Available online on 15.02.2024 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Review Article

Contrasting Evidences Between High Cholesterol Levels and The Risk of Cardiovascular Disease: A Review

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Article Info:

Article History:

Received 21 Dec 2023
Reviewed 10 Jan 2024
Accepted 29 Jan 2024
Published 15 Feb 2024

Cite this article as:

Saleh A, Esievo KAN., Contrasting Evidences Between High Cholesterol Levels and The Risk of Cardiovascular Disease: A Review, Journal of Drug Delivery and Therapeutics. 2024; 14(2):233-239

DOI: http://dx.doi.org/10.22270/jddt.v14i2.6399

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Abstract

Introduction: The cholesterol hypothesis had been kept alive for decades by reviewers who used statistics that excluded the results from unsuccessful trials and ignored numerous contradictory observations. High dietary cholesterol intake had been associated with development of cardiovascular diseases (CVD) and mortality, for over several decades, without a direct link between the CVD and high serum cholesterol level. Hence, people avoided healthy cholesterol-rich diets due to the fear of developing CVD. The relationship between elevated plasma cholesterol and CVD and criteria for appropriate methods for screening patients with elevated cholesterol had remained a source of medical debates. Lack of decrease in overall mortality rates in patients without clinical coronary disease in whom aggressive lowering of cholesterol was achieved might have contributed to the lack of consensus on this most important issue.

Methodology: With information derived from search engines, such as Elsevier, Springer, PubMed, Science Direct, Medline, Google Scholar and a library search for articles published in peer-reviewed journals.

Results: Several research results showed that association between total serum cholesterol, its components and CVD is weak, absent or inverse implying that consuming healthy high cholesterol diets may not be harmful to health.

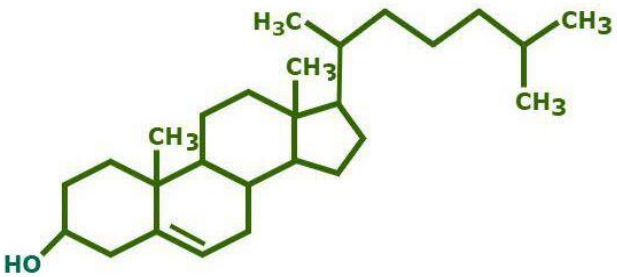
Conclusion: This review provides evidence contrasting the links between elevated plasma cholesterol and CVD, and demonstrated that elevated cholesterol concentrations, rather, improve quality of life and life expectancy. In addition, most prevalent methods for cholesterol quantification in biological samples and foods, utilizing new technologies, such as Ambient Ionization Mass Spectrometry are summarized, along with other components of cholesterol.

Keywords: Cholesterol, CVD, Quantification

Introduction

Cholesterol, a fat-like or waxy organic molecule made of sterol backbone with hydroxy group and 8 carbon atoms had been reported in the blood stream, body organs, nerve fibres and all animal products[1,2,3,4]. Available reports[5,6,7,8,9,10] showed that cholesterol, which composed of about 30% of all animal cell membranes, was required to build and modulate membrane fluidity over the range of physiological temperatures, maintain membranes integrity, cells flexibility, signaling and nerve conductivity. More than 90% of cellular cholesterol, located in the plasma membrane[11,12], served as a precursor of all steroid hormones including adrenal gland hormones, cortisol and aldosterone, progesterone, estrogens and testosterone and their derivatives[13,14,15], vitamin D and bile salt, responsible for intestinal digestion of dietary fats and absorption of fat-soluble vitamins A, D, E and K[16,17,18,19].

Cholesterol could either be synthesized *de novo* by many cells of the body (endogenous) or supplied from the diet (exogenous) and for the latter, the major dietary factors leading to hypercholesterolaemia include high intakes of cholesterol itself, saturated fats and excessive calories. The liver was reported as one of the major sites of endogenous synthesis of cholesterol, from a wide variety of foods, especially saturated fats[20].



Formular: $C_{27}H_{46}O$ Molecular mass: 386.65

Figure1: Structure of cholesterol,

The liver had long been reported to tightly regulate its pool of cholesterol by influencing the input from dietary cholesterol, the biosynthesis, secretion and uptake from plasma

lipoproteins, the conversion of cholesterol into bile, and the reuptake of biliary cholesterol and bile acids from the intestine to the liver[21]. All animal-based foods contained cholesterol in varying amounts[22] from the ability of all animal cells to manufacture cholesterol. Major dietary sources of cholesterol were listed as red meat, egg yolks and whole eggs, liver, kidney, giblets, fish oil, butter and human breast milk[23]. According to Sonawane *et al.*[23] and De Smet *et al.*[24], synthesis of cholesterol in plant cells occurred as a precursor for other compounds, such as phytosterols and steroidal glycoalkaloids, with cholesterol remaining in plant foods only in minor amounts or absent. Some plant foods, such as avocado, flax seeds and peanuts, contain phytosterols, competing with cholesterol for absorption in the intestines, thereby reducing the absorption of both dietary and bile cholesterol[25]. Cholesterol and other blood fats are carried in the blood by proteins and after the combination of these proteins and fats, lipoproteins were the end products[26]. There are 2 main types of Lipoproteins: low density lipoprotein (LDL) or 'bad cholesterol' and high-density lipoprotein (HDL) or 'good cholesterol'. The LDL was reported to carry cholesterol from the liver, through the blood stream to the cells and arteries of the body[27,28] a phenomenon that was incriminated in promoting atherosclerosis by stimulating production of haematopoeitic stem/progenitor cells[29]. Hence, laboratory measurement of HDL was considered more significant for clinical diagnosis. Whereas, HDL removes cholesterol from the blood stream rather than depositing it in the arteries[30].

Physiology of Cholesterol

The esterification of fat via chylomicrons led to introduction of cholesterol into the blood[31], which causes its poor absorption by the gut. The body also compensated for absorption of ingested cholesterol by reducing its own cholesterol synthesis[32]. For these reasons, cholesterol in food, 7 to 10 hours after ingestion, has little, if any effect on concentrations of cholesterol in the blood. However, during the first 7 hours after ingestion, as absorbed fats are being distributed around the body within extracellular water by the various lipoproteins, the concentrations increased[33] with the liver excreting it via bile in a non-esterified form into the digestive tract. Behrman and Gopalan[34] reported that about 50% of the excreted cholesterol was reabsorbed by the small intestine back into the bloodstream. According to Jones[35], increased dietary cholesterol absorption led to decreased endogenous synthesis, thus, maintaining cholesterol balance. While dietary triglycerides were almost completely absorbed, only 30-50% of the cholesterol present in the intestinal lumen was absorbed[36,37,38].

Pathophysiology of cholesterol

Atherosclerotic process commenced with endothelial damage, dysfunction of endothelial cells and consequential increase in LDL particle which permeated through the vascular wall and got trapped by cellular matrix in the intima with smooth muscle cells migration into the lesion ultimately encapsulation and protection of the newly formed plaque from exposure occurred; the plaque may occlude or reduce laminal flow of blood causing ischaemia or ruptured to form thrombus and could lead to unstable angina and/or acute myocardial infarction (AMI)[39,40].

Contrasting Research on Cholesterol Values and the Controversies

Early last century, Anichkov observed that rabbits fed pure cholesterol dissolved in sunflower oil developed atherosclerotic lesions, whereas the control rabbits fed just sunflower oil did not[41,42,43]; the latter was largely rejected with no followed-up attempt. Serious research on the role of cholesterol in atherosclerosis got under way when laboratories later tried to reproduce the results using dogs and rats but failed to link cholesterol rich diet with the development of atherosclerosis, because dogs and other carnivores metabolize cholesterol differently from rabbits and other herbivores. This led to the dismissal of Anichkov's results speculating that rabbits were not a good model for human physiology and not relevant to humans and other animals. For half a century, a high level of total cholesterol (TC) or LDL cholesterol (LDL-c) was considered as the major cause of atherosclerosis and cardiovascular diseases (CVD) which implied that individuals with high TC should have a higher risk of death from CVD[44]. The hypothesis that high TC causes CVD was introduced in the 1960s by Framingham Heart Study group with a 30-year follow-up study which reported that for each 1 mg/dL drop in TC per year, there was an 11% increase in coronary heart disease (CHD) and total mortality[45]. In contrast, about 3 years later, the American Heart Association and the U.S. National Heart, Lung and Blood Institute jointly published a summary, concluding that a 1.0% reduction in an individual's TC resulted in an approximately 2.0% reduction in CHD risk'[46]. This apparent controversy is supported by the notion that high blood cholesterol levels mainly caused CVD was found debatable because people with low levels were just as atherosclerotic as those with high levels and their risk of developing CVD was reported to be the same or higher[47]. Much earlier, Landé and Sperry[48] found, in correlation with age, that unselected low TC people were as atherosclerotic as high TC people. Indeed, there was a lack of association between LDL-c and degree of atherosclerosis[49]. In addition, an Austrian study that included 67,413- and 82,237-men and women, respectively, follow up for many years, revealed that TC was weakly associated with CHD mortality for men, except for those within the 50 to 64 years bracket, while for women, it was weakly associated among those below 50 years old with no association after that age[50]. There was no association between TC and other CVD-induced mortality, except that low TC was inversely associated with CVD mortality for women above the age of 60 years. If high LDL-c causes CVD, LDL-c of untreated patients with CVD should be higher than normal. If the speculation that high LDL-c caused CVD is accepted, it would be expected that LDL-c of untreated CVD patients should be higher than normal, but in contrast, a large American study, that included almost 140,000 patients with AMI revealed that their LDL-c at the time of admission into the hospital, was actually lower than normal[51]. Al-Mallah *et al.*[52] reported similar results, but lowered the patients' LDL-c even more, a 3 -year follow-up revealed that total mortality among those with LDL-c below 105 mg/dL (2 mmol/L) was twice as high compared to those with a higher LDL-c, adjustment for confounding variables (14.8% vs. 7.1%, p = 0.005), notwithstanding. Weak association between TC and degree of atherosclerosis occurred in patients admitted into a hospital[53] who might have included patients with familial hypercholesterolemia (FH). As the percentage of such patients in cardiology department was much higher than that obtained in the general population, a bias might have been introduced. If high TC constituted the major cause of atherosclerosis, an exposure–response should be expected to follow in cholesterol-lowering drug trials; for example, the arteries of those whose lipid values were lowered should benefit the more. The calculated exposure–response in a review of 16 angiographic exercise induced cholesterol-lowering trials, revealed a correlation in only one present. Lewington[54], in a meta-analysis performed by the prospective studies collaboration, there was an association between TC and cardiovascular mortality in all ages and both sexes; the risk decreased with increasing age and became minimal after the age of 80 years. This appears contrasting, since atherosclerosis and CVD were considered mainly the diseases

of elderly, which implied that the cholesterol hypothesis, expectedly, would predict that the disease should be more prevalent in the elderly than in the young, which disagreed with the above finding.

Epidemiological analysis revealed that lack of association existed between dietary cholesterol and/or egg intake and CVD risk, generally in population[55,56] in contrast to a consistent relationship that existed between egg intake and CVD in Diabetics[15,55,56]; this risk in the diabetics was ascribed to phosphatidylcholine content of eggs[57] and not the dietary cholesterol that are more poorly absorbed in obese and insulin-resistant populations compared to lean individuals[58]. The phosphatidylcholine intake was linked to the gut microbial-dependent production of trimethylamine N-oxide (TMAO), a metabolite that promoted atherosclerosis in hyperlipidemic mouse model with an association with CVD risk in human cohort studies[59]. Indeed, a systematic review and meta-analysis of 17 cohort studies examining the relationship between dietary cholesterol and CVD[60] dietary cholesterol was significantly associated with heart disease, ischaemic stroke or haemorrhagic stroke. In addition, no association existed between LDL-c and coronary calcification in a study that included 304 patients[61]. However, a notable exception occurred in 1779 healthy individuals without conventional risk factors for CVD, where LDL-c was significantly higher among those with subclinical atherosclerosis (125.7 vs.117.4 mg/dL), an association that did not prove causation, as mental stress, for instance, was able to raise cholesterol by 10–50% within half an hour[62,63], in addition to causing atherosclerosis by mechanisms other than an increase in LDL-c; for instance, via hypertension and increased platelet aggregation. In Japan, the prevalence of heterozygous FH was found to be 5.7% (1/17.5) in a total of 359 patients with acute coronary syndrome, but more prominent in younger patients who were less than 60 years old (7.8%)[64].

However, Zhong *et al.*[65] reported associations of dietary cholesterol (egg consumption) with incident CVD and mortality using 29 615 adults pooled from 6 prospective cohort studies in the US with a median follow-up of 17.5 years; each additional 300 mg of dietary cholesterol consumed per day was significantly associated with higher risk of incident CVD and each additional half an egg consumed per day was significantly associated with higher risk of incident and all-cause mortality in a dose-response manner. This study had several limitations. First, appropriate interpretation of the study findings required consideration of measurement error for self-reported diet data. Instead of using different dietary assessment tools to create heterogeneities for data analyses, the study relied on single measurement of egg and dietary cholesterol consumption. Exposure misclassification might be of concern, but results were similar when censoring participants at different time points[65].

In animals, cholesterol is produced endogenously with only about 25% of the serum cholesterol derived from the diet. An average 70 kg adult syntheses about 850 mg cholesterol per day. If this individual is to consume 400 mg/day of dietary cholesterol and absorb 60% that amounts to only 22% of cholesterol coming from the diet. Furthermore, the values are skewed even more towards cholesterol biosynthesis in overweight and obese individuals[58] studies evaluating the association of low LDL-c with safety outcomes, Faselis *et al.*[66] found that achieving an LDL-c of 40-50 mg/dL seems to be safe, and importantly might offer CV beneficial effects.

The writing committee of the studies of Lewington[54] and Collins *et al.*[67], published a meta-analysis of 61 prospective observational studies that involved about 900,000 adults with the conclusion that TC was associated with CHD mortality in all ages and both sexes. In contrast, Ravnskov *et al.*[68], found the opposite in a systematic review of 19 cohort studies that involved more than 68,000 elderly individuals above 60 years of age. In a larger cohort study consisting 118,160 subjects greater than 50 years, individuals with the highest LDL-c levels were found to live even longer than those on statin treatment[69]. In addition, Hamazaki *et al.*[70] reported that numerous Japanese epidemiological studies found high LDL-c was not a risk factor for CHD mortality in women of any age, and further, there was an inverse association between TC and all-cause mortality, irrespective of age and sex. Benn[71] used genetic variants in proprotein convertase subtilisin-kexin type 9 (PCSK9) and a variant in HMGCR, to examine the causal effect of low LDL-c levels on risk of neurological disease and did not observe any associations with increased risk of Alzheimer's disease, vascular dementia, any dementia, and Parkinson's disease in 111, 194 individuals from the general population. Data on 380 genetic variants from the International Genomics of Alzheimer's Project were, however, suggestive of a causal effect of low LDL-c levels in reducing the risk of Alzheimer's disease.

Researchers have over the years, repeatedly fed laboratory animals large amounts of cholesterol in their diets[72,73,74], expecting this to produce vascular accidents. It did not, which showed that despite the presence of cholesterol in atheromatous plaques these lesions are not caused by eating cholesterol. These animal studies have demonstrated that inflammatory processes tightly regulate the developmental processes of lesions, with significant contributions from both adaptive and innate immune processes[75,76,77,78]. Other supports for the above hypotheses were derived from the fact that CVD might be caused by infections and that LDL directly bound to inactivate a broad range of micro-organisms and their toxic products[44,79,80]. Another explanation for an inverse association between LDL-c and mortality was the report that high LDL-c, protected against cancer, as many cancers were caused by viruses[81]. Furthermore, cholesterol lowering experiments on rodents resulted in cancer[82]. In addition, 9 cohort studies including more than 1440,000 individuals followed for 10-30 years found an inverse association between cancer and TC, measured at a start of the study and more patients with cancer were on cholesterol lowering treatment, irrespective of their age and sex[53]. For these reasons, it will be of value to state that there is an inverse association or lack of association between cholesterol and CVD and mortality.

Measurements of Cholesterol Level

Various analytical methods for determination of cholesterol levels (Table 1) have been developed including Gas Chromatography-Mass Spectrometry (GC-MS), Gas Chromatography Isotope-Dilution Mass Spectrometry (GC-ID-MS), Liquid chromatography-mass spectrometry (LC-MS), Electros Pray Ionization Tandem Mass Spectrometry (ESI-MS), Electros pray Ionization tandem mass spectrometry (ESI-MS), Matrix-assisted laser desorption ionization-time of flight (MALDI-MS), and Matrix-assisted laser desorption/ionization-ion-mobility mass spectrometry (MALDI-IM-MS), with serum and plasma as the acceptable specimens[83,84] and Ambient ionization mass spectrometry techniques (AIMS). The AIMS had been proposed as alternative approaches for high-throughput screening as they did not require tedious sample pretreatments. However, with the new diagnostic technique or imaging modality such as the performance of intravascular photoacoustic imaging, which is an emerging plaque detection technique that provides lipid specific chemical information from an arterial wall with great optical contrast and long acoustic penetration depth[85]. Direct Analysis in Realtime Mass Spectrometry (DART-MS) was developed for rapid, cost-

effective and increase the understanding of physiological processes and disease pathology towards a more precise absolute quantitation of cholesterol. Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) and Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI) techniques were used in tissue sections, to observed spatial distribution of cholesterol and its derivatives. Mass spectrometry-based molecular imaging increases the understanding of physiological processes and pathology towards to a more precise absolute quantitation of molecules in it.

Table 1: Methods for cholesterol measurements

Method	Principles	Ref
Modified Abell-Kendall method	Saponification and colour development	86
Fluorometric-enzymatic assay	Hydrosis of cholesterol to produce hydrogen peroxide (H_2O_2) which can be quantified	87
GC-MS	Separation of cholesterol using the density and dispersal in gas medium and quantification by mass spectroscope	88, 89
GC-ID-MS	Comparing the sample with a standard cholesterol of known concentration which is an isotopically labelled cholesterol in gas medium and quantification using mass spectroscope	90
LC-MS	Separation of cholesterol using the density and distribution or dispersal in a liquid phase and quantification with mass spectroscope	91
ESI-MS	Conversion of cholesterol to ionic form and quantification	90
MALDI-MS	Removal of cholesterol, its dehydration, ion mobility and ultimate quantification using mass spectroscopy	92
MALDI-IM-MS	Coating of cholesterol with metal to enhance its ion mobility and quantification with mass spectroscope	93
DESI-MSI	Formation of cholesterol ion and quantification	94
DART-MS	Dehydration of cholesterol and the spectrometric quantification	95,96
MALDI-MSI	Embedding cholesterol in a matrix the spectrometric imaging and quantification in tissues	97
DESI-MSI	Reaction of cholesterol with Betained aldehyde to produce coloured a background in organs that can be imaged	98

Conclusion

The multifaceted path by which cholesterol became linked to CHD is one of the greatest biomedical stories of the 20th century. The current review does not support the notion that dietary cholesterol increases the risk of CVD. The idea that high cholesterol levels in the blood constitute the main cause of CVD is doubtful because people with low levels become just as atherosclerotic as people with high levels and their risk of suffering from CVD is the same or higher. In reality, the accelerated vascular damage seen in CVD is likely to be a consequence of more brittle arterial cell walls, as cholesterol to be a component of them which modulates their fluidity, conferring flexibility and hence resistance to damage from the ordinary hydrodynamic blood forces. In the absence of efficient receptors for LDL cholesterol, cells will be unable to use this component adequately for the manufacture of normally resilient arterial cell walls, resulting in accelerated arteriosclerosis. Eating high cholesterol diet is harmless, shown by its failure to produce vascular accidents in laboratory animals, but its avoidance causes malnutrition from lack of fat-soluble vitamins, especially vitamin D. Analytical differences may account for variations in laboratory results that may account for variations in interpretations.

Conflicts of interest

None.

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