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Saturated fat and CVD: importance of inter-individual variation in the response of serum low-density lipoprotein cholesterol

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The aim of this review is to provide an overview of the history in support of the role of dietary saturated fatty acids (SFA) in the development of cardiovascular disease (CVD), and the controversy and consensus for the evidence in support of guidelines to remove and replace SFA with unsaturated fatty acids. The review will also examine the existence, origins, and implications for CVD risk of variability in serum LDL-cholesterol in response to these guidelines. While the quality of supporting evidence for the efficacy of restricting SFA on CVD risk has attracted controversy, this has helped to increase understanding of the inter-relationships between SFA, LDL-cholesterol and CVD, and reinforce confidence in this dietary recommendation. Nevertheless, there is significant inter-individual variation in serum LDL-C in response to this dietary change. The origins of this variation are multi-factorial and involve both dietary and metabolic traits. If serum biomarkers of more complex metabolic traits underlying LDL-responsiveness can be identified, this would have major implications for the targeting of these dietary guidelines to LDL-responders, to maximise the benefit to their cardiovascular health.

Key words: SFA: Serum LDL-cholesterol: CVD

A brief history of dietary saturated fats and CVD: *'If you don't know where you've come from, you don't know where you're going'*[1]

Dietary SFA and CVD share a long and protracted history. Leonardo de Vinci (1452–1519) was one of the first to observe the restriction of blood flow by the narrowing of coronary arteries in his anatomical dissections. However, these early observations of what we now know to be coronary atherosclerosis, the most common form of CVD, were not linked to the pathogenesis and fatal outcomes of this condition until the end of the 18th century. The first scientific evidence to implicate diet as a cause of CVD would not emerge until the beginning of the 20th century, with the first recommendations to restrict intake of SFA following 60 years later. In the mid-18th century, medical history attributed the rise in the prevalence of the first symptom of CHD angina pectoris, to dietary changes brought about by the industrialisation of agriculture and food production[2]. First described in 1765 as a 'disorder of the breast', angina was eventually linked to the increased availability and consumption of animal fats, sugar and salt. Advances in transportation also led to more sedentary lifestyles and an escalation of obesity and the then-unknown diseases of hypertension, type-2 diabetes,

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and CVD. While Edward Jenner (1749–1823) and colleagues are credited with linking angina to the hardening of the arteries, at this time, and for the next 150 years, this degenerative condition was considered to be an inevitable consequence of ageing and went untreated.

The first true insight into the dietary origins of CVD came with the serendipitous findings of a Russian medical student Nicolai Anitschkow, whose original findings[3] have been elegantly summarised in the first of a series of reviews on '*An interpretive history of the cholesterol controversy*' by Professor Daniel Steinberg[4]. When studying the effects of high protein diets in rabbits, in response to claims these could be toxic, Anitschkow inadvertently discovered that the high content of dietary cholesterol in these diets accelerated the development of atherosclerotic lesions in large arteries. In subsequent cholesterol-feeding studies, he established that dietary cholesterol promoted the rapid development of atherosclerotic lesions in a dose-response fashion, primarily because the high intake of cholesterol led to extremely high level of blood cholesterol in this particular animal species (12–24 mmol/l)[3,4]. However, Anitschkow also observed that this effect could not be reproduced in non-herbivorous animals, presumably because these species are adapted to eating dietary fat and can convert excess dietary cholesterol into bile acids, which are excreted in faeces[4]. Although exposure to lower intakes of dietary cholesterol, as consumed by humans, could promote atherosclerosis over a much longer period of time, extrapolation of this disease process in cholesterol-fed rabbits to humans seemed unlikely. It would transpire over the next 50 years, that it was SFA, typically in animal fats, and *not* dietary cholesterol that was the main harbinger of atherosclerotic CVD in humans, primarily by raising the concentration of blood cholesterol.

Pioneering research in the USA would follow, in three very different experimental areas. This research would provide strong and consistent evidence for associations between dietary SFA, serum cholesterol, and CVD that would galvanise opinion on the role of SFA in CVD and form the backbone of the diet-heart hypothesis. In 1950, low-density lipoprotein (LDL) was first isolated from the serum of patients with CVD by John Gofman and colleagues, who showed that LDL in these patients was linked to the severity of the disease[5]. It was reported soon after that blood cholesterol could be raised and lowered by increasing and decreasing the relative proportion of SFA to unsaturated fatty acids (UFA), respectively, in liquid meals[6,7]. These observations, from a series of tightly controlled metabolic ward studies in two independent centres, concurred with the findings from the cross-cultural and migration studies of the epidemiological biochemist, Dr Ancel Keys and colleagues. Keys reported significant positive correlations between the intake of dietary fat, and later SFA, and incidence of CVD in different countries[8], and in Japanese migrants adopting a Western diet in the USA[9]. He concluded that SFA in diets raise, and polyunsaturated fats (PUFA) lower blood cholesterol, and on a mass basis, the effect of SFA on blood cholesterol was two-fold greater than that of PUFA[10]. Keys went on to examine variation in the blood cholesterol response to diet among individuals[11], and to formulate equations for predicting how the relative proportion of these fatty acids influenced blood cholesterol. These equations were adapted later by Hegsted[12], and are still used in clinical practice today. In 1961, the strength of this evidence was considered sufficient for the American Heart Association to issue the first dietary guidelines to reduce risk of heart attack and stroke in patients at risk of these cardiac endpoints by reducing their intake of total fat and SFA[13]. This recommendation was accompanied by the caveat that there was still no supporting evidence from intervention studies for the efficacy of these guidelines. Such evidence would follow, but not before this recommendation was rolled-out to the general public in 1965[14].

Controversy and consensus surrounding the cholesterol and diet-heart hypothesis. *If you don't know where you're going, any road will get you there*[15]

Cholesterol 'lipid' hypothesis

A causal relationship between an elevated concentration of serum LDL and development of atherosclerotic CVD, especially in the large coronary arteries, is now supported by strong and consistent evidence from randomised controlled trials, cohort, and Mendelian randomisation studies[16]. Since the majority of cholesterol in blood (~60–70 %) is transported in LDL, measurement of total blood cholesterol mostly reflects cholesterol carried in this serum lipoprotein and is denoted as LDL-cholesterol or 'LDL-C'. The greatest support for the causal role of elevated serum LDL-C in the development of CVD comes from randomised controlled trials, which have provided unequivocal evidence for the benefits of 'statin' drugs in reducing the risk of fatal and non-fatal cardiac events e.g. heart attacks and strokes. A primary mode of action of statins (HMGCoA-reductase inhibitors) is to inhibit the endogenous synthesis of cholesterol in the body, and by doing so, increase the uptake of circulating LDL, thereby reducing serum LDL-C. The dramatic effect of statins on serum LDL-C and cardiac endpoints made it possible to show that the magnitude of reduction in serum LDL-C was directly proportional to the reduction in CVD risk (1 mmol/l reduction in LDL-C being associated with an estimated 24 % reduction in coronary events)[17]. The impact of the reduction in serum LDL-C on CVD risk was also shown to be without threshold. In other words, the lower the serum LDL-C, the lower the CVD risk, though linear regression indicates that LDL should cease to promote coronary atherosclerosis at a serum concentration of 1 mmol/l or below.

Elevated serum LDL-C is the principal characteristic of common moderate hypercholesterolaemia and is estimated routinely for assessing the CVD risk associated with this condition by the Friedwald formula (total serum cholesterol - HDL-C - (TAG/2·2 as an estimate of cholesterol in VLDL mmol/l))[18]. Serum LDL-C can also be measured directly on LDL isolated from serum by a host of physiochemical techniques[19] e.g. ultra-centrifugation, electrophoresis and selective anion precipitation.

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Other measures of LDL of major clinical relevance, include the concentration of its principal protein, apoprotein B (apo B). Since each LDL particle carries a single polypeptide of apo B, the concentration of this protein conveys information about the number of LDL particles. When serum apo B is elevated (> 1·3 g/l, 'hyper-apobetalipoproteinaemia'), this measurement is likely to provide a more discriminating marker of CVD risk than serum LDL-C, because the former provides a measure of the total number of atherogenic lipoprotein particles in blood (LDL plus lipoprotein remnants)[20]. Elevated concentrations of serum LDL-C and apo B are *not* mutually exclusive conditions and can be expressed together in a single individual. The conditions may also have distinct metabolic and genetic origins and convey different information about CVD risk associated with LDL. Raised serum LDL-C in moderate hypercholesterolaemia typically reflects an abundance of LDL of intermediate particle size and density often referred to as LDL-II (hydrated density 1·034–1·044 g/ml; diameter 27·0–25·5 nm). This type of LDL transports the greatest proportion of cholesterol relative to its total mass, and as such, is the principal transporter of cholesterol relative to its larger (less dense) and smaller (more dense) counterparts (LDL-I and III respectively)[21]. On the other hand, a raised number of LDL particles (LDL-apo B) is invariably associated with elevated serum TAG, TAG-rich lipoproteins, and abundance of smaller and denser LDL (LDL-III, hydrated density 1·044–1·060 g/ml; diameter 24·2–21·8 nm). While all LDL, regardless of size, density or composition, holds the potential to promote atherosclerosis, a predominance of small, dense LDL, that is intimately associated with raised serum TAG, is associated with greater atherogenicity than other subtypes of LDL[22]. In clinical practice, greater emphasis in the risk management of elevated serum LDL is given to LDL-C[23], but this is arguably inappropriate given the superiority of serum apo B in discriminating CVD risk[24].

Diet-heart hypothesis

As described previously, the evidence in support of the 'diet-heart hypothesis' that emerged in the 1950s, was followed by a number of randomised controlled trials, which attempted to test the hypothesis by removing and replacing SFA, mainly with PUFA, in various settings and populations[25]. While the majority of these early trials were statistically underpowered to produce clinically significant effects on CVD events, they were consistent in demonstrating a lowering of serum LDL-C. On the back of this growing body of evidence, the UK introduced its first guidelines to limit the intake of total fat and SFA intake to no more than 35 % and 10 % of total energy, respectively, in 1983[26].

Improvements in the recognition and medical treatment of CVD risk factors, and prevention of CVD-related death have made major contributions to the substantial decline in CVD-related mortality over the last 60 years. However, the extent to which the dietary guideline to restrict intake of SFA has contributed to this decline, is difficult if not impossible to determine with any accuracy. In the absence of definitive evidence for the efficacy of restricting SFA, it is perhaps surprising that this dietary guideline went unchallenged for so long. This situation would change with the increasing popularity of secondary forms of analysis in the form of systematic reviews and meta-analyses. A purpose of these methods when applied to prospective trials and intervention studies, is to provide more definitive evidence for associations and causal relationships, respectively. In, the context of the diet-heart hypothesis, these types of study would help to strengthen the evidence for a dose-response relationship between dietary SFA and serum LDL-C (1 % TE SFA corresponding to 0·046–0·056 mmol/l of LDL-C)[27], which was later refined to incorporate the replacement macronutrient (1 g of SFA removed and replaced by a PUFA serum LDL-C will be reduced by 0·05 mmol/l)[28]. On the other hand, they would bring disrepute upon the recommendation to restrict intake of SFA to prevent CVD.

A number of systematic reviews and meta-analyses, especially of prospective cohort trials, and reanalysis of data from intervention studies exumed from the 1970s, could find no significant evidence for a direct relationship between dietary SFA and CVD/CHD[29–33]. While other meta-analyses did report significant reductions in cardiac events in response to the removal and replacement of SFA[34,35], the negative outcome from the former studies would fuel a surge of opposition against guidelines to restrict intake of dietary SFA. This situation prompted an urgent reappraisal of the strength and quality of supporting evidence by expert working groups in the UK and the World Health Organisation. After assessing the totality of evidence, these groups were unanimous in concluding that the original guidelines were still valid. With some minor variations, these stated that SFA should not exceed 10 % of total energy and should be replaced by UFA[36,37].

Various explanations have been proposed to explain the negative outcome of studies, which could find no significant evidence to link SFA with endpoints of CVD. These explanations included flaws in study design and data analysis, and confounding factors in old studies, such as trans fatty acids. Though intake of trans-fats in hydrogenated vegetable oils and spreads used in intervention studies was considerable in the 1970s, this was not considered in the data analysis and represents a major confounder of the beneficial effects of SFA replacement with UFA on serum LDL-C. Also, studies that are still upheld as providing evidence to refute the diet-heart hypothesis, gave little or no address to the macronutrient that was replacing SFA or significant inter-individual variation in serum LDL-C response to this dietary exchange.

Variation in serum LDL-C response to dietary cholesterol and SFA

In keeping with Anitschkow's early experimental finding in rabbits, inter-individual variation in serum LDL-C in humans, and the genetic and metabolic origins of this variation, was first described in response to high intakes of

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dietary cholesterol in egg-feeding studies[38–40]. The phenomenon of hyper- and hypo-responsiveness in serum LDL-C to dietary cholesterol was shown to be attributed to a variable capacity to absorb cholesterol in the gut[41]. This was a secondary response to the primary action of excess dietary cholesterol, which was to reduce the uptake of LDL into cells from the blood by decreasing the activity of cell surface LDL receptors. This effectively lowers free cholesterol in the cell, stimulating an increase in the endogenous synthesis of cholesterol[42]. The responsiveness of serum LDL-C to added dietary cholesterol was also shown to be inversely related to habitual intake of dietary cholesterol, with serum LDL-C increasing between < 0·05 and 0·25 mmol/l in response to the consumption of an additional 200 mg cholesterol (approximately equivalent to one large hen's egg) in individuals consuming no dietary cholesterol or up to 500 mg/d, respectively[43]. Translation of these findings to free-living groups and populations was problematic for several reasons. Firstly, the intakes of dietary cholesterol in free-living groups and populations were much lower than in experimental egg-feeding studies. Secondly, dietary SFA was known to exert similar effects to dietary cholesterol in raising serum LDL-C and be associated with cholesterol in certain foods and meal patterns. Thirdly, dietary SFA is also consumed in relatively much greater amounts than cholesterol (grams *v.* milligrams), increasing the former's contribution to raising serum LDL-C, several fold.

When the independent effects of dietary cholesterol on the variable serum LDL-C response were distinguished from the effects of SFA, by feeding participants high cholesterol on a low SFA diet, hyper- and hypo-responsiveness in LDL-C was reproduced[44,45]. It had been shown previously that this phenomenon was reproducible on a second identical diet[46], with hyper- and hypo-responsiveness representing extreme ends of a continuous spectrum of change in serum LDL-C, rather than discrete phenotypes[47].

Inter and intra-individual variation in serum LDL-C has been characterised in response to various dietary exchanges and the NCEP step 2 diets[48,49]. Variation in serum LDL-C in the order of between 0·5–1·5 mmol/l to +0·5 mmol/l, has also been reported more recently in a number of well-controlled dietary interventions in response to the removal and replacement of SFA with UFA[50,51] (Fig. 1), and addition of SFA as dairy fat[52].

Unlike the concentration of serum TAG, which fluctuates significantly over 24 hours in response to the ingestion of dietary fat and secretion of TAG in VLDL from the liver, serum LDL-C shows no significant diurnal variation. Its concentration can vary over a period of weeks to months in response to subtle changes in diet and lifestyle within the same individual[53]. This *intra*-individual variation has been reported to weaken the association between dietary SFA and blood cholesterol in cross-sectional studies, as the strength of the link between these two variables is attenuated as intra-variation in serum LDL-C exceeds its variation between individuals (inter-variation)[54]. However, this is not the case in intervention studies, where inter-variation in serum LDL-C between

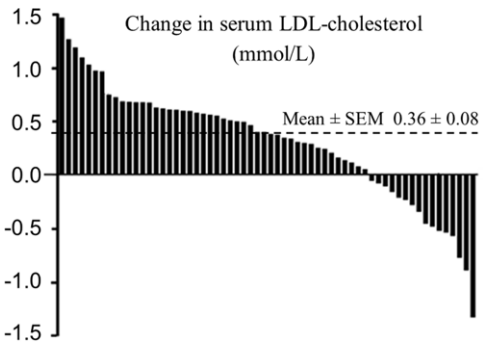

Fig. 1 Individual variation in serum LDL-cholesterol in response to a high SFA diet (17·6 ± 0·4% total energy (mean ± SEM) relative to habitual diet (SFA 11·5 ± 0·5 % total energy) in men and women (*n* 65) at increased risk of CVD. A mean increase in the intake of SFA of 6·1% total energy produced variation in serum LDL-cholesterol ranging from +45 to –20%. Data taken from the DIVAS study (51). Figure adapted from Griffin, *et al.*(55)

individuals is of an order of magnitude greater than intra-variation (0·5–1·5 mmol/l), and has the potential to exert a major influence on CVD risk management and attainment of treatment targets for serum LDL-C (Fig. 2).

Variance in dietary compliance can never be fully excluded as a contributing factor to the variation in serum LDL-C in intervention studies. However, while evidence of good dietary compliance tends to exclude this possibility, the reproduction of hyper- and hypo-responsiveness in LDL-C in the same individual on a second, identical diet, is recognised as a defining characteristic of this phenomenon that supports underlying causes other than dietary compliance[55].

Factors influencing variation in serum LDL-C response to the removal and replacement of dietary SFA

Numerous factors of dietary and biological origin interact to influence the impact of dietary SFA on CVD. Examples of dietary factors include the nature of replacement macronutrient for SFA, variable effects of specific SFA on serum LDL-C, and composition of whole, SFA-containing foods[55]. The latter can involve interactions between SFA and other components in food, which alter the absorption and bioavailability of SFA. Biological factors relate to genetic and metabolic effects on the digestion, absorption and subsequent fate of dietary SFA, and all aspects of lipid and lipoprotein metabolism that determine the concentration of serum LDL-C. As a dependent variable, that is fundamental to the origin and modification of variation in serum LDL-C, diet should take precedence over other factors. Similarly, in this context, metabolic factors should take precedence over genetic traits, as representing the collective outcome of an incalculable number of effects from genetic polymorphisms, nutrient-gene interactions and epi-genetic phenomena that lie beyond the scope of this review.

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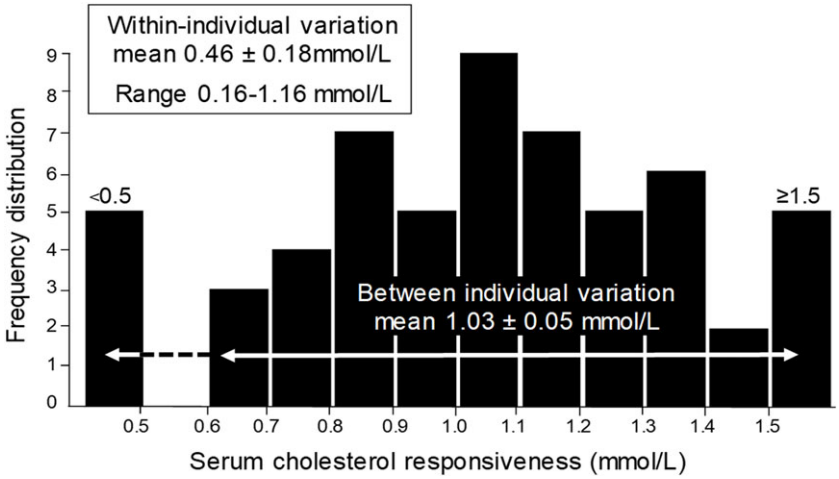

Fig. 2. Frequency distribution of variation in serum cholesterol between individuals (inter) as compared within individuals (intra) in 58 metabolically healthy men, in response to six consecutive dietary interventions (Data taken from reference 48). The diets differed by the quality of a macronutrient 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 reference 48

Dietary factors

Further to Ancel Keys' early recognition of the greater potency of SFA over PUFA in lowering blood cholesterol[10], a dose-response relationship has been shown to exist between all dietary fatty acids and serum LDL-C, with the iso-energetic substitution of carbohydrate with trans-FA and SFA raising, and MUFA and PUFA lowering serum LDL-C[56].

For a diet to remain edible and palatable, the available options for replacing dietary SFA are with another type of dietary fat, carbohydrate or possibly protein, any of which will result in a lowering of serum LDL-C. Rationale for the emphasis of replacing SFA with UFA over carbohydrate in dietary guidelines is based on the weight of evidence in favour of UFA, and especially *cis* PUFA, in the lowering of serum LDL-C. This dietary exchange also avoids the potential for the overconsumption of free sugars, in place of complex carbohydrates (fibres and wholegrains). As a non-essential component of our diet, free sugars contribute to energy intake, and therefore body weight, when over consumed. Over consumption (> 20–25 % TE) has also been associated with adverse effects on cardio-metabolic health[57].

Individual SFAs exert differential effects in raising serum LDL-C, relative to carbohydrate, in the order of increasing carbon chain length (C12:0, lauric acid > C14:0, myristic acid > C16:0 palmitic acid)[58]. In contrast, stearic acid (C18:0), a ubiquitous fatty acid in many foods, is relatively neutral in its effects on serum LDL-C, either because of its slower absorption and thus lower bioavailability from certain foods and/or its rapid desaturation to C18:1 in the body[59].

There is discrepancy in the evidence for the association between SFA, as a single nutrient in certain SFA-rich foods, and CVD risk. Epidemiological studies suggest that fermented dairy foods in particular, including cheese and yoghurts, are associated with a relatively lower risk of CVD than would be predicted on the basis of their SFA content[60,61]. While these foods could simply be markers of other factors related to lower CVD risk, intervention studies investigating an equivalent amount and quality of SFA in cheese compared with butter, reported a lower capacity to raise serum LDL-C[62]. This effect may be explained, in part, by other components in these products, such as calcium interacting with SFA to form insoluble salts that are not absorbed but excreted. The food matrix and form in which SFA is stored in foods may also influence its bioavailability in food. For example, the SFA in cream is encapsulated by a milk globular membrane, which reduces its capacity to raise serum LDL-C relative to homogenised butter fat, by impeding the absorption of SFA in the gut[63].

Metabolic factors – control of cholesterol homeostasis

The concentration of serum LDL-C is determined chiefly by the rate at which it is removed from the blood, and not by the rate at which it is synthesised from VLDL. This is governed by the activity/abundance of cell surface LDL receptors, which bind and internalise LDL into the cell[42]. The abundance, and thus activity of LDL receptors is controlled by the rate of transcription of the LDL-receptor gene, which is up and down-regulated as the amount of free, unesterified cholesterol in the cell decreases or increases, respectively. The variable size of this 'regulatory pool' of free cholesterol effectively acts as a sensing mechanism for the cell to maintain adequate cholesterol status for its biological roles. Since all cells can synthesise cholesterol, the size of this regulatory pool of free cholesterol is determined by the balance between endogenous synthesis and uptake via LDL-receptors. There are other, non-receptor-mediated routes of uptake of LDL, but these routes are not subject to the same metabolic regulation. One possible explanation for the opposing effects of dietary SFA and UFA in raising and lowering serum LDL-C is via the differential effects of

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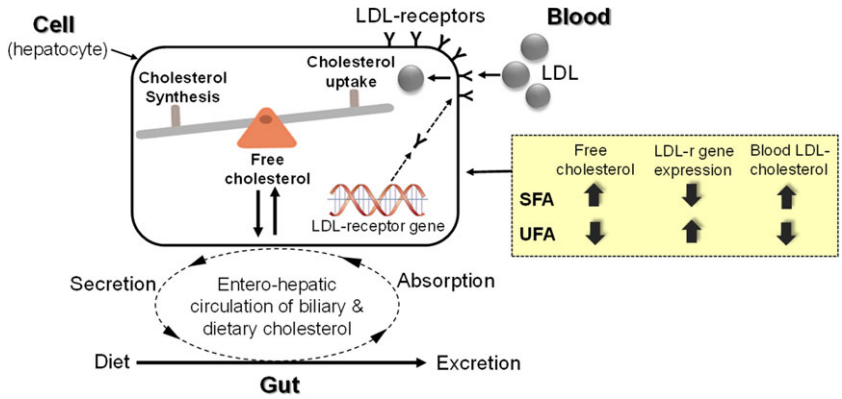

Fig. 3. LDL-receptor pathway, showing the reciprocal relationship between cholesterol synthesis and uptake from the blood via LDL-receptors and uptake via absorption from the gut, and effects of dietary SFA and UFA on the 'regulatory pool' of free cholesterol

these fatty acids on the amount of free cholesterol in the regulatory pool[64]. While dietary SFA tends to maintain the pool of free cholesterol, UFA promotes esterification of free cholesterol, and reduction in the size of the regulatory pool, resulting in an upregulation of transcription of the LDL-receptor gene and lowering of serum LDL-C. This effect is mediated through the preferential affinity of the enzyme that esterifies free cholesterol (acyl-cholesterol-acyltransferase or 'ACAT') for UFA rather than SFA. Changes in the size of the regulatory pool, as determined by interplay between endogenous cholesterol synthesis and receptor-mediated uptake of LDL, trigger secondary, reciprocal changes in the reabsorption of cholesterol of dietary and biliary origin in the gut. i.e. cholesterol absorption is either increased or decreased to replenish or deplete the size of the hepatocyte regulatory pool, respectively[65] (Fig. 3).

An intriguing revision of this model by Kiss & Sniderman has major implications for our understanding of the origins of variation in serum LDL-C response to dietary SFA[66].

In the original model of Brown and Goldstein, the gene expression and thus activity of LDL-receptors is controlled by the reception of cholesterol from serum LDL into the regulatory pool[42]. Alternatively, Kiss & Sniderman propose that LDL only exerts control over the size of the regulatory pool because the original model was conceived in cell cultures of fibroblasts incubated with LDL[66]. Under physiological conditions, the liver is actually exposed to several different sources of cholesterol from serum lipoproteins (chylomicron and VLDL remnants, LDL and HDL). In the revised model, the liver partitions these different sources of lipoprotein cholesterol into discrete pathways in the endosome of the hepatocyte. Critically, the cholesterol from LDL is 'shunted' into the production of VLDL and not the regulatory pool, which receives cholesterol predominantly from chylomicron remnants, while cholesterol from HDL feeds into the production of bile acids[66]. By shifting emphasis away from cholesterol derived from serum LDL, this alternative model refocuses attention on the impact of SFA removal on the clearance of serum TAG, and specifically, chylomicrons and their remnants in the postprandial phase as a potential origin of variation in serum LDL-C. Moreover, because this dietary change typically reduces serum HDL-C, an effect which also shows significant inter-individual variation (unpublished observation), this may have similar implications for effects on serum LDL-C via the reduced production and circulation of bile acids.

Effect of serum TAG and TAG-rich lipoproteins

As the two principal lipophilic molecules circulating in blood within serum lipoproteins, TAG and cholesterol are related through their synthesis, transport and storage. As such, variation in serum LDL-C in response to changes in the intake of SFA could be mediated through effects on the concentration of serum TAG, as determined by the rate at which TAG-rich lipoproteins are synthesised and cleared from the circulation, chiefly in the postprandial phase.

In theory, serum LDL can be produced directly from the liver, though the bulk is produced by the lipolytic breakdown of VLDL. In simple terms, the rate at which LDL is synthesised from VLDL should be determined by the amount of VLDL that is produced and secreted into the blood from the liver. However, serum lipoproteins are structurally and metabolically heterogeneous and exist as discrete subfractions with metabolic characteristics that are unique to their particle size and composition[67]. For example, the extent to which serum VLDL is converted to LDL depends on the predominant subtype or subfraction of VLDL that is secreted from the liver. Similarly, different subfractions of LDL can be produced from different subfractions of VLDL[67]. The composition and distribution of VLDL and LDL subfractions are largely determined by the total concentration of serum TAG. When serum TAG is elevated above 1·5 mmol/l, the liver tends to produce and secrete a larger, TAG-rich VLDL ($VLDL_1$), which can be partially lipolysed to become a lipoprotein remnant, and/or facilitate the remodelling of LDL into smaller, dense particles (LDL-III)[68]. Conversely, when serum TAG is below this concentration, the VLDL produced is smaller, carries less TAG ($VLDL_2$)

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and is more likely to be converted into larger LDL particles, of intermediate particle size (LDL-II) and larger LDL-I if serum TAG is extremely low (< 1 mmol/l). The LDL of intermediate size that is produced from this conversion is the principal transporter of cholesterol, as described previously, and is likely to be most LDL-receptor active subfraction of LDL[69].

The predominant subtype of VLDL that is produced in the liver and secreted into the serum is determined by the amount of TAG in the liver[67]. This is governed by the amount of pre-formed TAG arriving in serum lipoproteins, especially in the postprandial phase (lipoproteins remnants of CM (CMR) and VLDL, LDL & HDL) or substrates for TAG synthesis (e.g. NEFA from adipose tissue) or to lesser degree, by the endogenous synthesis of TAG by the liver itself (*de novo* lipogenesis). There is evidence to suggest that the replacement of dietary SFA, especially with a MUFA, will be associated with a decrease in serum TAG and accelerate clearance of TAG-rich lipoproteins (CMR) in the postprandial phase[70,71]. If the magnitude of this dietary effect on serum TAG is variable between individuals, it is reasonable to speculate this could contribute to variation in the decrease of serum LDL-C, either through an upregulation of LDL receptors (reduced delivery of cholesterol from CMR to the 'regulatory pool' of free cholesterol) and/or reduced input from VLDL.

Microbiota & enterohepatic circulation of bile acids/salts and biliary and dietary cholesterol

Factors that influence and/or interfere with the tight regulation of the entero-hepatic circulation of bile acids and salts, and cholesterol originating from the diet and bile, will exert an impact on whole-body cholesterol homeostasis, and serum LDL-C. The capacity to actively reabsorb bile salts/acids and cholesterol of dietary and biliary origin in the gut is of crucial importance in restoring free cholesterol to the liver cells for the production of bile acids[72]. At the same time, it repletes the regulatory pool of free cholesterol, down-regulating LDL-receptor activity. Conversely, any interference that reduces the reabsorption of bile acids, releases a suppression on the transcription of the rate-limiting enzyme for the production of bile acids (7-α hydroxylase), depleting the regulatory pool of free cholesterol and upregulating LDL-receptor activity. The sequestration and enhanced excretion of bile acids is the mechanism by which the first, cholesterol-lowering drugs and dietary fibres like *β*-glucans, lower serum LDL-C[73,74]. Interrupting the reabsorption of cholesterol in the gut has a similar effect in depleting the regulatory pool of free cholesterol and upregulating LDL receptors. This mechanism underlies the LDL-C-lowering effects of plant sterols and stanols, which compete for the uptake of biliary and dietary cholesterol[75]. Variance in the efficiency of these processes between individuals, and other factors that can influence them, such as the microbiota, are all potential contributors to variation in serum LDL-C response to SFA.

The composition of the gut microbiota adjusts rapidly in response to changes in diet, to accommodate its nutritional and metabolic requirements, but reverts back to its original state when the habitual diet is restored[76]. These changes in the microbiota could be influential in contributing to variation in serum LDL-C response to the removal and replacement of SFA through various mechanisms. Examples include the variable capacity of the microbiota to interrupt the entero-hepatic circulation of bile acid/salts via the action of the microbial enzyme, bile salt hydrolase, which deconjugates bile salts back to less polar and less well-absorbed bile acids, which are then excreted[77]. The extent to which the microbiota convert dietary and biliary cholesterol to less well-absorbed faecal steroids (e.g. coprastanol) could also contribute to variation in serum LDL-C by the mechanisms described above[78]. The microbiota also produces short-chain fatty acids from the fermentation of dietary fibres, and secondary bile acids, which produce pleotropic effects on fat metabolism and cholesterol homeostasis[79,80].

Genetic factors

The scientific literature is replete with studies of associations between single nucleotide polymorphisms, blood lipids, and diet, but the associations are weak, and effect sizes are small and clinically insignificant. Polymorphism in the apoprotein E gene provides the best example of a common genetic trait that impacts on variation in serum LDL-C in populations, and in response to dietary cholesterol and SFA[81].

A common, single base-nucleotide polymorphism in the apo E gene, results in three isoforms of apoprotein E, which differ by a single amino acid. This is sufficient to alter the charge on the protein and elicit subtle differences in the metabolic characteristics of the serum lipoproteins in which the different protein isoforms are carried. Apo E is found in serum TAG-rich lipoproteins and HDL, and functions as a ligand for several cell surface receptors, including the LDL-receptor. As such, variants of apo E show differential effects on the uptake of lipoprotein cholesterol cholesterol into cells[82]. This can influence the size of the regulatory pool of free cholesterol in cells, which makes a significant contribution to the variance in serum LDL-C in populations[81]. Its impact on variation in serum LDL-C in response to dietary cholesterol and SFA is weaker. Carriers of the apo E4 and E2 variants, tend to have a greater and lesser capacity for facilitating the uptake of lipoprotein (cholesterol) into cells, producing relatively lower and higher LDL-receptor activity, respectively. Carriers of E4 tend to express a higher serum LDL-C than E2 carriers and be more responsive to changes in the intake of SFA and cholesterol, as a result of their relatively suppressed LDL-receptor pathway. Carriers of E4 are also higher absorbers of cholesterol in the gut and synthesise less cholesterol than E2 carriers[83].

Relevance of inter-individual variation in serum LDL-C response to CVD risk

The reduction in risk of cardiac events has been shown to be directly proportional to the magnitude of LDL-C

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lowering, (14 % *v.* 28 % or 1:1). This effectively means that the targeting of dietary guidelines to reduce and replace SFA in an LDL-C responsive individual (reduction in serum LDL-C of ~1 mmol/L) will produce a reduction in risk of cardiac events that is 2-fold or greater (24 % reduction in risk of cardiac arrrest, 22 % combined reduction in cardiac arrest plus stroke) than a non-responsive individual (reduction in LDL-C ≤ 0·5 mmol/L), irrespective of the baseline serum LDL-C[84].

Elevated total serum cholesterol is associated with a high *absolute* risk but low *attributable* risk (absolute risk × prevalence of serum cholesterol concentration in population) of CVD[85]. It follows, at a high concentration of serum cholesterol (e.g. 7·8 mmol/L) there is a high absolute risk of a cardiac event (~90 %), but at a lower, moderately raised concentration of serum cholesterol (e.g. 5·8 mmol/L), which is much more prevalent in populations, the absolute risk is much lower (~20 %) than the attributable risk. Although the absolute risk is less at this lower, more prevalent concentration of serum cholesterol, even a measure of serum LDL-C can be relatively poor at predicting this level of risk of a future cardiac event. What clinicians often refer to as a 'grey area' of diagnosis and treatment (serum LDL-C ~2–3 mmol/L), will invariably include a significant proportion of a population for which the identification of the responsiveness of serum LDL-C to dietary SFA, and thus targeting of dietary guidelines to LDL responsive individuals, is highly relevant.

It is a general phenomenon that the extent of reductions in serum metabolite in response to treatment, tends to be positively associated with the baseline concentration of that metabolite. This also applies to blood lipids (serum LDL-C and TAG), such that the greatest reductions in serum LDL-C are achieved in those individuals with the highest concentrations at the start of treatment. In the absence of knowledge about the responsiveness of serum LDL-C to SFA, a sensible approach would be to target those with the highest serum LDL-C, who are at the greatest CVD risk, with more intensive diet and lifestyle modification. However, while these individuals are likely to show a significant reduction in serum LDL-C, this approach ignores a greater proportion of individuals in a population with only moderately raised serum LDL-C and lower absolute CVD risk, but whose LDL-C may be responsive to eating less SFA.

Conclusions

The diet-heart hypothesis has been the cornerstone of dietary guidelines to prevent CVD for over 60 years. The supporting evidence for these guidelines has withstood serious criticism, which in retrospect was necessary to help increase our understanding of the complex inter-relationship between diet, blood cholesterol and CVD. While it is reasonable to expect a dietary guideline to be supported by definitive evidence of reduced disease mortality from intervention studies, placing the burden of proof on this requirement for the impact of SFA on CVD seems somewhat erroneous, given the irrefutable evidence for the effect of restricting SFA intake in reducing serum LDL, a risk factor causally related to the development and endpoints of CVD.

Inter-individual variation in serum LDL-C in response to the removal and replacement of SFA is a common phenomenon with complex multi-factorial origins and major implications for the efficacy of our existing guidelines in reducing CVD risk. The Reading Imperial Surrey Saturated fat Cholesterol Intervention study (ClinicalTrials. Gov registration No. NCT03270527; ISRCTN16727) is a two-phase research project, aiming to identify serum biomarkers of more complex metabolic traits that underlie LDL-C responsiveness to these dietary guidelines. Its outcomes will hopefully help to advance the targeting of these guidelines to those who stand to gain the most benefit to their cardiovascular health.

Conflicts of interests

B.A.G. has no declarations of interest. J.A.L. is Deputy Chair of the UK Scientific Advisory Committee for Nutrition (SACN) and was a member of the Saturated Fats and Health Working Group for SACN. The content of this review is independent of SACN's activities.

Authorship

B.A.G. presented on the topic of this review at the Nutrition Society's Summer Conference in Liverpool in July 2023. He drafted the manuscript and is ultimately responsible for its content. J.A.L. contributed to the content, review and editing of the draft manuscript.

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