

# Dietary cholesterol and egg yolks: Not for patients at risk of vascular disease

J David Spence MD FRCP<sup>1</sup>, David JA Jenkins MD PhD FRCP<sup>2</sup>, Jean Davignon MD MSc FRCP<sup>3</sup>

JD Spence, DJA Jenkins, J Davignon. Dietary cholesterol and egg yolks: Not for patients at risk of vascular disease. *Can J Cardiol* 2010;26(9):e336-e339.

A widespread misconception has been developing among the Canadian public and among physicians. It is increasingly believed that consumption of dietary cholesterol and egg yolks is harmless. There are good reasons for long-standing recommendations that dietary cholesterol should be limited to less than 200 mg/day; a single large egg yolk contains approximately 275 mg of cholesterol (more than a day's worth of cholesterol). Although some studies showed no harm from consumption of eggs in healthy people, this outcome may have been due to lack of power to detect clinically relevant increases in a low-risk population. Moreover, the same studies showed that among participants who became diabetic during observation, consumption of one egg a day doubled their risk compared with less than one egg a week.

Diet is not just about fasting cholesterol; it is mainly about the postprandial effects of cholesterol, saturated fats, oxidative stress and inflammation. A misplaced focus on fasting lipids obscures three key issues. Dietary cholesterol increases the susceptibility of low-density lipoprotein to oxidation, increases postprandial lipemia and potentiates the adverse effects of dietary saturated fat. Dietary cholesterol, including egg yolks, is harmful to the arteries.

Patients at risk of cardiovascular disease should limit their intake of cholesterol. Stopping the consumption of egg yolks after a stroke or myocardial infarction would be like quitting smoking after a diagnosis of lung cancer: a necessary action, but late. The evidence presented in the current review suggests that the widespread perception among the public and health care professionals that dietary cholesterol is benign is misplaced, and that improved education is needed to correct this misconception.

**Key Words:** Atherosclerosis; Cholesterol; Coronary; Diet; Eggs; Stroke

Recent media reports reflect the remarkable effectiveness of the sustained propaganda campaign of the egg producers' lobby. Not only in Canada, but around the world, the public, nutritionists (1-3) and even physicians (4) are increasingly accepting of the notion that dietary cholesterol is not important. Even the Heart and Stroke Foundation has been taken in (5), quoting directly from the egg marketers' propaganda in a brochure distributed to British Columbia and Ontario households in February 2010, which is Heart Month! It is timely, therefore, to review the evidence that dietary cholesterol is important, and that regular consumption of egg yolks cannot be recommended for patients with cardiovascular disease or for those at high risk of cardiovascular disease.

## RECENT MEDIA REPORTS

In the past year, two studies funded by egg marketing agencies led to media reports promoting the benefits of eggs. The first (6) was a British study of healthy young people from Surrey who were on a weight loss and exercise regimen. This study showed that egg consumption did not

## Le cholestérol alimentaire et les jaunes d'œuf : Pas pour les patients vulnérables à une maladie vasculaire

Une idée fausse et généralisée se répand au sein du public canadien et des médecins, qui pensent de plus en plus que la consommation de cholestérol alimentaire et de jaunes d'œuf est inoffensive. Les recommandations de longue date qui préconisent de limiter le cholestérol alimentaire à moins de 200 mg/jour reposent sur de bonnes raisons. Un seul gros jaune d'œuf contient environ 275 mg de cholestérol (plus que la portion quotidienne de cholestérol). Même si certaines études ont démontré que la consommation d'œufs n'est pas nuisible chez les personnes en santé, ce résultat peut découler de l'absence de capacité à déceler des augmentations pertinentes sur le plan clinique au sein d'une population à faible risque. De plus, les mêmes études ont révélé que chez les participants devenus diabétiques pendant la période d'observation, la consommation d'un œuf par jour doublait leur risque par rapport à la consommation de moins d'un œuf par semaine.

Le régime ne vise pas à éviter le cholestérol, mais surtout les effets postprandiaux du cholestérol, des gras saturés, du stress oxydant et de l'inflammation. Le fait de se concentrer à tort sur les lipides à jeun occulte trois enjeux. Le cholestérol alimentaire accroît la susceptibilité des lipoprotéines à faible densité à l'oxydation, accroît la lipémie postprandiale et potentialise les effets secondaires des graisses saturées alimentaires. Le cholestérol alimentaire, y compris les jaunes d'œuf, est nuisible pour les artères.

Les patients vulnérables aux maladies cardiovasculaires devraient limiter leur consommation de cholestérol. Le fait d'arrêter de consommer des jaunes d'œuf après un accident vasculaire cérébral ou un infarctus du myocarde s'apparenterait à arrêter de fumer après un diagnostic de cancer du poumon : c'est un geste nécessaire, mais entrepris tardivement. D'après les données probantes présentées dans la présente analyse, la perception généralisée du public et des professionnels de la santé selon laquelle le cholestérol alimentaire est un mal bénin est une idée fausse, et une meilleure information s'impose pour la corriger.

increase levels of fasting cholesterol. The lack of relevance of an effective weight loss and exercise program to most patients at risk of vascular disease seemed to escape the commentators. The second was a Canadian study (7), which showed that eggs contain a substance that inhibits angiotensin-converting enzyme. This study led to media reports that eggs could be beneficial, even though the study was in vitro, with no established relevance to human disease. The eagerness of the media to report benefits of egg consumption suggests that such stories are of interest because they are surprising reversals of accepted wisdom. However, the consequences of high cholesterol intake in those at increased risk of cardiovascular disease who are sedentary and not losing weight – especially when already consuming relatively high levels of saturated fat – give reason for concern.

## RECOMMENDATIONS TO LIMIT CHOLESTEROL INTAKE

Although the low-fat diet originally recommended by the National Cholesterol Education Program (NCEP) was probably not the optimal

<sup>1</sup>Stroke Prevention & Atherosclerosis Research Centre, Robarts Research Institute, London; <sup>2</sup>Risk Factor Modification Centre, St Michael's Hospital, Toronto, Ontario; <sup>3</sup>Clinique de nutrition métabolisme et athérosclérose, Institut de recherches cliniques de Montréal, Montreal, Quebec

Correspondence: Dr J David Spence, Stroke Prevention & Atherosclerosis Research Centre, 1400 Western Road, London, Ontario N6G 2V2.

Telephone 519-663-3113, fax 519-663-3018, e-mail dspence@robarts.ca

Received and accepted for publication April 26, 2010

diet (8), there were good reasons for its recommendation that patients at risk of cardiovascular disease should limit their cholesterol intake to less than 200 mg/day (in both the NCEP Step 2 diet [9] and the American Heart Association dietary recommendations [10]). The general population was advised to limit cholesterol intake to less than 300 mg/day (9,10). More recently, the "Healthy People 2000" goal (11) of a dietary cholesterol intake of 200 mg/day or less for the general United States population appeared to be obtainable based on trends seen in the National Health and Nutrition Examination Surveys (12). A single egg yolk contains approximately 215 mg to 275 mg of cholesterol (depending on the size). The yolk of a large egg provides more than the 210 mg of cholesterol in a Hardee's Monster Thickburger (Hardee's Food Systems Inc, USA), which contains two-thirds of a pound of beef, three slices of cheese and four strips of bacon (13). A single egg yolk thus exceeds the recommended daily intake of cholesterol.

### IT IS NOT ALL ABOUT FASTING CHOLESTEROL LEVELS

A common misconception is that because statin drugs lower fasting cholesterol levels by approximately one-half and a low-fat diet only lowers cholesterol levels by approximately 10% (14), there is no point in worrying about diet – simply take a statin, and it will be okay to eat anything! Nothing could be further from the truth. Although statins lower coronary artery disease risk by 25% to 40% and possibly more, it has been estimated that diet may account for 85% of coronary risk (15), and in the best of circumstances, statins still leave a residual coronary risk in the order of 70% in major clinical trials (16).

In human subjects, a high intake of dietary cholesterol increased fasting low-density lipoprotein (LDL) levels by approximately 10% (17) in a dose-dependent manner (18). A 10% increase in fasting cholesterol levels may not seem like much, but in the first study to show that diet and cholestyramine reduced coronary risk, a 12% reduction of fasting LDL levels reduced coronary risk by 19% (19). Even the relatively permissive Step 1 American Heart Association diet (300 mg/day of cholesterol and 30% of calories from fat) reduced fasting LDL levels by approximately 10%, compared with a typical western diet (14).

Furthermore, diet is not all about fasting lipids; it is mainly about the three-quarters of the day that we are in the nonfasting state (20). Fasting lipids can be thought of as a baseline; they show what the endothelium was exposed to for the last few hours of the night. Then we get up and eat breakfast. For the next several hours, the endothelium is exposed to sugars, fats, cholesterol and free radicals from that meal, on top of the baseline. Then we have lunch followed by supper and by the time we retire to bed, we have been in a nonfasting state for approximately 18 h of the day. This is determined without even considering snacks between meals.

A focus on fasting LDL and dietary cholesterol levels per se has obscured three important issues. The first is that dietary cholesterol increased susceptibility of LDL to oxidation by 37% (21) in one study and by 39% in another (22). The latter study was performed with cooked egg yolks fed for periods of 32 days. The second issue is that the consumption of more than 140 mg dietary cholesterol in a single meal markedly increases postprandial lipemia (23). Third, dietary cholesterol potentiates the adverse effects of dietary saturated fat (the bacon and egg effect), as discussed below.

In a study of normolipidemic young men (52 Caucasian and 32 non-Caucasian), Fielding et al (24) compared the effects of diets high or low in saturated and polyunsaturated fat (polyunsaturated/saturated fat ratio 0.8 versus 0.3). The study also compared diets high versus low in cholesterol (200 mg versus 600 mg). At the lower cholesterol intake, the high saturated fat diet had only a modest effect on LDL cholesterol level in Caucasians (increase of 6 mg/dL [0.16 mmol/L]) and no effect in non-Caucasians. However, the diet with 600 mg cholesterol and high in saturated fat led to a substantial

mean increase in LDL cholesterol level, which was significantly greater in Caucasian than in non-Caucasian subjects (increase of 31 mg/dL [0.82 mmol/L] versus 16 mg/dL [0.41 mmol/L],  $P < 0.005$ ). In contrast, 600 mg of cholesterol with increased polyunsaturated fat gave a mean LDL level increase of 16 mg/dL (0.42 mmol/L) – lower than that found when the same high cholesterol intake was coupled with increased saturated fat. Variation in cholesterol level rather than the proportions of saturated and polyunsaturated fat had the most influence on LDL cholesterol levels. Among non-Caucasians in this study, it was the only significant factor.

### DIETARY CHOLESTEROL, POSTPRANDIAL FAT AND OXIDATIVE STRESS

The principal reason for measuring serum cholesterol levels in the fasting state is to minimize variability (25). Postprandial levels of lipids are at least as predictive of risk as fasting lipid levels (26,27); the difficulty is in standardizing the conditions in which they are measured.

In mouse models, both a western diet and a high-cholesterol diet induce endothelial dysfunction (28). In human subjects, endothelial function is impaired for approximately 4 h after consumption of a high-fat/high-cholesterol meal; this effect is probably due to oxidative stress (29), because the effect is mitigated by the antioxidant supplements (30).

The effects of dietary cholesterol on serum cholesterol are, in part, dependent on the diet and the characteristics of the individual consuming the cholesterol. Dietary cholesterol has a much greater effect on people consuming a low-cholesterol diet, with a threshold effect as shown by Connor et al (31). In their 1961 study, Connor et al also showed that egg yolk, containing 240 mg of cholesterol, had a greater hyperlipidemic effect than pure crystalline cholesterol dissolved in oil. In people consuming a low-cholesterol diet, egg yolk intake increased fasting serum cholesterol level by 40 mg/dL (1.04 mmol/L) (31). This finding was substantiated later by Mattson et al (32) and Sacks et al (33), who found a 12% increase in LDL cholesterol level with egg consumption.

The relationship of the apolipoprotein (apo) E phenotype with the effects of dietary cholesterol was analyzed in a Finnish study (34). At baseline, there were no statistically significant differences in lipid concentrations between the phenotype groups. The cholesterol-rich diet induced significant increases in total cholesterol levels, LDL cholesterol levels and apoB in all apoE groups ( $P < 0.001$ ). The magnitudes of these increases were similar in groups E3/2, E3/3 and E4/3, in which total cholesterol concentration rose by 13%, 18% and 12%, respectively. Stronger responses were observed in the small group of E4/4 subjects, in whom the increases in total cholesterol levels, LDL cholesterol levels and apoB were more than doubled, compared with all the other phenotypes studied (34). Knopp et al showed that the increase in fasting LDL cholesterol level associated with egg consumption was greater in subjects without insulin resistance (35) and in those with combined hyperlipidemia (36).

### EGG YOLKS AND POSTPRANDIAL INFLAMMATION

Feeding of egg yolks induces inflammation characterized by elevations of C-reactive protein and serum amyloid A, but this effect is more pronounced in lean individuals without insulin resistance (37). Mechanisms of the inflammatory effects of dietary cholesterol have been investigated in animal models. Dietary cholesterol worsens macrophage accumulation in adipose tissue and atherosclerosis in LDL-receptor-deficient mice (38), and increases systemic inflammation (39). Dietary cholesterol also induces monocyte chemoattractant protein-1, contributing to hepatic steatosis (40).

A Mediterranean diet improves endothelial function compared with a western diet (41,42), probably because in addition to being lower in cholesterol and animal fat, it has higher levels of antioxidants and lower levels of free radicals (43).

## ADVERSE EFFECTS OF DIETARY CHOLESTEROL

Despite widespread belief to the contrary, it is simply not true that dietary cholesterol is harmless. Concern about dietary cholesterol has been developing over the past 40 years. This concern is based on the careful and independent conclusions of Ancel Keys and Mike Hegsted, who formulated our two most commonly used equations (44,45) relating dietary saturated and polyunsaturated fat and cholesterol to serum cholesterol.

There is also no doubt that cholesterol feeding is a causal factor in animal models of atherosclerosis. Such models include rhesus monkeys fed a diet rich in egg yolks that developed hypercholesterolemia, xanthomatosis and atherosclerosis (46,47). In 1908, Ignatowski showed that meat – containing the pro-oxidant iron – fed to adult rabbits, or milk and egg yolks fed to weanling rabbits, induced atherosclerosis (48). In 1913, Anitschkov established the cholesterol-fed rabbit as a model for dietary atherosclerosis (49). Cholesterol feeding in rabbits not only causes cholesterol accumulation in plasma and the arterial wall but also promotes inflammation and cell proliferation, which may be blocked by the anti-inflammatory agent cortisone to prevent the formation of raised atherosclerotic lesions (50).

The human diet contains large quantities of oxidized fatty acids and oxidized cholesterol because a large portion of the fat and cholesterol in the diet is often prepared in fried, heated or processed form (51); as much as 12% of dietary cholesterol is in the oxidized form (39). Salonen et al (52) showed that lipid oxidation independently increased progression of carotid intima-media thickness. It seems likely that it is oxidized cholesterol in the diet that is harmful.

Dietary cholesterol increased coronary risk in both the Ireland-Boston Diet-Heart Study (53) and the Western Electric study (54). These results showed good agreement between the Keys dietary score, which emphasized saturated fat, and the Hegsted score, which emphasized dietary cholesterol.

Possible confounding of cholesterol intake from eggs with other risk factors, such as smoking, have been raised as a concern. However, the careful work of Markus et al (55) showed that cholesterol intake, independent of smoking and other risk factors, increased carotid intima-media thickness.

## EGG CONSUMPTION AND CARDIOVASCULAR RISK

The proponents of egg consumption repeatedly point out (eg, in advertisements in last year's Medical Post, and in a letter to the Globe and Mail last year) that two large epidemiological studies (56,57) showed no harm from egg consumption in healthy people. However, they fail to mention that both those studies showed that in participants who became diabetic during observation, consumption of one egg a day markedly increased cardiovascular risk compared with less than one per week (56,57). The first study – the Health Professionals Study – included physicians and nurses, and showed a doubling of cardiovascular disease in men who became diabetic during the course of the study. It also showed a significant increase of new-onset diabetes with regular egg consumption (56).

The second study (57) also showed a doubling of cardiovascular risk with regular egg consumption in participants who became diabetic during the course of the study, but not in people who remained healthy and free of diabetes.

In these two studies, failure to show harm from eggs in healthy people is likely an issue of statistical power: in healthy people, a larger study with longer follow-up would be required; in diabetic patients who have a coronary risk equivalent to that of patients with coronary artery disease, the statistical power was adequate to show a harmful effect of egg yolks. A recent reanalysis of the smaller Physicians' Health Study (58) did not show an increase in cardiovascular disease, but did show that regular egg consumption doubled all-cause mortality. Two recent studies (59,60) also showed that consumption of eggs increased new-onset diabetes, independent of other dietary factors.

Furthermore, those following a lifelong lacto-ovo vegetarian diet have a markedly reduced risk of cardiovascular disease (by approximately 40% in men and approximately 50% in women) (61). However, this group also has an absence of heme iron in the diet as a pro-oxidant, together with an increased fibre- and plant sterol-induced loss of cholesterol as bile acids and fecal cholesterol.

## CONCLUSION

There is no question that egg white is classed as a valuable source of high-quality protein. Egg yolks, however, are not something that should be eaten indiscriminately by adults without regard to their global cardiovascular risk, genetic predisposition to heart attacks and overall food habits. Diabetic patients are a case in point. At a time when inactivity, obesity and diabetes are increasing internationally, together with the western diet and lifestyle, it seems very unwise to reverse the long-standing recommendations on limiting cholesterol consumption. Reduced cholesterol intake formed a major part of the advice given over the past four decades, during which time a marked reduction in cardiovascular disease has been recorded. In our opinion, stopping egg consumption after a myocardial infarction or stroke would be like quitting smoking after lung cancer is diagnosed: a necessary act, but late.

**ACKNOWLEDGEMENTS:** The authors are indebted to Dr David Fitchett for the reference to Hardee's Monster Thickburger.

**CONFLICT OF INTEREST:** None of the authors receives funding from purveyors of margarine or eggs. Dr Spence and Dr Davignon have received honoraria and speaker's fees from several pharmaceutical companies manufacturing lipid-lowering drugs, and Dr Davignon has received support from Pfizer Canada for an annual atherosclerosis symposium; his research has been funded in part by Pfizer Canada, AstraZeneca Canada Inc and Merck Frosst Canada Ltd.

## REFERENCES

1. Beck L. Heart health concerns? You can still get cracking. The Globe and Mail, April 16, 2008.
2. Beck L. Cracking nutrition myths. The Globe and Mail, March 4, 2009.
3. Taylor V. Regular eggs 'no harm to health'. British Heart Foundation, BBC news, November 2, 2009. <<http://news.bbc.co.uk/2/hi/health/7882850.stm>> (Accessed on April 12, 2010).
4. McDonald BE. The Canadian experience: Why Canada decided against an upper limit for cholesterol. J Am Coll Nutr 2004;23(6 Suppl):616S-20S.
5. Insider. Eggs: They can be part of a healthy diet! Heart and Stroke Foundation, February 10, 2010.
6. Harman NL, Leeds AR, Griffin BA. Increased dietary cholesterol does not increase plasma low density lipoprotein when accompanied by an energy-restricted diet and weight loss. Eur J Nutr 2008;47:287-93.
7. Majumder K, Wu J. Angiotensin I converting enzyme inhibitory peptides from simulated in vitro gastrointestinal digestion of cooked eggs. J Agric Food Chem 2009;57:471-7.
8. Willett WC, Stampfer MJ. Rebuild the food pyramid. Sci Am 2003;1:64-71.
9. Adult Treatment Panel III. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-421.
10. Krauss RM, Eckel RH, Howard B, et al. Revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. J Nutr 2001;131:132-46.
11. Healthy People 2000: National Health Promotion and Disease Prevention Objectives. United States Department of Health and Human Services, 1991.
12. Ernst ND, Sempos CT, Briefel RR, Clark MB. Consistency between US dietary fat intake and serum total cholesterol concentrations: The National Health and Nutrition Examination Surveys. Am J Clin Nutr 1997;66(4 Suppl):965S-72S.

13. Hardee's Monster Thickburger. <<http://www.hardees.com/menu/lunch-and-dinner/23-lb-monster-thickburger/Hardee's>> (Accessed on August 22, 2009).
14. Ginsberg HN, Barr SL, Gilbert A, et al. Reduction of plasma cholesterol levels in normal men on an American Heart Association Step 1 diet or a Step 1 diet with added monounsaturated fat. *N Engl J Med* 1990;322:574-9.
15. Hu FB, Stampfer MJ, Manson JE, et al. Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med* 1997;337:1491-9.
16. Steinberg D, Glass CK, Witztum JL. Evidence mandating earlier and more aggressive treatment of hypercholesterolemia. *Circulation* 2008;118:672-7.
17. Sacks FM, Salazar J, Miller L, et al. Ingestion of egg raises plasma low density lipoproteins in free-living subjects. *Lancet* 1984;1:647-9.
18. Ginsberg HN, Karmally W, Siddiqui M, et al. A dose-response study of the effects of dietary cholesterol on fasting and postprandial lipid and lipoprotein metabolism in healthy young men. *Arterioscler Thromb* 1994;14:576-86.
19. The Lipid Research Clinics Coronary Primary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA* 1984;251:365-74.
20. Spence JD. Fasting lipids: The carrot in the snowman. *Can J Cardiol* 2003;19:890-2.
21. Levy Y, Maor I, Presser D, Aviram M. Consumption of eggs with meals increases the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. *Ann Nutr Metab* 1996;40:243-51.
22. Schwab US, Ausman LM, Vogel S, et al. Dietary cholesterol increases the susceptibility of low density lipoprotein to oxidative modification. *Atherosclerosis* 2000;149:83-90.
23. Dubois C, Armand M, Mekki N, et al. Effects of increasing amounts of dietary cholesterol on postprandial lipemia and lipoproteins in human subjects. *J Lipid Res* 1994;35:1993-2007.
24. Fielding CJ, Havel RJ, Todd KM, et al. Effects of dietary cholesterol and fat saturation on plasma lipoproteins in an ethnically diverse population of healthy young men. *J Clin Invest* 1995;95:611-8.
25. Hegsted DM, Nicolosi RJ. Individual variation in serum cholesterol levels. *Proc Natl Acad Sci U S A* 1987;84:6259-61.
26. Steiner G. Triglyceride-rich lipoproteins and atherosclerosis, from fast to feast. *Annals Med* 1993;25:431-5.
27. Gronholdt ML, Nordestgaard BG, Nielsen TG, Sillelsen H. Echolucent carotid artery plaques are associated with elevated levels of fasting and postprandial triglyceride-rich lipoproteins. *Stroke* 1996;27:2166-72.
28. Terasaka N, Yu S, Yvan-Charvet L, et al. ABCG1 and HDL protect against endothelial dysfunction in mice fed a high-cholesterol diet. *J Clin Invest* 2008;118:3701-13.
29. Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. *Am J Cardiol* 1997;79:350-4.
30. Plotnick GD, Corretti MC, Vogel RA. Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. *JAMA* 1997;278:1682-6.
31. Connor WE, Hodges RE, Bleiler RE. The serum lipids in men receiving high cholesterol and cholesterol-free diets. *J Clin Invest* 1961;40:894-901.
32. Mattson FH, Erickson BA, Kligman AM. Effect of dietary cholesterol on serum cholesterol in man. *Am J Clin Nutr* 1972;25:589-94.
33. Sacks FM, Salazar J, Miller L, et al. Ingestion of egg raises plasma low density lipoproteins in free-living subjects. *Lancet* 1984;1:647-9.
34. Lehtimäki T, Moilanen T, Solakivi T, Laippala P, Ehnholm C. Cholesterol-rich diet induced changes in plasma lipids in relation to apolipoprotein E phenotype in healthy students. *Ann Med* 1992;24:61-6.
35. Knopp RH, Retzlaff B, Fish B, et al. Effects of insulin resistance and obesity on lipoproteins and sensitivity to egg feeding. *Arterioscler Thromb Vasc Biol* 2003;23:1437-43.
36. Knopp RH, Retzlaff BM, Walden CE, et al. A double-blind, randomized, controlled trial of the effects of two eggs per day in moderately hypercholesterolemic and combined hyperlipidemic subjects taught the NCEP step I diet. *J Am Coll Nutr* 1997;16:551-61.
37. Tannock LR, O'Brien KD, Knopp RH, et al. Cholesterol feeding increases C-reactive protein and serum amyloid A levels in lean insulin-sensitive subjects. *Circulation* 2005;111:3058-62.
38. Subramanian S, Han CY, Chiba T, et al. Dietary cholesterol worsens adipose tissue macrophage accumulation and atherosclerosis in obese LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 2008;28:685-91.
39. Subramanian S, Chait A. The effect of dietary cholesterol on macrophage accumulation in adipose tissue: Implications for systemic inflammation and atherosclerosis. *Curr Opin Lipidol* 2009;20:39-44.
40. Rull A, Rodriguez F, Aragones G, et al. Hepatic monocyte chemoattractant protein-1 is upregulated by dietary cholesterol and contributes to liver steatosis. *Cytokine* 2009;48:273-9.
41. Vogel RA, Corretti MC, Plotnick GD. The postprandial effect of components of the Mediterranean diet on endothelial function. *J Am Coll Cardiol* 2000;36:1455-60.
42. Fuentes F, Lopez-Miranda J, Sanchez E, et al. Mediterranean and low-fat diets improve endothelial function in hypercholesterolemic men. *Ann Intern Med* 2001;134:1115-9.
43. Mancini M, Parfitt VJ, Rubba P. Antioxidants in the Mediterranean diet. *Can J Cardiol* 1995;11(Suppl G):105G-9G.
44. Keys A. Serum cholesterol response to dietary cholesterol. *Am J Clin Nutr* 1984;40:351-9.
45. Hegsted DM. Serum-cholesterol response to dietary cholesterol: A re-evaluation. *Am J Clin Nutr* 1986;44:299-305.
46. Andrus SB, Mann GV. Xanthomatosis and atherosclerosis produced by diet in an adult rhesus monkey. *J Lab Clin Med* 1956;48:533-50.
47. Gresham GA, Leat WM, Howard AN, Jennings IW. Pathological changes in pigs reared on semi-synthetic diets containing no fat, beef tallow and maize oil. *Br J Exp Pathol* 1964;45:128-34.
48. Kritchevsky D. Dietary protein, cholesterol and atherosclerosis: A review of the early history. *J Nutr* 1995;125(3 Suppl):589S-93S.
49. Finking G, Hanke H. Nikolaj Nikolajewitsch Anitschkow (1885-1964) established the cholesterol-fed rabbit as a model for atherosclerosis research. *Atherosclerosis* 1997;135:1-7.
50. Friedman M, Byers S, St George S. Cortisone and experimental atherosclerosis; effects upon administration. *Arch Pathol* 1964;77:142-58.
51. Staprans I, Pan XM, Rapp JH, Feingold KR. Oxidized cholesterol in the diet is a source of oxidized lipoproteins in human serum. *J Lipid Res* 2003;44:705-15.
52. Salonen JT, Nyyssonen K, Salonen R, et al. Lipoprotein oxidation and progression of carotid atherosclerosis. *Circulation* 1997;95:840-5.
53. Kushi LH, Lew RA, Stare FJ, et al. Diet and 20-year mortality from coronary heart disease. The Ireland-Boston Diet-Heart Study. *N Engl J Med* 1985;312:811-8.
54. Shekelle RB, Shryock AM, Paul O, et al. Diet, serum cholesterol, and death from coronary heart disease. The Western Electric study. *N Engl J Med* 1981;304:65-70.
55. Markus RA, Mack WJ, Azen SP, Hodis HN. Influence of lifestyle modification on atherosclerotic progression determined by ultrasonographic change in the common carotid intima-media thickness. *Am J Clin Nutr* 1997;65:1000-4.
56. Hu FB, Stampfer MJ, Rimm EB, et al. A prospective study of egg consumption and risk of cardiovascular disease in men and women. *JAMA* 1999;281:1387-94.
57. Qureshi AI, Suri FK, Ahmed S, Nasar A, Divani AA, Kirmani JF. Regular egg consumption does not increase the risk of stroke and cardiovascular diseases. *Med Sci Monit* 2007;13:CR1-CR8.
58. Djousse L, Gaziano JM. Egg consumption in relation to cardiovascular disease and mortality: The Physicians' Health Study. *Am J Clin Nutr* 2008;87:964-9.
59. Liese AD, Weis KE, Schulz M, Tooze JA. Food intake patterns associated with incident type 2 diabetes: The Insulin Resistance Atherosclerosis Study. *Diabetes Care* 2009;32:263-8.
60. Djousse L, Gaziano JM, Buring JE, Lee IM. Egg consumption and risk of type 2 diabetes in men and women. *Diabetes Care* 2009;32:295-300.
61. Fraser GE. A comparison of first event coronary heart disease rates in two contrasting California populations. *J Nutr Health Aging* 2005;9:53-8.