Evidence Paper

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Heart Foundation™

EGGS AND THE HEART

This paper summarises the evidence which forms the basis of the Heart Foundation's position on eggs and heart health

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ABBREVIATIONS

CHD	Coronary heart disease
CVD	Cardiovascular disease
FFQ	Food frequency questionnaire
HDL	High density lipoprotein cholesterol
HR	Hazard ratio
IHD	Ischaemic heart disease
LDL	Low density lipoprotein cholesterol
MI	Myocardial infarction
P:S	Polyunsaturated to saturated fatty acid ratio
RCT	Randomised controlled trial
RR	Relative risk
TC	Total cholesterol
TC:HDL	Total cholesterol to HDL cholesterol ratio
TG	Triglycerides
TMAO	Trimethylamine-N-oxide
USDA	United States Department of Agriculture
VLDL	Very low density lipoprotein cholesterol

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BACKGROUND

Cholesterol is a waxy substance that is a type of lipid. It forms part of cell membranes and is used in the formation of brain cells, nerve cells, bile acids, preformed Vitamin D and hormones. While some cholesterol in our body is essential, the type and amount of cholesterol in our blood correlates with risk of heart disease.

There are two sources of cholesterol in our bodies. The main source is endogenous cholesterol made in our bodies or recycled by the liver. The type and amount of cholesterol our body makes is influenced by the food we eat. This is our 'blood cholesterol', for example LDL-cholesterol and HDL-cholesterol. This cholesterol is packaged up in lipoproteins that act as transporters around the body. The second source is 'dietary cholesterol' from eating animal products that already contain cholesterol. These two forms of cholesterol need to be kept distinct.

This umbrella review assesses the impact of dietary cholesterol from the food we eat, especially from eggs, and updates the earlier Heart Foundation position paper from 1999. It considers whether recommendations should continue to be made to limit dietary cholesterol from eggs for people at high risk of heart disease. Systematic reviews, meta-analyses and recent RCTs assessing the impact of eggs on heart disease or risk factors are included.

INTRODUCTION

Egg yolks are high in cholesterol and are a major source of dietary cholesterol. The New Zealand Heart Foundation has previously recommended that people at high risk of heart disease limit egg intake to three eggs (yolks) per week. Whilst its original (1999) evidence paper found dietary cholesterol had little effect on blood cholesterol levels and risk of heart disease, there were two main reasons for continuing the advice to limit egg intake:

- Dietary cholesterol may contribute to a small increase in lipids in susceptible people, for example those with combined hyperlipidaemia.

- Dietary cholesterol has little impact on blood cholesterol levels within the context of a diet lower in saturated fat. However, dietary cholesterol and saturated fat act synergistically. It was thought that intakes of dietary cholesterol higher than 300mg/day were a more important determinant of total and LDL cholesterol when saturated fats accounted for more than 15% of total energy intake.[1] Average saturated fat intakes at the time were high enough to consider it prudent to advise limiting eggs for people at high risk of heart disease.

It should be noted that these recommendations were not for the general healthy population, for whom no recommendations have been made by the NZ Heart Foundation around limiting egg intake.

OTHER ORGANISATIONS RECOMMENDATIONS

Since the original Heart Foundation position paper was published, most organisations around the world have liberalised their advice on eggs. The Australian Heart Foundation now recommend up to six eggs per week as part of a balanced diet for people with heart disease, and the British Heart Foundation removed restrictions on egg intake.

In the United States, the draft 2015 USDA dietary guidelines are in line with recent American Heart Association recommendations and state:

> *Previously, the Dietary Guidelines for Americans recommended that cholesterol intake be limited to no more than 300 mg/day. The 2015 DGAC will not bring forward this recommendation because available evidence shows no appreciable relationship between consumption of dietary cholesterol and serum cholesterol, consistent with the conclusions of the AHA/ACC report.*[2]

The latest American Heart Association and American College of Cardiologists position is that “there is insufficient evidence to determine whether lowering dietary cholesterol reduces LDL-cholesterol”.[3] They were unable to identify a good quality systematic

review or meta-analysis that met their inclusion criteria, thus leading to a rating of insufficient evidence.

New Zealand context

The Adult Nutrition Survey 2008/09 found usual median daily intakes of dietary cholesterol were 316mg for males and 219mg for females. Intakes were higher for Māori and Pacific peoples, with no difference by level of socio-economic deprivation.[4]

Eggs and egg dishes contributed 13% of dietary cholesterol, poultry 12%, beef and veal 9%, milk 8%, fish and seafood 8%, bread-based dishes 7% and pork 5%. A medium-sized egg contains around 202mg of dietary cholesterol.

Saturated fatty acids have a greater effect on blood cholesterol levels than dietary cholesterol, and their intake was on average 36.5g per day for males and 25.8g per day for females. This equated to 13.1% of daily energy intake. Intakes were higher than average for Māori, but similar for Pacific peoples.

Apart from their dietary cholesterol content, eggs are an inexpensive source of protein and other nutrients such as carotenoids, vitamin D, B12, selenium and choline.

The Ministry of Health's Eating and Activity Guidelines for adults do not give any specific recommendations around limiting egg intake.

Considerations when interpreting studies on eggs

Dietary context and confounders

- The dietary context in studies is often not well defined, and the surrounding diet could moderate the influence of eggs. For example, if the background diet is high or low in dietary cholesterol, saturated fat,[5] or fibre.[6,7] If the background diet is unhealthy, then consumption of eggs may do little to alter disease risk.

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- It is difficult to discern the effect of dietary cholesterol from that of dietary fat as they are typically found in the same foods.[6]
- Dietary cholesterol has a smaller influence on LDL cholesterol when saturated fat intake is low (or the P:S ratio is high). It has been estimated adding 100mg of dietary cholesterol (half an egg) to a diet high in saturated fat would increase LDL by 0.06mmol/L compared to 0.04mmol/L for a diet low in saturated fat.[5]
- In cohort studies, especially earlier studies, adjustments were often not made for potential confounders.[8,9] Saturated fat intakes often increase alongside dietary cholesterol, and diets higher in animal products may be lower in fruit and vegetables. Eggs are often eaten as part of a cooked breakfast with foods such as bacon, sausages or white bread with butter, all of which could affect blood cholesterol levels and heart disease risk. There are also lifestyle factors that may be more common in people who eat a greater number of eggs that could influence risk, such as smoking (in men) or low levels of physical activity.[10-12]
- There is a level of measurement error in dietary assessment. Cohort studies mainly used self-reported intake, food questionnaires seldom collect detailed information on eggs, there is often no way of differentiating between egg white and egg yolk intake, or accounting for eggs in food or mixed dishes.[13]

Cholesterol absorption and synthesis

- The body adjusts its absorption and production of cholesterol to try and maintain a stable pool of cholesterol in the body.[6]
- If dietary intake of cholesterol is moderate to high, absorption and production of cholesterol is reduced. Thus, LDL-cholesterol is more sensitive to low rather than high intakes of dietary cholesterol.[6]
- Furthermore, increases in dietary cholesterol might increase cholesterol removal from the body through the reverse cholesterol transport pathway.[14-16]
- The cholesterol raising effect of eggs is strongest at cholesterol intakes of less than 400mg/day.[17]

- If dietary intake of cholesterol is low, the body increases production and absorption.[6]
- Dietary restriction and increased meal frequency have been found to reduce cholesterol synthesis.[6]

Blood cholesterol type

- A high egg intake can increase LDL cholesterol but it also increases HDL cholesterol, and therefore may have only a small effect on the TC:HDL ratio.[5,16-18,19,20] However, HDL may not increase as much as LDL so the overall effect could still be adverse, although this is not consistent between studies.[4,15,21] A meta-analysis estimated the equivalent of an egg a day would increase the TC:HDL ratio by 0.04 units, increasing risk of myocardial infarction by 2.1%.[5]
- Egg consumption may increase formation of large buoyant LDL, which has been associated with being less atherogenic than small dense LDL, although both are still considered atherogenic to some degree.[16,19,20,22,23] Some of these studies were also in a carbohydrate restricted diet which could influence results.
- Spence contends that fasting lipid measurements conceal the true effect on the endothelium as so much of our time is spent in the post-prandial state. Cholesterol peaks after six to eight hours, whereas studies often measure fasting levels.[24,25]

Genetic factors and hypo/hyper responders

- There are large differences in cholesterol absorption between people, and differences by age and sex. Absorption of cholesterol can range from 20% to 80% in healthy subjects on a moderately low-cholesterol diet, and increases with aging.[6]
- Some people have a greater response to dietary cholesterol intake (hyper-responders) whilst others have little response (hypo-responders) to dietary cholesterol.[14,15] Some of this difference may be due to intra-individual fluctuations, rather than differing response levels.[26]
- Up to one-quarter of the population are considered hyper-responders to dietary cholesterol because their cholesterol levels

increase more acutely than non-responders, with up to a three-fold difference in effect.[21]

- Genetic factors such as presence of certain alleles and phenotypes can increase sensitivity to dietary cholesterol.[6 27]
- However, even in hyper-responders, both LDL and HDL cholesterol increase and the effect on the TC:HDL ratio may not be statistically significant.[14,15] In contrast, Weggemans calculated that the rise in HDL was not sufficient to compensate for the rise in LDL cholesterol (see Evidence Table 2).[5]

Special populations

- At high intakes, people with insulin resistance could be less sensitive to egg-induced increases in LDL and HDL cholesterol than those who are insulin sensitive,[17] potentially due to reduced cholesterol absorption.[28] Cholesterol synthesis is increased and absorption decreased in insulin resistance and type 2 diabetes, and this is independent of obesity.[29]
- Obesity and overweight may increase rate of cholesterol synthesis.[6]
- Dietary cholesterol and egg intake have been associated with increased CVD risk in people with type 2 diabetes in most studies that have assessed them. Results are inconsistent in relation to whether higher egg intakes increase risk of getting type 2 diabetes in the first place.[9]
- Studies of populations already experiencing some form of heart disease or at high risk of heart disease are likely to be on medication, which will reduce their risk of experiencing heart disease outcomes, and may partially disguise beneficial effects of dietary effects.

TMAO and choline

- Eggs are a good source of the B-vitamin choline, an essential nutrient for foetal development. Preliminary research suggests that microbes can metabolise choline to TMAO (trimethylamine-N-oxide).[30] High levels of TMAO in the plasma have been associated with CVD in humans in epidemiological studies, and animal studies suggest this is due to direct involvement in the

atherosclerotic process, rather than acting as a biomarker.[31,33] Studies have shown increased formation of TMAO with consumption of two or more eggs in a meal.[30,32,33] On the contrary, choline deficiency has also been linked to atherosclerosis.[34] Further research is required to confirm the findings and determine their full implications.

Other

- There is a very small amount of mechanistic data that implies dietary cholesterol could be involved in the inflammatory response and atherosclerosis.[35,36]
- Many intervention studies have assessed high cholesterol intakes. An Institute of Medicine review of 50 clinical studies found more than half used changes in cholesterol dose of more than 500mg/day, which is far higher than average intakes.[37]
- The egg industry has funded many of the intervention studies and reviews. No sensitivity analyses were identified that assessed any differences between industry funded and non-industry funded research.

Evidence overview: Epidemiological research on eggs and risk of heart disease

The studies included in this section are all prospective cohort studies. The advantage of these types of studies is that they can look at disease outcomes, rather than risk factors, as well as the time-sequence of events. However, they are unable to determine if associations with a disease outcome are due to cause and effect. They are prone to some level of measurement error and confounding from other variables. As an example, results from early studies suggesting a link between dietary cholesterol and risk of CVD[38] likely reflected the effect of saturated fat rather than dietary cholesterol, as most did not control for confounders.[8]

Two recent meta-analyses of prospective cohort studies found no evidence that an egg a day effects CHD or CVD outcomes (see

Evidence Table 1).[39,40] However, they did find increased risk in people with type 2 diabetes. An earlier systematic review also found that once dietary confounders were taken into account, there was no association with one or more eggs per day and risk of CHD in people without diabetes.[8]

A re-analysis of data from three of the studies included in these reviews (Nurses' Health study, Health Professionals Follow-Up study, NHANES data) estimated that one egg per day contributed to less than 1% of heart disease mortality.[41]

A third meta-analysis of cohort studies had a contradictory finding (Evidence Table 1). It concluded there was an association between egg consumption and CVD, with a 19% increased relative risk in the highest compared to lowest egg intake groups.[42] In comparison to the other meta-analyses, this study included case-control and cross-sectional studies, which are weaker study designs. However, excluding the one cross-sectional study on CVD only reduced the relative risk to 16%. The studies included did differ, with about half overlapping with Shin et al,[39] but only three studies overlapping with the analysis by Rong et al.[40] Seventeen percent of the weighting in the analysis came from a study by Nettleton which was not included in the other meta-analyses. It found increased risk of heart failure with higher egg intake.[43] The other study on heart failure included in this review also found increased risk with seven or more eggs per week.[44]

Larsson et al assessed the effect on heart failure in two Swedish cohorts. Consumption of greater than 1 egg/day was associated with increased risk of heart failure in men only (a 32% increased relative risk). Risk was even higher at 2 eggs/day. Results were not altered by diabetes history.[45]

Berger et al conducted the most recent systematic review and meta-analysis on dietary cholesterol and CVD, including both cohort and intervention studies (which are discussed in the next section).[46] It included 17 cohorts and 19 trials published between 1979 and 2013 in people without existing CVD (cohort studies) and with normal lipid concentrations (intervention studies). They noted inconsistent findings

between studies and generally poor methodological quality, both of which limited the ability to draw robust conclusions. Cohort studies did not identify any association between dietary cholesterol and increased risk of CVD. Due to the sparse data, a meta-analysis of cohort studies was not conducted. Few of the studies adjusted for dietary factors, and all used a single baseline assessment of diet.[46]

Whilst this evidence paper is looking at cardiovascular outcomes, it should be noted that a re-analysis of the Physicians' Health Study found consumption of more than six eggs per week was associated with a 23% increased risk of mortality after adjusting for confounders. Consumption of less than six eggs per week was not associated with increased risk. There was no association at any level of intake with myocardial infarction or stroke,[11] but there was a 28% increased risk of heart failure.[44] However, no dietary intake data was reported and adjustments were not made for saturated fat intake. Potential flaws in the statistical analysis have been identified.[47]

Although there is still need for caution, overall, epidemiological evidence suggests six to seven eggs per week does not increase heart disease risk, with the exception of type 2 diabetes and possibly heart failure. A proposed mechanism for the increased risk with type 2 diabetes is increased intestinal cholesterol absorption due to molecular and genetic changes in type 2 diabetes.[48]

Tran et al suggested caution in interpreting the findings from meta-analyses on type 2 diabetes because of challenges in combining the data. They were unable to conduct a meta-analysis because of variations between studies in outcome definition, measurement of exposure, assessment of diabetes status, inconsistent reporting of diabetes treatment, controlling for confounders, and follow-up time.[9]

Evidence overview: Intervention studies on eggs and risk factors

There have not been any intervention studies investigating the effect of dietary cholesterol or eggs on heart disease outcomes. Although this would provide the strongest evidence, the length, cost, and

number of participants required makes it prohibitive to undertake such studies. Instead, intervention studies have assessed the impact on intermediary risk factors for heart disease such as raised lipids, inflammation, and metabolic syndrome.

Blood cholesterol

Weggemans et al conducted a meta-analysis of RCTs to 2001 in order to determine the effect of dietary cholesterol on the TC:HDL ratio. Eleven of these studies were metabolic ward studies where diet can be more carefully controlled. For each additional egg consumed in a day, the TC:HDL ratio was predicted to increase by 0.04 units, implying a small increased risk of MI of 2.1% (see Evidence Table 2).[5]

Griffin et al conducted a systematic review of randomised controlled trials of dietary cholesterol and blood lipids published between 2003 and 2013.[49] Twelve studies were included in the review, seven of which controlled for background diet (in that they were told to follow a certain diet), and four of those aimed at weight loss. The authors noted a high degree of variability in background diet, subject characteristics and study design between the studies. Eggs (1-3 per day) were predominantly used as the source of dietary cholesterol. Interventions lasted from four to 12 weeks and were generally small in size. Baseline TC levels were in the range of 4.37 to 6.31mmol/L. They found only a very modest effect on cholesterol levels, which appeared to be limited to population subgroups. Where there were statistically significant increases in LDL cholesterol, this was only in sub-groups who were genetically susceptible[27,28] or in hyperresponders.[20] These sub-groups were identified in post-hoc analysis rather than pre-defined, and results should therefore be treated as tentative. However, one study did find a significant decrease in LDL cholesterol with an egg-free substitute in comparison to two eggs per day.[50] There were statistically significant effects on HDL in five studies, but they were small. Many studies did not assess the TC:HDL ratio. In the five studies that did there was no significant effect, except in a sub-group of hyper-responders.[51] There was little effect on triglycerides. No trends were identified for intakes of up to three eggs per day or length of intervention.[49]

A review for the US Dietary Guidelines Review in 2010 included both epidemiological and intervention studies. It found moderate evidence from epidemiological studies that related dietary cholesterol to cardiovascular disease. Consuming one egg per day was not associated with coronary heart disease or stroke in healthy adults, whereas higher intakes were. As noted earlier, this advice was not carried forward into the 2015 Guidelines.[52] There were three RCTs included in the US Dietary Guidelines Review not included in the review by Griffin. One reported on the same study population as an included article.[28,53] The remaining two were rated neutral quality and showed no statistically significant effect of egg intake on blood lipids.[54,55]

In the meta analysis by Berger, discussed in the previous section, intervention studies showed a statistically significant increase in total cholesterol (0.62mmol/L), LDL cholesterol (0.37mmol/L), HDL cholesterol (0.18mmol/L), and the LDL to HDL ratio (0.17), with no effect on triglycerides or VLDL in intervention compared with control groups. There was no evidence of a linear relationship between dietary cholesterol and lipids. Cholesterol intakes in the intervention group were higher than what is typically consumed, ranging from 500mg to 1400mg/day. The quality of intervention studies was rated good or fair, representing low to medium risk of bias.[46]

Since the above reviews, six further studies have been published focused on heart disease risk factors[16,56-61] three of which reported on the same study[16,58,59], and five of which were funded by the egg industry (see Evidence Table 2).

Fuller et al studied people with type 2 diabetes and found no adverse effect of dietary cholesterol on lipids, in contrast to other studies in this population.[56] It has been cautioned not to over-interpret the results of this study, in the context of other research that has suggested increased risk in this population group.[62]

Papers by Blesso and Andersen reported on the same study of people with metabolic syndrome and a carbohydrate-restricted diet. A large number of measurements were made, with statistically significant

findings for three eggs per day and improvements in HDL and VLDL levels, HDL composition, tumour necrosis factor and serum amyloid A.[16,59]

Baumgartner compared the effect of an extra egg per day with an egg in a buttermilk drink, and concluded that buttermilk modified the effect of dietary cholesterol in the egg yolk.[60] Daily consumption of an egg increased TC and LDL cholesterol in women only, but not HDL, TG, inflammatory markers or endothelial function. Consuming the egg with buttermilk ameliorated the increase in LDL cholesterol.

None of the individual studies or studies included in the most recent reviews specifically investigated populations at high risk or with existing heart disease. Weggemans meta-analysis from 2001 included two studies with hyperlidipaemic participants, which found opposing effects on cholesterol. There were five studies in hyperlipidaemic participants which did not meet their selection criteria, but which did not suggest any different effects to those with normal cholesterol levels.[5]

Endothelial function

A crossover trial with 40 hyperlipidaemic men and women found that whilst two eggs per day for six weeks had no effect on endothelial function (measured through flow-mediated dilation), egg substitute improved it.[50]

Summary

The overall level of evidence on eggs and blood cholesterol or risk of heart disease is inconsistent, and there are strong limitations to many of the studies. There are differences in findings between cohort and intervention studies, and a high level of industry funded research.

Whilst the evidence is not clear enough to say that there is no association between dietary cholesterol and heart disease risk, there is also not strong enough evidence to warrant continuing recommendations to limit egg intake to three per week. A prudent

recommendation is a limit of six to seven eggs per week for people at increased risk of heart disease.

The weight of evidence suggests that eggs have only a very small effect on blood cholesterol levels, especially at normal levels of intake. For most people, any such increased risk is not likely to be clinically meaningful.

There has been a limited amount of research since 1999 in people with hyperlipidaemia, and epidemiological research suggests the prudent limit for them is six to seven eggs per week.

When sub-groups of hyper-responders to dietary cholesterol were identified, the increases in blood cholesterol levels were still small. Advice for hyper-responders would be best based on individual response to egg intake.

In summary, based on the available evidence, for people at risk of heart disease intake of less than six to seven eggs per week is unlikely to have any substantive influence on heart disease risk. Recommendations for the general population should remain that eggs can be included as part of a heart healthy eating pattern. The exception is people with type 2 diabetes, for whom eggs may increase risk, and possibly people with heart failure.

This review focuses specifically on eggs, however the greatest effect on reduction of heart disease risk will be from an overall heart-healthy pattern of foods. Thus, care should be taken with the company that eggs keep, that is, the combination of foods often eaten with eggs such as processed meats, white bread and/or butter. A heart healthy dietary pattern is high in vegetables and fruit, contains whole grains in place of refined grains, legumes, nuts and other sources of healthy fats such as oily fish, and can contain non-processed meats or poultry and/or dairy.

EVIDENCE TABLE 1: META-ANALYSES OF EPIDEMIOLOGICAL STUDIES ON EGGS AND HEART DISEASE RISK

Study name and type	Population and dietary context	Intervention and comparator	Key outcomes	Notes
Shin 2013: systematic review and meta analysis of prospective cohort studies[39]	22 cohorts from 15 studies. Majority of studies in Western countries, with the remainder in Asia. Age range 20-90 years with an average 11.3 years of follow up.	≥1 egg/day vs <1 egg/week	CVD: HR 0.96 (95%CI 0.88, 1.05) IHD mortality: HR 0.98 (95%CI 0.77, 1.24) Type 2 diabetes: HR 1.42 (95%CI 1.09, 1.86) CVD, in people with diabetes: HR 1.69 (95%CI 1.09, 2.62)	Most studies assessed intake by self-reported questionnaire. Unable to standardise egg consumption across all studies. No significant heterogeneity and no evidence of publication bias. The female cohort from Hu et al not included for no apparent reason (no significant association with diabetes in this group). Small number of studies in diabetes.
Rong 2013: dose-response meta-analysis of prospective cohort studies[40]	6 studies with 9 independent reports including 3,081,269 person years and 5847 incident cases of CHD. No prior diagnoses of CVD at baseline. Follow up from 8 to 20 years. Two cohorts	Increase of one egg per day	CHD: RR 0.99 (95% CI 0.85, 1.15) Diabetes sub-group: RR 1.54 (95% CI 1.14, 2.09)	Food frequency questionnaires used to assess intake in all studies (with potential for measurement error and misreporting). Some FFQs only assessed frequency and not volume. Only four studies undertook repeat dietary measures. All included studies were rated high quality. No heterogeneity identified between

Study name and type	Population and dietary context	Intervention and comparator	Key outcomes	Notes
	were Japanese and the remainder from the United States.			studies and no evidence of substantial publication bias. Only a small number of studies in people with diabetes.
Li 2013: meta-analysis of cohort, case-control and cross-sectional studies[42]	14 studies with 320,788 subjects, with 12 reporting risk of CVD (11 cohort studies and 1 cross-sectional study). The majority of studies were in the US. Mean age ranged from 33 to 75 years, with follow up from 6.1 years to 20 years in cohort studies.	Highest vs lowest egg intake >1 egg day vs <1 egg/week	CVD: RR 1.19 (95% CI 1.02-1.38) Dose-response for 4 eggs/week: RR 1.06 (95% CI 1.03-1.10) Risk of CVD in participants with diabetes: RR 1.83 (95% CI 1.42 to 2.37) Risk of diabetes: RR 1.66 (95% CI 1.41 to 2.00) *Sub-group analysis:* Risk of CVD in cohort studies (n=11): RR 1.16 (95% CI 1.00 to 1.34) Risk of CVD in non-cohort studies (n=1): RR2.59 (95% CI 1.11 to 6.04) (p=0.06 for the sub-group difference)	Included case control and cross-sectional studies. Sub-group analysis found higher risk in Western countries outside the US. Only two studies adjusted for saturated fat intake, and two adjusted for fibre intake. Two studies allowed self-reported outcome measures. All assessed diet with a FFQ or questionnaire. No evidence of publication bias.

EVIDENCE TABLE 2: INTERVENTION STUDIES ON EGGS AND HEART DISEASE RISK FACTORS

Study name and type	Population and dietary context	Intervention and comparator	Key outcomes	Notes
McNamara 2000: pooled analysis of crossover trials published between 1960 and 1999[21]	167 cholesterol feeding studies with 3,519 participants. Diverse population groups: nor-mocholesterolaemic, hypercholesterolaemic, young to elderly, men and women. Included metabolic ward studies, controlled feeding trials, and free-living trials. Background diets were low to high in total fat and low to high P:S ratios. Dietary cholesterol ranged from 100-300mg to 3-5g.	Eggs vs egg substitute	Up to 1200mg/day dietary cholesterol, TC increases, but is attenuated at high levels. Estimated change to 100mg/day dietary cholesterol challenge: TC: 2.2mg/dL (95%CI 1.9-2.5) (0.06mmol/L) LDL: 2.07mg/dL (95% CI 2 to 2.73) (0.05mmol/L) HDL: 0.44mg/dL (95% CI 0.34 to 0.55) (0.01mmol/L) *Hyper-responders*: TC: 3.9±0.6mg/dL (95% CI 2.5 to 5.3) (p=0.0002) (0.1mmol/L) LDL: 2.84±0.66mg/dL (p=0.02) (0.07mmol/L) HDL: 0.69±0.16mg/dL (p>0.05) (0.02mmol/L) *Hypo-responders*: TC: 1.4±0.2mg/dL (95% CI 1.0 to 1.9) (0.04mmol/L)	The USDA Nutrition Evidence Library review for the 2010 US Dietary Guidelines graded this study as having strong methodological quality, and listed it as a pooled analysis. However, there is no detail in the referenced paper on review methodology or data analysis and it is not possible to determine if the review was systematic or how the data was combined and analysed. It appears to be an analysis of all studies in a database. Due to the lack of methodological data in available reports, this study was not included in the narrative of this review. Funded by the egg industry.

Study name and type	Population and dietary context	Intervention and comparator	Key outcomes	Notes
			LDL: 0.76±0.25mg/dL (0.02mmol/L) HDL: 0.50±0.14mg/dL (0.01mmol/L)	
Weggemans 2001: meta-analysis of crossover or parallel RCTs[5]	17 studies with 556 participants (422 men and 134 women) from United States, Europe and South Africa aged 18-75 years, mean BMI 21 to 28 an mean baseline cholesterol 4.05 to 5.92mmol/L. 11 studies were metabolic ward studies with all food included; 5 were in free-living participants	Increase of 100mg cholesterol/day	TC:HDL ratio: +0.02 units (95% CI 0.01 to 0.03) TC: +0.056mmol/L (95% CI 0.046 to 0.065) HDL: +0.008mmol/L (95% CI 0.005 to 0.01) The equivalent of one egg a day would imply an increased risk of myocardial infarction of 2.1%	The USDA Nutrition Evidence Library review for the 2010 US Dietary Guidelines graded this study as having strong methodological quality. It found that the greatest adverse effect on cholesterol was in subjects with the lowest TC:HDL ratios. Many of the studies utilised high dietary cholesterol intakes.
Fuller 2015: RCT[56]	12 week RCT with 140 overweight or obese participants with pre-diabetes or type 2	2 eggs/day for six days per week vs <2 eggs/week (between group difference in dietary cholesterol was	HDL, TC, LDL, TG and glycaemic control: no mean differences between groups	Small baseline differences in TC and HDL, with higher values in the egg group. Study may have been underpowered. Lipids only measured once. Weight, age and statin use not considered.[62] Funded

Study name and type	Population and dietary context	Intervention and comparator	Key outcomes	Notes
	diabetes	337.2mg)		by the egg industry.
Katz 2015: RCT, crossover trial[57]	32 adults with established coronary artery disease. Mean age 67 years, 6 women, 26 men	Three arms: Breakfast with 2 eggs vs breakfast with ½ cup egg substitute vs high-carbohydrate breakfast (control) for six weeks each with a four week washout	Flow-mediated dilation: no difference between groups TC: no difference between groups BP: no difference between groups Body weight: no difference between groups	Funded by the egg industry
Blesso 2013[16]; Andersen 2013[59]; Blesso 2013[58]: single-blind RCT	37 middle-aged men and women with metabolic syndrome, and an average BMI of 30.5	3 whole eggs/day vs yolk-free substitute with both groups on a carbohydrate restricted diet (planned 25-30% energy, achieved 41%) for 12 weeks	*Blesso (lipids)*: Statistically significant between group differences were (egg vs substitute): HDL (mg/dL): +8.6 vs +4.7 Large HDL particles (μmol/L): +1.7 vs +0.6 VLDL (nmol/L): -15.9 vs -0.3 Medium VLDL (nmol/L): -9.3 vs +4.0 No differences in insulin, HOMA-IR, triglycerides, lipid sub-fractions, or apolipoproteins *Andersen (HDL composition)*: The egg group	Ad libitum diet; intention-to-treat analysis not used; 24% energy reduction from baseline in both groups and 4% weight loss (thus have only reported between group differences in lipids). Participants in the egg group were more insulin resistant than in the control group. A large number of biochemical measures were taken (only statistically significant results included in outcomes). Funded by the egg industry.

Study name and type	Population and dietary context	Intervention and comparator	Key outcomes	Notes
			improved some aspects of HDL composition, and this effect was more favourable in less severe metabolic syndrome *Blesso (inflammation)*: reductions in plasma tumour necrosis factor-α and serum amyloid A seen in the egg group only	
Baumgartner 2013: RCT[60]	Three-arm parallel intervention with 97 healthy men and women for 12 weeks	Three arms: Control (n=20) habitual intake of 1-2 eggs/week One extra egg/day (n=57) One extra egg yolk in 100ml buttermilk/day (n=20)	During the intervention, mean dietary cholesterol intakes in women were 232 vs 431 vs 442mg/d and in men 275 vs 415 vs 459mg/d. TC (+0.24±0.62mmol/L) and LDL (+0.17±0.50mmol/L) increased in women in the egg group from baseline but not HDL, TC:HDL or TG. Other lipids and markers of liver and endothelial function were unchanged.	Slight increase in saturated fat intake in the egg group and decrease in the egg-drink group. The difference was statistically significant in women only.

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