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What We Know, Think We Know, or Are Starting to Know

Ah, dietary cholesterol. That most exacting of nutrients, an intercorrelated conundrum with 70yrs of research and yet, we are not much further along in reconciling the evidence compared to the metabolic ward studies of the 1950's.

There are 3 ways to think about the role of dietary cholesterol as it pertains to potential risk of cardiovascular disease [CVD]:

- The absolute impact of dietary cholesterol on LDL-C, as the causal intermediate risk factor for CVD
- The expected impact of those changes in LDL-C on CVD outcomes
- The tight relationships between dietary cholesterol, total energy, saturated fat and unsaturated fats, and dietary fibre [which plays a role in cholesterol recycling]

Ancel Keys and colleagues conducted a range of experiments with diets ranging from entirely free of dietary cholesterol to 700mg/d, and with diets with 0g dietary fat up to 150g dietary fat, carefully controlling for energy and different sources of fats ⁽¹⁾. In a 1958 review ⁽²⁾ of his and others' work, Keys stated: *"The ensemble points strongly to the conclusion that, other things being equal, the serum cholesterol level in adult man is independent of the cholesterol intake over the range of zero to at least 700mg daily."*

This was *not* to state that dietary cholesterol had *no* effect on blood cholesterol levels at all, but that the variations in blood cholesterol levels themselves were primarily influenced by other factor factors, total dietary fat and composition in particular ⁽²⁾. For example, when they fed participants 15g of fat per day and zero dietary cholesterol, blood cholesterol dropped by about 80% of baseline values; however, when they added up to 500mg of dietary cholesterol to 15g fat, the same results were observed. The changes in dietary cholesterol were achieved by adding egg yolks to the diet.

Dietary cholesterol could never truly be left out, however, because the Keys and Hegsted Equations developed to predict the effects of saturated fat and polyunsaturated fat on blood cholesterol levels could not be fully determined unless dietary cholesterol was included in the equations ^(1,3). Indeed, despite the greatest magnitude of change in blood cholesterol levels being observed from saturated fat, and to a lesser extent polyunsaturated fat, it was known that dietary cholesterol does have an effect in and of itself.

A number of studies have sought to update the evidence for the effects of dietary cholesterol. In a 1997 analysis of 395 metabolic ward studies, Clarke et al. ⁽⁴⁾ showed that a reduction of 200mg/d dietary cholesterol would be predicted to lower LDL-C by 0.13mmol/L [5.01mg/dL]. A more recent 2019 ⁽⁵⁾ study conducted a meta-regression analysis [where data is combined from multiple primary sources to estimate the relationship between two variables, e.g., dietary cholesterol and blood cholesterol], and found that a 100mg/d change in dietary cholesterol would be predicted to alter LDL-C by 0.11mmol/L [4.52 mg/dL]. That analysis investigated dose-responses, and found that the greatest magnitude of change in blood cholesterol levels occurred in a range of 0-400mg/d, after which the effect on blood cholesterol levels plateaued.

The magnitude of effect in these studies has been derived from isocaloric trials, i.e., studies not designed for weight loss. However, while weight loss may improve blood cholesterol levels, the magnitude of effect is relatively small: 0.2mmol/L [8mg/dL] per 10kg weight loss ⁽⁶⁾. The present study conducted a secondary analysis* of the associations between changes in dietary cholesterol intake, and changes in blood lipids, from a large weight loss diet trial.

*Geek Box: Post-hoc Secondary Analysis

The literal translation of the Latin term 'post-hoc' is 'after this'. Post-hoc means something which occurs after an event. A post-hoc analysis is therefore an analysis which is undertaken after a trial has concluded, using data from that trial, to look at a question that was not pre-specified. So of course, while all data analysis is conducted after a study is concluded, the key feature of a post-hoc analysis is that it is secondary to the study, and the question it is addressing was not a primary research question. For example, the DIETFITS trial was designed to test the effects of baseline insulin secretion and genetic factors on response to either low-carbohydrate or lowfat diets. It was not designed to test the effects of different percentages of saturated fat on cardiovascular risk factors. However, the researchers had data on both diet and blood lipids. Thus, they could do a post-hoc analysis secondary to the intervention, looking at the relationship between saturated fat and blood lipids. However, it is important to distinguish that because it is a secondary analysis and not part of the pre-planned study, in effect a post-hoc analysis is an observational study. It can look at relationships, but not causality. Post-hoc analyses are very useful, and informative, tools in research, particularly where a well-conducted controlled trial generates a rich data set. Like all research designs, it is important to understand their place, and what they can show (associations) and cannot show (causation).

The Study

The DIETFITS study was a randomised controlled trial with 609 men and women aged 18-50 and BMI of 28-40 whom were randomised to either a healthy low-fat diet [HLF] pattern or a healthy low-carb diet [HLC] pattern. The primary aims of the trial were to test the effects of baseline insulin secretion or genetics influenced weight loss on either diet over 12-months.

Participants on either diet were not given prescriptions for energy intake, or percentage targets for any macronutrient. The intervention began with instructions to maximally reduce either total fat [HLF] or carbs [HLC], following which individuals could find a level of intake that they felt could be maintained indefinitely. Both groups were counselled to:

- a. maximise vegetable intake;
- b. minimise intake of refined flours, added sugars, and trans fats;
- c. focus on whole foods that were minimally processed, nutrient-dense, and aim to prepare food at home where possible.

All participants were counselled over the 12-month duration of the intervention by dietitians and health educators, and both diet groups received 22 diet-specific education sessions over the study period. The study was single-blinded, with data collectors blinded to diet group allocation. Data collection, including blood sampling for examination of blood lipid levels, occurred at baseline, 3-months, 6-months, and 12-months. At each of these data collection points, dietary intake was assessed via three unannounced 24-hour dietary recalls using the multiple-pass interview method.

For this present study, a post-hoc secondary analysis was performed using data from subjects on the HLC diet [n = 208] to examine whether changes in dietary cholesterol intake were associated with changes in LDL-C, HDL-C, and triglycerides. Only subjects with complete diet and blood lipid data for the entire intervention period were included. Subjects were divided into tertiles of dietary cholesterol intake according to their 12-month change in dietary cholesterol intake, from highest to lowest [i.e., highest change, moderate change, lowest change]. For completeness, the analysis was repeated for the LF diet group. **Results:** Participants on average were aged 40yrs and 39yrs in the HLC and HLF groups, respectively. Average LDL-C levels at baseline were 3.0mmol/L [116mg/dL] and 2.8mmol/L [111mg/dL] in the HLC and HLF groups, respectively. Baseline dietary cholesterol intake was 322mg/d and 320mg/d in the HLC and HLF groups, respectively. 50% of the HLC group consumed >300mg/d dietary cholesterol at baseline.

For the overall group differences, the HLC group increased dietary cholesterol by 137mg/d, compared to an 82mg/d decrease in the HLF group. The following findings set out the results by tertile of change in dietary cholesterol intake.

Change in Dietary Cholesterol and Correlations with LDL-C:

- HLC:
 - **Lowest:** Dietary cholesterol decreased by 102mg/d, for an average daily intake at 12-months of 312mg/d. This correlated with an increase in LDL-C of 0.07mmol/L [2.8mg/dL] in this group.
 - **Middle:** Dietary cholesterol increased by 112mg/d, for an average daily intake at 12-months of 420mg/d. This correlated with an increase in LDL-C of 0.06mmol/L [2.7mg/dL] in this group.
 - **Highest:** Dietary cholesterol increased by 401mg/d, for an average daily intake at 12-months of 647mg/d. This correlated with an increase in LDL-C of 0.13mmol/L [5.1mg/dL] in this group.

The 12-month changes in dietary cholesterol in the HLC group were not significantly correlated with changes LDL-C, HDL-C, or triglycerides.





Figure from paper illustrating (top) the change in dietary cholesterol intake in each tertile of dietary cholesterol change [from left to right], and (bottom) the related change in LDL-C levels after 12-months of consuming the healthy low-carb diet. The 'x' in each boxplot represents the mean level, while the horizontal line represents the median; as you can see, both are practically identical, which indicates that the mean was not overly influenced by outlier values. The size of the box itself, from top to bottom, represents the 75th percentile and 25h percentile, respectively; again you can see these were largely similar, reflecting the lack of average differences between groups despite substantially different dietary cholesterol intakes. The 'whiskers' either side of the box represent 1.5 x the interquartile range [the box], which shows range of values higher or lower than the 75th percentile and 25h percentile and 25h percentile, respectively.

• HLF:

- **Lowest:** Dietary cholesterol decreased by 263mg/d, for an average daily intake at 12-months of 181mg/d. This correlated with a decrease in LDL-C of 0.10mmol/L [3.9mg/dL] in this group.
- **Middle:** Dietary cholesterol decreased by 61mg/d, for an average daily intake at 12-months of 216mg/d. This correlated with a decrease in LDL-C of 0.02mmol/L [0.8mg/dL] in this group.
- **Highest:** Dietary cholesterol increased by 79mg/d, for an average daily intake at 12-months of 316mg/d. This correlated with a decrease in LDL-C of 0.04mmol/L [1.6mg/dL] in this group.

The 12-month changes in dietary cholesterol in the HLF group were not significantly correlated with changes LDL-C, HDL-C, or triglycerides.

12-month Change in LDL-C per 100mg Increase in Dietary Cholesterol:

- **HLC:** Without adjusting for any other factors, the 12-month change in LDL-C levels for each 100mg increase in dietary cholesterol was 0.009mmol/L [0.37mg/d]. Adjusting for age, gender, baseline dietary cholesterol, and 12-month changes in saturated fat and body weight, that change in LDL-C per 100mg dietary cholesterol was 0.0002mmol/L [0.01mg/dL].
- **HLF:** Without adjusting for any other factors, the 12-month change in LDL-C levels for each 100mg increase in dietary cholesterol was 0.03mmol/L [1.17mg/dL]. Adjusting for age, gender, baseline dietary cholesterol, and 12-month changes in saturated fat and body weight, that change in LDL-C per 100mg dietary cholesterol was 0.01mmol/L [0.57mg/dL].

Thus, there was no significant correlation with changes in LDL-C per 100mg increase in dietary cholesterol in either the HLC or HLF groups.



Figure from the paper illustrating (*left*) the average dietary cholesterol intake at each data collection point in the study [i.e., baseline, 3-months, 6-months, 12-months]. The solid royal blue plot line is the lowest tertile of change in dietary cholesterol; the red dashed plot line is the middle tertile; the green dashed plot line is the highest tertile. The figure on the right illustrates the average LDL-C level measured at each of those data collection points. As can be clearly seen, while the dietary intake increased significantly by 401mg in the highest tertile group, this increase was almost entirely within the first 3-months of the study. However, while LDL-C was measured at its highest at 3-months, it appears to have declined by 6-months and plateaued by 12-months.

The Critical Breakdown

Pros: Randomisation method was appropriate [computer generated] and was conducted by a statistician not involved in the study. Participants did not find out which diet they had been assigned to until after baseline data collection was completed. The trial emphasised overall diet patterns, rather than targeting specific thresholds of intake. The dietary quality in both diet groups was high. Both groups received equally intensive counselling, a positive as nutrition trials may often be biased toward the intervention group. The trials had nearly 50:50 men and women, had a large sample size, and had excellent retention for a weight loss trial. The present analysis contained a large sample of 208 in the HLC diet group with full dietary data and lipid measurements for the entire study.

Cons: The present study was a *post-hoc* secondary analysis, and is therefore a correlative study and does not demonstrate potential cause-effect relationships. As a secondary analysis, the authors were unable to distinguish whether the results were due to the reduction in refined carbohydrate and added sugars that occurred on the HLC diet, which could have explained the lack of overall impact on blood lipids. It would have been helpful to know whether the three 24hr diet recalls included, for example, two weekdays and one weekend, given typical variances in diet that occur thereto. It would also have been informative if change in energy intake was added as a variable in the regression analysis. While not a necessarily a major 'Con', the participants baseline LDL-C levels were not particular high, i.e., but this reflects the fact that the DIETFITS intervention was primarily a weight loss trial, not a trial assessing CVD risk.

Key Characteristic

One of the methodological barriers with RCTs in nutrition is the relatively narrow range of variability in nutrient intake in participants at baseline. This often means that, after randomisation, there isn't that much of a difference in levels of intake between two comparison groups, and it can mean it is difficult to see meaningful differences between groups. To compound this, most trials will simply report on the mean change in a group as a whole. For example, we know from the present study that for the overall group mean differences, the HLC group increased dietary cholesterol by 137mg/d, compared to an 82mg/d reduction in the HLF group.

Many studies would just leave it at that. However, the present study divided the diet groups into tertiles, based on changes in the exposure of interest, i.e., dietary cholesterol. While it is standard practice in nutritional epidemiology to rank participants according to levels of intake of an exposure [whether that is a food or nutrient], it is seldom done in RCTs. This allowed for important insights into the varying levels of change in dietary cholesterol, both increases and decreases, and the potential relationship with other dietary factors.

However, as the authors note, division into tertiles creates differences between groups that may influence the findings, e.g., differences in baseline dietary cholesterol levels; this is why the regression analysis was important, because the effects of changes in dietary cholesterol on LDL-C levels could be analysed while adjusting for factors like age, gender, baseline dietary cholesterol, and 12-month changes in saturated fat and body weight.

Interesting Finding

The change in egg consumption over the 12-months, and the contribution of daily egg consumption to dietary cholesterol intake in the HLC group, showed that participants in the highest tertile of dietary cholesterol change increased consumption from 1 to 2 whole eggs per day. This occurred in the context of substantial reductions in both sugars and refined grains in this group. We certainly know that refined grains and sugars may have their own effects on blood lipids, particularly on post-prandial triglycerides, and potential knock-on effects for LDL particle size ⁽⁷⁾. Did the reduction in refined grains and sugars attenuate the effects of profoundly increasing dietary cholesterol intake by 401mg/d in the highest HLC tertile? Maybe. While this finding is interesting, unfortunately it is a secondary outcome in a *post-hoc* secondary analysis, meaning that there is no further analysis to determine whether the change in refined grain and sugar intake was associated with change in LDL-C levels.



Figure from the paper illustrating the change in egg consumption at each data collection point in the study in, from *left to right*, the lowest, middle, and highest tertile change in dietary cholesterol.



Figure from the paper illustrating the change in sugar consumption at each data collection point in the study in, from *left to right*, the lowest, middle, and highest tertile change in dietary cholesterol.

Relevance

The primary finding of the present secondary analysis is that a range of substantial changes in dietary cholesterol intake of up to 401 mg/d did not correlated with changes in LDL-C or other blood lipid biomarkers. The r-value from the Pearson's correlation analysis [-1 is a perfect negative correlation, 0 is no correlation at all, and 1 is a perfect positive correlation] for the change in dietary cholesterol on the HLC diet was r = 0.04, and on r = 0.09 on the HLF diet. Per 100 mg/d increase in dietary cholesterol, there was no significant change in LDL-C levels.

What to make of this, in light of the expected differences in blood lipid levels from differences in dietary cholesterol intake?

The first point is that these studies attempt to determine effects of dietary cholesterol *independent* of other variables, which does not necessarily reflect the fact that the effect of dietary cholesterol is mostly *dependent* on other variables. Secondly, despite their sophisticated analyses, they are not immune from methodological issues. Remember that for analyses like these, the output data reflects the input data. The Vincent et al. ⁽⁵⁾ meta-regression was based on the reported variation in effect of dietary cholesterol on blood cholesterol in the primary included studies, but the variance is going to be influenced by the type of exposure and comparison in the primary study. For example, the primary studies included:

- A comparison between lean red meat and white meat in free living healthy subjects [Arch Intern Med. 1999;159(12):1331-8.]
- 2 eggs per day in subjects with elevated blood cholesterol [J Am Coll Nutr. 1997;16(6):551-61.]
- The effects of different forms and levels of hydrogenated fats [N Engl J Med. 1999;340(25):1933-40.]
- The effects of 20% of energy from walnuts [N Engl J Med. 1993;328(9):603-7.]

Thus, the variance itself was likely influenced by the substantial differences in primary interventions, and even adjusting for different fat types etc. in the ultimate analysis doesn't change the fact that, to quote Sander Greenland; *"No statistical technique can compensate for fundamental limitations of the input data."*

The meta-analysis of metabolic ward studies by Clarke et al. ⁽⁴⁾ and predicted effects from dietary cholesterol were derived from isocaloric interventions, with no change in total energy intake or body weight. Thus, the effect of changing dietary cholesterol by 200mg/d was predicted to lower LDL-C by 0.13mmol/L [5.01mg/dL] *without* changes in energy intake. We could then expect that the energy deficit in the DIETFITS study and related weight loss would lead to *more* reductions in LDL-C, on top of changes in dietary cholesterol.

So, what of weight loss? This is important, because the HLC and HLF groups lost 6.3kg and 5.6kg of body weight, respectively, over 12-months. In the analysis in the present study, change in body weight did not predict the changes in LDL-C associated with each 100mg/d increase in dietary cholesterol. The magnitude of change in LDL-C remains divorced from expected changes

from either reductions in body weight, changes in dietary cholesterol, or a combination of both. Consider the HLF tertile which achieved the lowest reduction in dietary cholesterol of 265mg/d; this group lowered energy intake by 723kcal/d, saturated fat by 19g/d, bodyweight by 7kg. If we were to just sum up the expected change in LDL-C from dietary cholesterol, saturated fat, and bodyweight [there are no predictions for total energy], this would roughly equate to an expected decrease in LDL-C of 0.45mmol/L [17.4mg/dL]. Although potentially confounded by the energy deficit, this would likely be expected to *enhance* the reduction in LDL-C. Yet, this group experienced a decline in LDL-C of a mere 0.10mmol/L [3.9mg/dL].

Consider that both the lowest tertile of the HLC group [102mg/d *decrease* in dietary cholesterol] and the middle tertile [112mg/d *increase*] exhibited an identical change in LDL-C levels. While the bodyweight changes and change in saturated fat were different between these tertiles, the analysis indicated that neither of these factors correlated with changes in LDL-C. The Vincent et al. regression predictions would suggest that LDL-C should have increased by 0.44mmol/L [18.08mg/dL] in the highest tertile, highlighting the discord between the isolated dietary cholesterol predictive changes and the effects of multiple dietary variables in a whole dietary pattern.

Another recent meta-regression analysis confined included studies to controlled feeding studies where the ratio of saturated to polyunsaturated fats [the 'P:S ratio'] was matched in *both* intervention and control groups; there was no significant association observed between dietary cholesterol ranges of 155 to 828mg/d and LDL-C ⁽⁸⁾. Clarke et al. showed that the impact of various types of fat on total blood cholesterol were observed with either over or under 300mg/d dietary cholesterol intake, although dietary cholesterol was nonetheless shown to have an impact on increasing LDL-C. This was Ancel Keys' insight: that with these other variables in the mix - energy, saturated fat, polyunsaturated fat, bodyweight - the effects of dietary cholesterol were likely to be relatively negligible ^(2,9).

We also know that there are substantial inter-individual variations in blood cholesterol levels, and the response of blood cholesterol to dietary cholesterol [influenced strongly by variation in absorption] ⁽¹⁰⁾. Future research should focus more on elucidating the factors influencing such inter-individual differences, rather than deriving crude predictive estimates of effects which may have no translational relevance to free-living, whole-diet contexts.

The predictive changes from the Clarke et al. and Vincent et al. analyses are often cited as providing biological plausibility for the observational studies which show an increase in risk associated with dietary cholesterol and/or eggs. However, this may be an over-simplistic extrapolation given the ambiguous associations for both exposures and health outcomes at the population level ^(8,11,12). It assumes that the predictive effect size holds true invariantly from absolute changes in dietary cholesterol may largely be independent of the absolute levels of dietary cholesterol intake. The present study also indicates a disconnect in the predicted changes in LDL-C from dietary cholesterol, particularly because it would be presumed that weight loss may have an *additive* effect. We are left to assume that weight loss and a modest energy deficit almost entirely attenuates any correlations between substantial increases in dietary cholesterol and effects on blood lipid levels, in particular LDL-C.

Application to Practice

There isn't a question in nutrition science to which I bow more in epistemic humility than dietary cholesterol and health effects. I remain far from persuaded that in isolation from the P:S ratio, fibre, and other related factors, dietary cholesterol poses any considerable risk for cardiovascular health. Yet I remain unpersuaded that it does not. The present study invites to conclude that in the context of a healthy dietary pattern and energy deficit, large changes in dietary cholesterol are not correlated with changes in LDL-C, and indeed that such changes that were observed must be explained by factors other than changes in dietary cholesterol. As a secondary analysis, we are not left with many answers as to what those other factors could be. This was Keys' s understanding. I appreciate my ambiguity on this topic may be frustrating. If I had to make a call, I would be inclined to think that at levels of dietary cholesterol habitually consumed in the population [~<300mg/d], in otherwise healthy people there remain lower hanging fruit to pick: reducing saturated fat from the current 13-14% to 8%, for example. However, for higher risk individuals in whom the maximal lowering of LDL-C from every angle possible is desirable, then also seeking to minimise dietary cholesterol may be warranted.

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