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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Declarations of interests.

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 RPM is Deputy Chair of the International Life Sciences Institute (ILSI) committee on 'Individual Saturated fatty acids and Cardiovascular Risk'.

1. Abstract

Background and Aims: Controversy over fat quality and cardiovascular disease risk stems from a series of meta-analyses of prospective cohort and randomised intervention trials, which found little evidence for a significant relationship between the intake of saturated fat and disease endpoints. Possible explanations for these null findings include difficulties inherent in estimating true food intake, the confounding effects of macronutrient replacement and food composition, and marked inter-individual variation in the response of serum LDL-cholesterol. The aim of this narrative review was to present evidence for the existence and origins of variation in serum LDL-cholesterol response to the replacement of dietary saturated fat, and its potential to explain the controversy over the latter. **Methods/Results:** The review provides evidence to suggest that variation in LDL-responsiveness may harbour significant potential to confound the relationship between saturated fat and atherosclerotic cardiovascular disease risk, thus undermining the effectiveness of the dietary guideline 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 dietary guidelines to LDL responsive individuals, who stand to gain a greater benefit to their cardiovascular health.

2. Introduction

Serum low density lipoprotein, saturated fat; consensus amidst controversy

Since the discovery of low-density lipoprotein (LDL) in 1955, knowledge of its now established 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 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 over the strength of evidence to support the link between raised LDL cholesterol and ASCVD [1], and more recently, dietary recommendations to lower serum LDL by reducing intake of saturated fatty acids (SFA) [2,3]. In 2017 and 2020 [4, 5], consensus panels for the European Atherosclerosis Society (EAS) concluded that LDL is a causal risk factor for the development of ASCVD. Simultaneously, independent expert scientific nutrition advisory committees confirmed the validity of dietary guidelines to reduce SFA, by their replacement with 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 debate over the validity of this recommendation was positive in reinforcing its relevance to human health, it also exposed weaknesses in the evidence for the impact of SFA on ASCVD, and urgent need for a better understanding of the complex relationship between SFA and 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 impact of inter-individual variation in the response of serum LDL to the reduction of SFA. The following narrative review examines the evidence for the origins of this variation in serum LDL-C, and its potential contribution to the controversy over fat quality and ASCVD. Emphasis has been placed on metabolic rather genetic determinants of this phenomenon, in areas where the evidence is sufficiently robust to be appraised. The roles of obesity and related conditions 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 review.

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

The concentration of serum LDL is most commonly represented by its cholesterol content ('LDL-C') but can also be expressed in terms of its total lipid and protein mass, or 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 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 from the European Cardiovascular Society and EAS, report that serum apo B provides the most accurate marker of ASCVD, by providing a measure of the total number of atherogenic lipoproteins in serum [9]. Moreover, serum apo B can be measured directly, inexpensively, and with greater accuracy and precision than LDL-C, which is mostly calculated indirectly from the Friedewald equation [10]. While these advantages confer greater all-round clinical utility 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 regulation of serum LDL and whole-body cholesterol homeostasis [13]. The lowering of serum 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 dietary management of elevated serum apo B [15].

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 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 of which is regulated by the amount of intra-cellular free cholesterol [13]. The size of the intra-cellular pool of free cholesterol is governed by the rate of cholesterol biosynthesis, export of cholesterol from the liver as bile acids and free cholesterol in bile, reabsorption of these bile 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 mass of intra-cellular cholesterol that is appropriate for the requirement of cells, and, at the same time, regulates and concentration of serum LDL [13]. However, because this traditional 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, it has been proposed that cholesterol entering the liver in LDL, HDL or chylomicrons has different fates. In this updated model, LDL-derived cholesterol is largely shunted into the 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 of bile acids. Most critically, it is the uptake of cholesterol into the liver in chylomicrons, and presumably their remnants, that enters the regulatory pool of intra-cellular cholesterol, and therefore is chiefly responsible for suppressing the activity of LDL-receptors [16]. This latter pathway has major implications for the metabolic coupling of serum LDL and triacylglycerol-rich lipoproteins and their remnants, and atherogenic roles of these lipoproteins in ASCVD.

3. 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 cholesterol, comes from tightly controlled, metabolic ward studies in the early 1950s, in which 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 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 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) [23-25], 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].

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 macronutrient with a substitute macronutrient to render the diet viable. In the case of SFA, the substitute macronutrients of choice are either unsaturated fatty acids (PUFA or MUFA), 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

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

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 inter-individual variation.

The most well documented example of inter-individual variation in serum LDL-C in response to a reduced intake of SFA in men and women, comes from the effects of the US 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 reductions in serum LDL-C and significant variation between individuals. Exposure to this diet from between 4.5-24 weeks was reported to produce changes in serum LDL-C ranging from +3 to -55% and +13 to -39% in men and women, respectively. In this case, 48% of this variation could be accounted for by baseline LDL-C concentration and age in men, and 13% to age in women **(Figure 1A)**. After taking into consideration variation in dietary compliance, and controlling for this and other extrinsic factors, significant variation was attributed to apo E 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; 'DIetary fat & 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 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 dietary SFA on cholesterol homeostasis between individuals.

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

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

Dietary guidelines to reduce disease risk are primarily designed for human populations that show inherent variability in risk susceptibility, dietary compliance, and response to dietary recommendations. When variation in an outcome measure (serum LDL-C) in response 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 demonstrate a significant effect of the intervention on that outcome measure. It is evident in each of the studies shown in **Figures 1 (A)** & **(B)** that the magnitude of inter-individual variation in response to SFA intake is greater than the mean response, which will effectively

reduce the significance of the dietary intervention [40]. Similarly, the amount of error and ability to demonstrate a significant association between two variables depends on the ratio of the intra to inter-variability in these variables. If intra-variation is greater than the inter-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 dietary SFA, this is not supported by observations of inter and intra-variation in LDL-C in response to diet. A comparison of inter and intra-individual variation in total serum cholesterol in 58 men, on six different dietary regimens for between 3-10 weeks, showed that inter-individual variation (between men) was nearly two-fold greater than variation within these men [42] **(Figure 2)**. Irrespective of this difference, it is likely that both inter- and intra-variation will attenuate the strength of associations between LDL-C, SFA and CVD, and reduce the strength of the statistical evidence on which dietary recommendations are based, even within dietary compliant cohorts. Identification of this variation in LDL response to SFA, together with an increased understanding of the metabolic origins of these traits, would 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.

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

The human liver and gut work in concert to regulate the rates of endogenous cholesterol synthesis and absorption, through a reciprocal mechanism that suppresses cholesterol synthesis in the liver in response to increased cholesterol absorption in the gut, and vice versa. This mechanism is largely driven by the inter-connecting entero-hepatic circulation that 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 the absorption of dietary cholesterol, and biosynthesis of cholesterol, chiefly in the liver, effectively controls the amount of free cholesterol (FC) within cells, which ultimately regulates the concentration of serum LDL-C by adjusting the uptake of LDL into cells via membrane LDL receptors. Expression of LDL-receptors is governed by a mechanism of inhibition feedback that modulates the transcription of the LDL-receptor gene by 'sensing' the level of intra-cellular free cholesterol. This mechanism also forms the basis of our understanding of the 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].

While it is often assumed that the 'push-pull' reciprocity between cholesterol biosynthesis 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. Imbalance in these processes would manifest as distinct metabolic phenotypes or 'metabotypes' characterised by either higher cholesterol synthesis (low absorption) or higher absorption (low synthesis). Evidence from metabolic studies for the existence of such metabotypes, who are respectively less and more sensitive to dietary cholesterol, may underlie the phenomenon of hypo and hyper-responsiveness of serum LDL-C to dietary cholesterol, which may, in part, be an inherited trait [46]. The relatively greater efficacy of LDL-lowering drugs that either inhibit cholesterol synthesis or block absorption in the gut (e.g. statins and ezetimibe) in synthesisers or absorbers of cholesterol, respectively, provides further evidence for the existence of these discrete metabotypes [47, 48]. Factors governing the absorption and synthesis of cholesterol are summarised in **Figure 3**.

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 LDL-C, reflect the fact that these dietary lipids share common determinants of cholesterol 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 SFA [49], dietary fatty acids and cholesterol are absorbed by different mechanisms. The bulk 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 proteins. However, since the absorption of both dietary lipids depends on the production and resorption of bile salts in the entero-hepatic circulation, the metabolism of bile acids provides a credible link between dietary SFA, cholesterol synthesis and absorption [47], which could 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 capacity of bile acids to emulsify dietary fat for absorption. Conversely, bacterial bile salt hydrolases (BSH) deconjugate primary bile salts in the gut, reducing their efficiency to 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 fat absorption, bile acid production drives the flow of bile. Bile acids also act as key cell signalling molecules, which serve as ligands for nuclear receptors that regulate the transcription of genes involved in lipid metabolism [52-54]. The gut microbiota shares a bi-directional relationship with dietary fat, by influencing the absorption of fat through bile salts, and, in turn, being modified by dietary fat. The BSH activity of certain bile acid-deconjugating lactobacilli and bifidobacteria may be especially relevant in the former respect, by reducing the absorption of dietary fat and lowering serum LDL-C, as shown in human intervention studies with probiotics [55]. The microbiota may also influence the effects of dietary SFA on serum LDL-C through the production of short chain fatty acids (SCFA) [56]. Acetate and propionate have been shown to stimulate and inhibit cholesterol biosynthesis, respectively. Propionate may also inhibit the uptake of acetate into hepatocytes, thus producing down-stream effects on cholesterol metabolism. In this respect, a high SFA diet has been reported to increase the excretion of SCFA, which attenuated the significant reduction in serum LDL-C when switching to a low SFA diet [56].

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 predominance of small, dense LDL, the bulk of LDL mass will reside in 'larger' cholesterol-rich subfractions. Mechanistically, there is evidence to suggest that larger LDL express a higher 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 larger LDL, respectively. However, this may not be the case if the uptake of cholesterol from 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 carbohydrate producing the opposite effect to SFA on large LDL [59]. Understanding how LDL particle size influences the effect of SFA replacement on serum LDL-C, and LDL particle number (LDL-apo B), has been difficult to establish, and may depend on the initial distribution of LDL particle size (LDL subclass phenotype), dietary exchanges, and threshold effects of SFA intake [62].

5.5 Genetic polymorphism in apoprotein E

Numerous common single nucleotide polymorphisms have been reported to influence the response of serum LDL-C to dietary fats, the address of which lies beyond the scope of this review [63-71]. Of all common genetic traits studied to date, two missense single nucleotide polymorphisms in the apoprotein E gene (rs429358 and rs7412 at codons 112 and 158, 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 to function as ligands for the binding of triacylglycerol-rich lipoproteins and their remnants, and HDL, to cell surface receptors, including LDL receptors. They are reported to account for 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 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 ε4 allele (apo E4 isoform) tend to have elevated serum LDL-C (5-10%) and are consistently more responsive to 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 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].

6. Future perspectives and conclusions

The cardiovascular risk that can be attributed to elevated serum LDL-C in a population is a function of the absolute risk (mortality associated with the concentration of raised LDL-C over 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 relatively low absolute risk in comparison to some other risk factors, such as blood pressure, making both total serum cholesterol and LDL-C poor discriminators of ASCVD risk within populations. Inter-individual variation in disease risk associated with elevated serum LDL-C and its variable response to treatment, including diet, will contribute to this low absolute risk. As such, a serum biomarker of serum LDL-C responsiveness to the replacement of dietary SFA would have major utility in increasing the power to discriminate disease risk, in this otherwise 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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Legends to Figures

Figure 1 (A)

Individual variation in serum LDL-cholesterol in response to a high SFA diet (17.6 ± 0.4% total energy (mean ± SEM) relative to habitual diet (SFA 11.5 ± 0.5 % total energy) in men and women (n=65) at increased risk of CVD in the 'DIVAS' study. A mean increase in the intake of

SFA of 6.1% total energy produced variation in serum LDL-cholesterol ranging from +45 to -20%. Data taken from [38].

Figure 1 (B)

Individual variation in serum LDL-cholesterol in response to a high SFA diet (16.0 ± 3.0% total energy (mean ± SD) relative to habitual diet (SFA 13.0 ± 3.5% total energy) in men and women (n=69) at risk of developing metabolic syndrome in the 'RISCK' study. A mean increase in the intake of SFA of 3.0% total energy produced variation in serum LDL-cholesterol ranging from to +30 to -30%. Data taken from [39].

Figure 2

Frequency distribution of variation in serum cholesterol between individuals (inter) as compared within individuals (intra) in 58 metabolically healthy men, in response to six 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 for PUFA) and carbohydrate (sugars exchanged with starch). For further details of diets see Ref. [42].

Figure 3

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