

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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Accepted Version

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Griffin, B. A., Mensink, R. P. and Lovegrove, J. A. (2021) Does variation in serum LDL-cholesterol response to dietary fatty acids help explain the controversy over fat quality and cardiovascular disease risk? *Atherosclerosis*, 328. pp. 108-113. ISSN 0021-9150 doi:
<https://doi.org/10.1016/j.atherosclerosis.2021.03.024> Available at <https://centaur.reading.ac.uk/99743/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1016/j.atherosclerosis.2021.03.024>

Publisher: Elsevier

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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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16

17 **Declarations of interests.**

18 JAL is Deputy Chair of the UK Government's Scientific Advisory Committee on Nutrition (SACN)
19 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**

28 **Background and Aims:** Controversy over fat quality and cardiovascular disease risk
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
31 the intake of saturated fat and disease endpoints. Possible explanations for these null
32 findings include difficulties inherent in estimating true food intake, the confounding
33 effects of macronutrient replacement and food composition, and marked inter-
34 individual variation in the response of serum LDL-cholesterol. The aim of this narrative
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
38 evidence to suggest that variation in LDL-responsiveness may harbour significant
39 potential to confound the relationship between saturated fat and atherosclerotic
40 cardiovascular disease risk, thus undermining the effectiveness of the dietary guideline
41 to replace saturated fat with unsaturated fat. **Conclusions:** the identification and
42 application of a simple biomarker of this phenomenon, would make it possible to tailor
43 dietary guidelines to LDL responsive individuals, who stand to gain a greater benefit to
44 their cardiovascular health.

45

46

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
51 (ASCVD) and target of cholesterol-lowering therapy, has made an incalculable contribution to
52 the reduction in morbidity and mortality from this disease worldwide. This remarkable
53 progress in medical science has occurred against a backdrop of controversy and scepticism
54 over the strength of evidence to support the link between raised LDL cholesterol and ASCVD
55 [1], and more recently, dietary recommendations to lower serum LDL by reducing intake of
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
58 ASCVD. Simultaneously, independent expert scientific nutrition advisory committees
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
61 strength of the effect of this dietary change in lowering serum LDL-cholesterol (LDL-C). While
62 debate over the validity of this recommendation was positive in reinforcing its relevance to
63 human health, it also exposed weaknesses in the evidence for the impact of SFA on ASCVD,
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
66 macronutrient which replaces SFA in the diet, SFA in whole foods and dietary patterns [8], and
67 impact of inter-individual variation in the response of serum LDL to the reduction of SFA. The
68 following narrative review examines the evidence for the origins of this variation in serum LDL-
69 C, and its potential contribution to the controversy over fat quality and ASCVD. Emphasis has
70 been placed on metabolic rather genetic determinants of this phenomenon, in areas where
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
73 cardiometabolic origins of variance in LDL, were considered to lie beyond the scope of the
74 review.

75

76

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
81 carries a single polypeptide chain of apo B-100, this protein conveys information about the
82 number of LDL particles. While both total serum cholesterol and apo B are informative with
83 respect to the association between LDL and cardiovascular risk, the most recent guidelines
84 from the European Cardiovascular Society and EAS, report that serum apo B provides the most
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
90 therapy, in part, because of its relatively greater prominence in the mechanism to explain the
91 regulation of serum LDL and whole-body cholesterol homeostasis [13]. The lowering of serum
92 LDL-C is also the main target for the dietary management of ASCVD risk, by approaches such
93 as the Portfolio Diet [14], though subtle differences exist between this approach and the
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
96 cholesterol, the uptake of which requires less energy than cholesterol biosynthesis. This view
97 is supported by a model of cholesterol homeostasis, whereby the cellular uptake of LDL is
98 regulated by the expression and activity of cell surface LDL-receptors, the gene-transcription
99 of which is regulated by the amount of intra-cellular free cholesterol [13]. The size of the intra-
100 cellular pool of free cholesterol is governed by the rate of cholesterol biosynthesis, export of
101 cholesterol from the liver as bile acids and free cholesterol in bile, reabsorption of these bile
102 acids and cholesterol in the gut, and uptake of serum LDL by LDL-receptors. Cholesterol
103 biosynthesis co-ordinates with these other processes in a reciprocal fashion, to maintain a
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

107 cholesterol homeostasis *in vivo* [16]. In a mutually inclusive update of this conventional model,
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
111 expression of LDL-receptors, and HDL-derived cholesterol is incorporated into the production
112 of bile acids. Most critically, it is the uptake of cholesterol into the liver in chylomicrons, and
113 presumably their remnants, that enters the regulatory pool of intra-cellular cholesterol, and
114 therefore is chiefly responsible for suppressing the activity of LDL-receptors [16]. This latter
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.

117

118 **3. Influence of dietary fatty acids on serum LDL-C; fundamental importance of** 119 **macronutrient replacement**

120 Arguably the strongest evidence to support dietary SFA as a modulator of total serum
121 cholesterol, comes from tightly controlled, metabolic ward studies in the early 1950s, in which
122 total serum cholesterol was manipulated by altering the relative proportions of dietary SFA
123 and unsaturated fatty acids, from animal and plant sources, within milk shakes [17-19]. These
124 findings were later supported by the outcome of epidemiological studies of Ancel Keys [20,
125 21], which laid the foundation for the 'diet-heart hypothesis' and earliest guideline to reduce
126 intake of total fat and SFA to prevent heart attacks in the USA in 1961 [22]. Further evidence
127 for the efficacy for this hypothesis would follow from randomised intervention trials (RCT) [23-
128 25], and the most comprehensive meta-analysis to date of RCTs, that showed a 27% reduction
129 in cardiovascular events in response to the replacement of SFA with polyunsaturated fat [26].

130 A fundamental principle that distinguishes the relatively subtle physiological effects of diet
131 from the pharmacological effects of drugs, is the obligation to replace a removed
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
135 have been shown to reduce serum LDL-C, in a dose-response fashion, with contributions to
136 these effects coming from both the removal of SFA, and the type and quality of substitute

137 macronutrient [27]. Isocaloric replacement of 1% energy from dietary SFA with PUFA, chiefly
138 in the form of linoleic acid, has been shown to be more effective in lowering serum LDL-C
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
141 mmol/L, P <0.001) or carbohydrate (mean change -0.033, 95% CI -0.039 to -0.027 mmol/L, P
142 <0.001) [28]. Nevertheless, increased demand for low fat diets and food products has
143 invariably favoured the replacement of SFA with carbohydrate in preference to unsaturated
144 fat in the USA and UK. The latter dietary exchange is estimated to be associated with an
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.

149 Other relevant dietary sources of variation in serum LDL-C, include the effects of specific
150 dietary fatty acids of variable chain length and capacity to raise and lower serum LDL-C [29],
151 and other constituents in whole foods (e.g. minerals, food matrix), meals, and dietary patterns
152 [30], which can alter the bioavailability and exposure to dietary SFA.

153

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

155 Serum LDL-cholesterol varies within (intra) and between (inter) individuals in response to
156 intrinsic factors (e.g. polymorphism and expression of genes, hormones) and extrinsic factors
157 (e.g. diet, behaviour), and interactions between the two. Estimates for the proportion of inter-
158 individual variation in serum LDL-C that can be ascribed to genetic heritability in and between
159 populations, though wide ranging (20-90%) [31], still accommodates a significant contribution
160 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

168 differing degrees of change at either end of a continuous spectrum of responses to dietary
169 cholesterol, rather than two discrete distributions or phenotypes [36]. In retrospect, the latter
170 would be unlikely in view of the multiple genes and metabolic variables contributing to inter-
171 individual variation.

172 The most well documented example of inter-individual variation in serum LDL-C in
173 response to a reduced intake of SFA in men and women, comes from the effects of the US
174 National Cholesterol Education Programme's (NCEP) Step 2 diet [37]. Low in total fat (18-29%
175 energy) and SFA (4-7% energy), the Step 2 diet has been shown to produce dramatic
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
183 increased intake of SFA in two randomised controlled intervention studies; 'Dietary fat &
184 VAScular function' 'DIVAS' study, **Figure 1(B)**, and 'Reading, Imperial, Surrey, Cambridge &
185 Kings' ('RISCK') study' [38, 39]. Rigorous control of confounding, extrinsic factors and dietary
186 compliance in these studies, provided further evidence to suggest that the variation in serum
187 LDL-C originated from intrinsic biological differences in the metabolic handling and impact of
188 dietary SFA on cholesterol homeostasis between individuals.

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
193 that show inherent variability in risk susceptibility, dietary compliance, and response to
194 dietary recommendations. When variation in an outcome measure (serum LDL-C) in response
195 to an intervention (replacement of dietary SFA) is greater between individuals than the
196 average response of the study population, this will reduce the ability of that study to
197 demonstrate a significant effect of the intervention on that outcome measure. It is evident in
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
204 [41]. While this has been reported to apply to the association between serum LDL-C and
205 dietary SFA, this is not supported by observations of inter and intra-variation in LDL-C in
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
215 enhance the effects of this dietary change in a more personalised dietary approach.

216

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
223 dietary fat and cholesterol [43]. As discussed previously, the reciprocal relationship between
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

231 esterification of intra-cellular cholesterol, as described in the pioneering work of John Dietschy
232 [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
235 the magnitude to which these variables can respond to each other and become misaligned.
236 Imbalance in these processes would manifest as distinct metabolic phenotypes or
237 'metabotypes' characterised by either higher cholesterol synthesis (low absorption) or higher
238 absorption (low synthesis). Evidence from metabolic studies for the existence of such
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

247 **5.3 Key role of bile acids in the absorption of dietary SFA and cholesterol**

248 The additive and even synergistic effects of dietary SFA and dietary cholesterol on serum
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
251 components may be helpful in explaining the origins of variation in serum LDL-C to dietary
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
254 the gut lumen is absorbed throughout the small intestine, via a series of regulatory transport
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.

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

262 secreted into the bile. This conjugation step enhances bi-polarity, which increases the
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
266 acids, the diversity of which is largely driven by the gut microbiota. In addition to facilitating
267 fat absorption, bile acid production drives the flow of bile. Bile acids also act as key cell
268 signalling molecules, which serve as ligands for nuclear receptors that regulate the
269 transcription of genes involved in lipid metabolism [52-54]. The gut microbiota shares a bi-
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**

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

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

292 association between serum LDL-C and ASCVD in populations, since for most people without a
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
296 replacing dietary SFA on LDL-receptor activity should be to selectively increase or decrease
297 larger LDL, respectively. However, this may not be the case if the uptake of cholesterol from
298 LDL has a minimal effect in regulating intra-free cholesterol and production of LDL receptors
299 *in vivo*. It could also be off-set by the nature of substitute macronutrient, with refined
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].

305

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
312 diet. These polymorphisms produce different isoforms of apoprotein E with variable capacity
313 to function as ligands for the binding of triacylglycerol-rich lipoproteins and their remnants,
314 and HDL, to cell surface receptors, including LDL receptors. They are reported to account for
315 up to 8-10% of variance in serum LDL-C in populations [72], primarily, by influencing the
316 regulatory pool of intra-cellular free cholesterol and activity of LDL-receptors, as described
317 previously. Apo E polymorphism has also been linked to variation in serum LDL-C response to
318 changes in dietary SFA and cholesterol [73, 74]. Most notably, carriers of the ϵ 4 allele (apo E4
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
321 lesser extent, dietary cholesterol elevate serum LDL-C by modulating intra-cellular cholesterol
322 and the expression of LDL receptors. Carriage of the apo E4 variant has also been shown to be

323 more effective in lowering serum LDL-C and apo B than wild type (E3/E3), when SFA is
324 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.

330 Moderately elevated serum cholesterol is extremely common in populations, but carries a
331 relatively low absolute risk in comparison to some other risk factors, such as blood pressure,
332 making both total serum cholesterol and LDL-C poor discriminators of ASCVD risk within
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.

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

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

348

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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)**

658 Individual variation in serum LDL-cholesterol in response to a high SFA diet ($16.0 \pm 3.0\%$ total
659 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
668 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'
674 relationship between the intestinal absorption and whole body synthesis of cholesterol, with
675 inputs from bile acid synthesis and excretion, and gut microbiota.

676