cholesterol and LDL-C poor discriminators of ASCVD risk within 332 333 populations. Inter-individual variation in disease risk associated with elevated serum LDL-C 334 and its variable response to treatment, including diet, will contribute to this low absolute risk. 335 As such, a serum biomarker of serum LDL-C responsiveness to the replacement of dietary SFA 336 would have major utility in increasing the power to discriminate disease risk, in this otherwise 337 diagnostically grey area.

While the impact of replacing SFA on serum LDL-C is considerably less than can be achieved with lipid-lowering drugs, the combination of several dietary bio-actives for LDL-lowering within dietary patterns, such as the Portfolio [13] and Mediterranean diets [76], can reduce serum LDL-C by up to 30%. In this context, the identification of serum LDL-C responsive individuals would increase efficacy, by the targeting of dietary advice to LDL-responsive individuals who stand to gain the most benefit.

In conclusion, the answer to the question 'Does variation in serum LDL-cholesterol response to
dietary fatty acids help in explaining the controversy over fat quality and cardiovascular
disease risk?' is likely to be 'yes', since this variation, together with its genetic and metabolic
origins, will attenuate the strength of statistical associations between LDL-C, SFA and ASCVD.

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352	References
353	[1] D. Steinberg, An interpretive history of the cholesterol controversy, part II: the early
354	evidence linking hypercholesterolemia to coronary disease in humans, J Lipid Res 46 (2005)
355	179-190.
356	
357	[2] Z. Harcombe, J.S. Baker, J.J. DiNicolantonio, F. Grace, B. Davies, Evidence from randomised
358	controlled trials does not support current dietary fat guidelines: a systematic review and
359	meta-analysis, Open Heart 3 (2016) e000409.
360	
361	[3] J.L. Heilsen, Dietary saturated fat and heart disease: a narrative review, Nutr Rev 78 (2020)
362	474-485.
363	
364	[4] B.A. Ference, H.N. Ginsberg, I. Graham, K.K. Ray, C.J. Packard, Bruckert E, et al., Low-
365	density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic,
366	epidemiologic, and clinical studies. A consensus statement from the European
367	Atherosclerosis Society Consensus Panel, Eur Heart J 38 (2017) 2459-2472.
368	
369	[5] J. Boren, M.J. Chapman, R.M. Krauss, C.J. Packard, J.F. Bentzon, C.J. Binder et al., Low-
370	density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological,
371	genetic, and therapeutic insights: a consensus statement from the European
372	Atherosclerosis Society Consensus Panel, Eur Heart J 41, (2020) 2313-2330.
373	
374	[6] World Health Organization. Draft guidelines on saturated fatty acid and trans-fatty acid
375	intake for adults and children. Public Consultation May to June 2018. https://extranet.who.
376	int/dataform/upload/surveys/666752/files/Draft%20WHO%20SFA-TFA%20guidelines_
377	04052018%20Public%20Consultation(1).pdf

379	[7] The Scientific Advisory Committee on Nutrition (SACN) report on saturated fats and
380	health, (2019) <u>https://www.gov.uk/government/publications/saturated-fats-and-health-</u>
381	sacn-report. (Accessed 20/11/2020).
382	
383	[8] A. Astrup, H.C.S. Bertram, J.P. Bonjour, L.C.P. de Groot, M.C.O. Otto, E.L. Feeney et al.,
384	WHO draft guidelines on dietary saturated and trans fatty acids: time for a new approach?
385	Brit Med J 366 (2019) I4137.
386	
387	[9] F. Mach, C. Baigent, A.L. Catapano, K.C. Koskinas, M. Casula, L. Badimon et al., ESC/EAS
388	Guidelines for the management of dyslipidaemias: lipid modification to reduce
389	cardiovascular risk. Eur Heart J 41 (2020) (2019) 111-188.
390	
391	[10] W.T Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density
392	lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin Chem
393	18, (1972) 499-502.
204	
394	
395	[11] C.N. Kohli-Lynch, G. Thanassoulis, A.E. Moran, A.D. Sniderman, The clinical utility of apoB
396	versus LDL-C/non-HDL-C, Clin Chim Acta 508, (2020) 103-108.
397	
208	[12] A.D. Spiderman, Did the ACC/AHA/AAC\/DR/AARA/ARC/ACDM/ADA/ACS/ADhA/ASPC/NI A
200	(IZ) A.D. Shiderhan, Did theACC/Ana/AACV/R/AAPA/ABC/ACPN/ADA/AGS/AFNA/AS-C/NLA
399	/PCNA cholesterol guidelines get apo B right?, J Clin Lipidol 13 (2019) 360-336.
400	
401	[13] M.S. Brown, J.L. Goldstein, A receptor-mediated pathway for cholesterol homeostasis,
402	Science 232 (1986) 34-47.
403	

404	[14] L. Chiavaroli, S.K. Nishi, T.A. Khan, C.R. Braunstein, A.J. Glenn, S.B. Mejia, et al.,
405	Portfolio dietary pattern and cardiovascular disease: A systematic review and meta-
406	analysis of controlled trials, Prog Cardiovasc Dis 61 (2018) 43-53.
407	
408	[15] V. Lamantia, A.D. Sniderman, M. Faraj, Nutritional Management of hyperapoB, Nutr Res
409	Rev 29 (2016) 202-233,
410	
411	[16] R.S. Kiss, A.D. Sniderman, Shunts, channels and lipoprotein endosomal traffic: a new model
412	of cholesterol homeostasis in the hepatocyte, J Biomed Res 31 (2017) 95-107.
413	
<b>414</b>	[17] I. W. Kinsell, I. Partridge, I. Boling, S. Margen, G. Michael, Dietary modification of serum
415	cholesterol and phospholinid levels. J Clin Endocrinol Metab 12 (1952) 909-913
413	
416	
417	[18] E.H. Ahrens Jr., D.H. Blankenhorn, T.T. Tsaltas, Effect on human serum lipids of substituting
418	plant for animal fat in diet, Proc Soc Exp Biol Med 86 (1954) 872-878.
419	
420	[19] R. Clarke, C. Frost, R. Collins, P. Appleby, R. Peto, Dietary lipids and blood cholesterol:
421	quantitative meta-analysis of metabolic ward studies, Brit Med J 314 (1997) 112-117.
122	
422	
423	[20] A. Keys, J.T. Anderson, F. Grande, Serum cholesterol in man: diet fat and intrinsic
424	responsiveness, Circulation19 (1959) 201-214.
425	
426	[21] A. Keys, J.T. Anderson, F. Grande, Serum cholesterol response to changes in diet. IV.
427	Particular saturated fatty acids in the diet, Metabolism 14 (1965) 776-787.
428	

[22] Report of the Committee for Medical and Community Program of the American Heart
Association, Dietary Fat and Its Relation to Heart Attacks and Strokes, Circulation 23 (1961)
133-136.

432

433 [23] P. Leren, The effect of plasma cholesterol lowering diet in male survivors of myocardial

434 infarction. A controlled clinical trial, Acta Med Scand 466 (Suppl) (1966) 1-92.

435

436 [24] S. Dayton, M.L. Pearce, H. Goldman, A. Harnish, D. Plotkin, M. Shickman, et al., Controlled
437 trial of a diet high in unsaturated fat for prevention of atherosclerotic complications, Lancet
438 2 (1968) 1060-1062.

439

[25] M. Miettinen, O. Turpeinen, M.J. Karvonen, R. Elosuo, E. Paavilainen, Effect of cholesterollowering diet on mortality from coronary heart disease and other causes. A twelve-year
clinical trial in men and women, Lancet 2 (1972) 835-838.

443

444 [26] L. Hooper, N. Martin, A. Abdelhamid, G.D. Smith, Reduction in saturated fat intake for
445 cardiovascular disease. Cochrane Database System Review, 101 (2015) 1938-1940.

446

[27] R. Micha, D. Mozaffarian, Saturated fat and cardiometabolic risk factors, coronary heart
disease, stroke, and diabetes: a fresh look at the evidence, Lipids 45 (2010) 893-905.

449

[28] R.P. Mensink, Effects of saturated fatty acids on serum lipids and lipoproteins: a
systematic review and regression analysis, Geneva: World Health Organization; 2016.
https://apps.who.int/iris/bitstream/handle/10665/246104/9789241565349-eng.pdf

453

454	[29] R.P. Mensink, P.L. Zock, A.D.M. Kester, M.B. Katan, Effects of dietary fatty acids and
455	carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and
456	apolipoproteins: a meta-analysis of 60 controlled trials, Am J Clin Nutr 77 (2003) 1146-
457	1155.
458	
150	
459	[30] A. Astrup, F. Magkos, D.M. Bier, J.T. Brenna, M.C.de Oliveira Otto, J.O. Hill, Saturated fats
460 461	Cardiol 76, (2020) 844-857.
462	
463	[31] G. Jermendy, T. Horvath, L. Littvay, R. Steinbach, A. L. Jermendy, A.D. Tarnoki et al., Effect
464	of genetic and environmental influences on cardiometabolic risk factors: a twin study,
465	Cardiovas Diabetol 10 (2011) 96.
466	
467	[32] A.C. Beynen, M.B. Katan, Inter-individual variation in the cholesterolemic response to
468	dietary cholesterol, Prog Clin Biol Res 188 (1985) 195-207.
469	
470	[33] M.B. Katan, A.C. Beynen, J.H. de Vries, A. Nobels, Existence of consistent hypo- and hyper-
471	responders to dietary cholesterol in man, Am J Epidemiol 123 (1986) 221-234.
472	
473	[34] A.C. Beynen, M.B. Katan, L.F. Van Zutphen, Hypo- and hyperresponders: individual
474	differences in the response of serum cholesterol concentration to changes in diet, Adv
475	Lipid Res 22, (1987) 115-171.
476	
477	[35] C. Cox, J. Mann, W. Sutherland, M. Ball, Individual variation in plasma cholesterol response
478	to dietary saturated fat, Brit Med J 311 (1995) 1260-1264.
479	
480	[36] A.J. Wallace, J.I. Mann, W.H. Sutherland, S. Williams, A. Chisholm, C.M. Skeaff, Variation in
481	plasma cholesterol response to dietary change, Nutr Metab Cardiovasc Dis 9 (1999) 176-
482	183.
483	

484	[37] E.J. Schaefer, S. Lamon-Fava, L.M. Ausman, J.M. Ordovas, B.A. Clevidence, J.T. Judd, et al.,
485	Individual variability in lipoprotein cholesterol response to National Cholesterol Education
486	Program Step 2 diets, Am J Clin Nutr 65 (1997) 823-830.
487	
488	
489	[38] K. Vafeiadou, M. Weech, H. Altowaijri, S. Todd, P. Yaqoob, K.G. Jackson, et al.,
490	Replacement of saturated with unsaturated fats had no impact on vascular function but
491	beneficial effects on lipid biomarkers, E-selectin, and blood pressure: results from the
492	randomized, controlled Dietary Intervention and VAScular function (DIVAS) study, Am J Clin
493	Nutr 102 (2015) 40-48.
494	
495	[39] S.A. Jebb, J.A. Lovegrove, B.A. Griffin, G.S. Frost, C.S. Moore, M.D. Chatfield MD, et al.,
496	Effect of changing the amount and type of fat and carbohydrate on insulin sensitivity
497	and cardiovascular risk: the RISCK (Reading, Imperial, Surrey, Cambridge, and Kings)
498	Trial <i>, Am J Clin Nutr</i> 92 (2010) 748-758.
499	
500	[40] D.R. Jacobs, Jr., J.T. Anderson, H. Blackburn, Diet and serum cholesterol - do zero
500	correlations negate the relationshin? Am I Enidemiol 110 (1979) 77-78
501	
502	
503	[41] D. Kromhout, J.M. Geleijnse, A.Menotti, D.R. Jacobs Jr., The confusion about dietary fatty
504	acids recommendations for CHD prevention, Brit J Nutr 106 (2011) 627-663.
505	
506	[42] D.R. Jacobs, Jr., J.T. Anderson, P. Hannan, A. Keys, H. Blackburn, Variability in individual
507	serum cholesterol response to change in diet, Arteriosclerosis 3 (1983) 349-356.
508	
509	[43] P.A.S. Alphonse, P.J.H. Jones, Revisiting human cholesterol synthesis and absorption: The
510	reciprocity paradigm and its key regulator. Lipids 51 (2016) 519-536.
511	

512	[44] J.M. Dietschy, L.A. Woollett, D.K. Spady, The interaction of dietary cholesterol and
513	specific fatty acids in the regulation of LDL receptor activity and plasma LDL-cholesterol
514	concentrations, Ann N Y Acad Sci. 676 (1993) 11-26.
515	
516	[45] J.M. Dietschy, Dietary Fatty Acids and the Regulation of Plasma Low Density Lipoprotein
517	Cholesterol Concentrations, J Nutr 128 (1998)444S-448S.
518	
519	[46] H. Gylling, T.A. Miettinen, Inheritance of cholesterol metabolism of probands with high or
520	low cholesterol absorption, J Lipid Res 43 (2002) 1472-1476.
521	
522	[47] S. Santosa, K.A. Varady, S. AbuMweis, P.J. Jones, Physiological and therapeutic factors
523	affecting cholesterol metabolism: does a reciprocal relationship between cholesterol
524	absorption and synthesis really exist?, Life Sci 80 (2007) 505-514.
525	
526	[48] P.E. Ziajka , M. Reis, S. Kreul, H. King, Initial low-density lipoprotein response to statin
527	therapy predicts subsequent low-density lipoprotein response to the addition of
528	ezetimibe, Am J Cardiol 93 (2004) 779-780.
529	
530	[49] M.B. Katan, M.A.M. Berns, J.F.C. Glatz, J.T.Knuiman, A. Nobels, J.H.M. de Vries,
531	Congruence of individual responsiveness to dietary cholesterol and to saturated fat in
532	humans, J Lipid Res 29 (1988) 883-892.
533	
534	[50] M.D. Wilson, L.L Rudel, Review of cholesterol absorption with emphasis on dietary and
535	biliary cholesterol, J Lipid Res 35 (1994) 943-955.
536	
537	[51] J.Y.L. Chian, Bile Acid Metabolism and Signaling, Compr Physiol. 2013 3 (2013) 1191-1212.
528	
220	
539	[52] G. Musso, R. Gambino, M. Cassader, Interactions between gut microbiota and host
540	metabolism predisposing to obesity and diabetes, Annu Rev Med 62 (2011) 361-380.
541	

542	[53] J.R. Swann, E. J. Want, F.M. Geier, K. Spagou, I.D. Wilson, J.E. Sidaway, et al., Systemic
543	gut microbial modulation of bile acid metabolism in host tissue compartments, Proc Natl
544	Acad Sci USA 108 Suppl 1 (2011) 4523-4530.
545	
546	[54] G. Porez, J. Prawitt, B. Gross, B. Staels, Bile acid receptors as targets for the treatment of
547	dyslipidemia and cardiovascular disease, J Lipid Res 53 (2012) 1723-1737.
548	
549	[55] M.L. Jones, C.J. Martoni, M. Parent, S. Prakash, Cholesterol-lowering efficacy of a
550	microencapsulated bile salt hydrolase-active Lactobacillus reuteri NCIMB 30242 yoghurt
551	formulation in hypercholesterolaemic adults Br J Nutr 107 (2012) 1505-1513.
552	
553	[56] C. Demigné , C. Morand, M.A. Levrat, C. Besson, C. Moundras, C. Rémésy, Effect of
554	propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated
555	rat hepatocytes, Br J Nutr 74 (1995) 209-219.
556	
557	[57] B.A. Griffin, Low-density lipoprotein heterogeneity, Baillière's Clin Endo Metab 9 (1995)
558	687-703.
559	
560	[58] R.M. Krauss, Lipoprotein subfractions and cardiovascular disease risk, Curr Op Lipidol 21
561	(2010), 305-311.
562	
563	[59] D.M. Dreon, H.A. Fernstrom, H. Campos, P. Blanche, P.T. Williams, R.M. Krauss, Change
564	in dietary saturated fat intake is correlated with change in mass of large low-density-
565	lipoprotein particles in men, Am J Clin Nutr 67 (1998) 828-836.
566	
567	[60] M. Yuan, R.T. Pickering, M. Singer, L. Moore, Dietary Saturated Fat Is Associated with
568	Larger LDL Particle Size and Reduced CVD Risk in Framingham Offspring Study, Curr
569	Develop Nutr 3 Suppl 1 (2019) 128-119.

571	[61] S. Lund-Katz, P.M. Laplaud, M.C. Phillips, M.J. Chapman Apolipoprotein B-100
572	conformation and particle surface charge in human LDL subspecies: implication for LDL
573	receptor interaction Biochem, 37 (1998) 12867-12874.
574	
575	[62] S. Chiu, P.T. Williams, R.M. Krauss, Effects of a very high saturated fat diet on LDL
576	particles in adults with atherogenic dyslipidemia: A randomized controlled trial, PLoS ONE
577	12 (2017) e0170664.
578	
579	[63] H. Gylling, M. Hallikainen, J. Pihlajamäki, J. Agren, M. Laakso, R.A. Rajaratnam, et al.,
580	Polymorphisms in the ABCG5 and ABCG8 genes associate with cholesterol absorption and
581	insulin sensitivity, J Lipid Res 45 (2004) 1660-1665.
582	
583	[64] S. Santosa, I. Demonty, A.H. Lichtenstein, J.M. Ordovas, P.J. Jones, Single nucleotide
584	polymorphisms in ABCG5 and ABCG8 are associated with changes in cholesterol
585	metabolism during weight loss. J Lipid Res 48 (2007) 2607-2613.
586	
587	[65] J.M. Anderson, A. Cerda, M.H. Hirata, A.C. Rodrigues, E.L. Dorea, M.M. Bernik, et al.,
588	Influence of PCSK9 polymorphisms on plasma lipids and response to atorvastatin
589	treatment in Brazilian subjects, J Clin Lipidol 8 (2014) 256-64.
590	
591	[66] A. Alsaleh, S.D. O'Dell, G.S. Frost, B.A. Griffin, J.A. Lovegrove, S.A. Jebb SA, et al., Single
592	nucleotide polymorphisms at the ADIPOQ gene locus interact with age and dietary intake
593	of fat to determine serum adiponectin in subjects at risk of the metabolic syndrome, Am J
594	Clin Nutr 94, 262-269.
595	
596	
597	[67] C.G. Walker, R.J. Loos, A.D. Olson, G.S. Frost, B.A. Griffin, J.A. Lovegrove, et al., Genetic
598	predisposition influences plasma lipids of participants on habitual diet, but not the
599	response to reductions in dietary intake of saturated fatty acids, Atherosclerosis 215, 421-
600	427.

601 602 [68] A. AlSaleh, S.D. O'Dell, G.S. Frost, B.A. Griffin, J.A. Lovegrove, S.A. Jebb, et al., Interaction 603 of PPARG Pro12Ala with dietary fat influences plasma lipids in subjects at cardiometabolic 604 risk, J Lipid Res 52, 2298-2303. 605 [69] U.S. Schwab, H.M. Maliranta, E.S. Sarkkinen, M. J. Savolainen, A. KesSniemi, M.I.J. 606 607 Uusitupa, Different Effects of Palmitic and Stearic Acid-Enriched Diets on Serum Lipids and 608 Lipoproteins and Plasma Cholesteryl Ester Transfer Protein Activity in Healthy Young 609 Women, Metabolism 45 (1996) 143-149. 610 611 [70] S. Jansen, J.López-Miranda, P. Castro, F. López-Segura, C. Marín, J.M. Ordovás, et al., 612 Low-fat and high-monounsaturated fatty acid diets decrease plasma cholesterol ester 613 transfer protein concentrations in young, healthy, normolipemic men, Am J Clin Nutr 72 614 (2000) 36-41. 615 616 [71] A.J. Wallace, J.I. Mann, W.H.F. Sutherland, S. Williams, A. Chisholm, C.M. Skeaff, et al., 617 Variants in the cholesterol ester transfer protein and lipoprotein lipase genes are 618 predictors of plasma cholesterol response to dietary change, Atherosclerosis 152 (2000) 619 327-333. 620 621 [72] P.W. Wilson, E.J. Schaefer, M.G. Larson, J.M. Ordovas, Apolipoprotein E alleles and risk of 622 coronary disease. A meta-analysis. Arterioscler Thromb Vasc Biol 16 (1996) 1250-1255. 623 624 [73] E. Sarkkinen, M. Korhonen, A. Erkkila, T. Ebeling, M. Uusitupa, Effect of apolipoprotein E 625 polymorphism on serum lipid response to the separate modification of dietary fat and 626 dietary cholesterol, Am J Clin Nutr 68 (1998) 1215-1222. 627

628	[74] A. Minihane, L. Jofre-Monseny, E. Olano-Martin, G. Rimbach, Apo E genotype,
629	cardiovascular risk and responsiveness to dietary fat manipulation, Proc Nutr Soc 66
630	(2207) 183-197.
631	
632	[75] B.A. Griffin. C.G. Walker. S.A. Jebb. C. Moore. G.S. Frost. L. Goff. et al., APOE4 Genotype
633	exerts greater benefit in lowering plasma cholesterol and apolipoprotein B than wild type
634	(E3/E3), after replacement of dietary saturated fats with low glycaemic index
635	carbohydrates, Nutrients 10 (2018) 1524.
636	
637	[76] R.J Widmer, A.J. Flammer, L.O. Lerman, A. Lerman, The Mediterranean diet, its
638	components, and cardiovascular disease, Am J Med 128 (2015) 229-238.
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650	Legends to Figures
651	Figure 1 (A)
652	Individual variation in serum LDL-cholesterol in response to a high SFA diet (17.6 $\pm$ 0.4% total
653	energy (mean $\pm$ SEM) relative to habitual diet (SFA 11.5 $\pm$ 0.5 % total energy) in men and
654	women (n=65) at increased risk of CVD in the 'DIVAS' study. A mean increase in the intake of

655 SFA of 6.1% total energy produced variation in serum LDL-cholesterol ranging from +45 to -20%.

656 Data taken from [38].

657 Figure 1 (B)

Individual variation in serum LDL-cholesterol in response to a high SFA diet (16.0 ± 3.0% total

energy (mean  $\pm$  SD) relative to habitual diet (SFA 13.0  $\pm$  3.5% total energy) in men and women

660 (n=69) at risk of developing metabolic syndrome in the 'RISCK' study. A mean increase in the

661 intake of SFA of 3.0% total energy produced variation in serum LDL-cholesterol ranging from to

662 +30 to -30%. Data taken from [39].

663

### 664 **Figure 2**

665 Frequency distribution of variation in serum cholesterol between individuals (inter) as

666 compared within individuals (intra) in 58 metabolically healthy men, in response to six

667 consecutive dietary interventions (data taken from Ref. [42]). The diets differed by the quality

of a macronutrient supplement (28% total energy) e.g. exchange in dietary fats (SFA exchanged

669 for PUFA) and carbohydrate (sugars exchanged with starch). For further details of diets see Ref.

670 [42].

671

### 672 Figure 3

673 Control of serum LDL-cholesterol and LDL-receptor expression via the reciprocal 'push-pull'

relationship between the intestinal absorption and whole body synthesis of cholesterol, with

675 inputs from bile acid synthesis and excretion, and gut microbiota.