NUTRITION

MARCH 2020

www.alineanutrition.com

TABLE OF CONTENTS

Bergeron N, Chiu S, Williams PT, M King S, Krauss RM. Effects of red meat, white meat, and nonmeat protein sources on atherogenic lipoprotein measures in the context of low compared with high saturated fat intake: a randomized controlled trial. Am J Clin Nutr. 2019;110(1):24–33.

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

We know from 395 tightly controlled metabolic ward studies that saturated fat has the most pronounced negative effect on blood cholesterol levels, while polyunsaturated fats have the most pronounced positive effect [1]. The early metabolic feeding studies in the 1950's broadly characterised fat as 'animal' or 'vegetable' fat and found that, while vegetable fat resulted in significant reductions in blood cholesterol levels, substituting the vegetable fat for animal fat resulted in significant increased blood lipids [2,3]. Reverting the patients to the high vegetable fat diet lowered the concentration of blood cholesterol once again [2,3].

We also know from the early epidemiology linking high population levels of blood cholesterol to heart disease, that high levels of saturated fat correlated strongly with blood cholesterol levels, but 'high' needs context; the Finnish averaged 19-23% energy from SFA, the Dutch 20%, the Croats 17%, the British 20%, the Americans 21% [4]. However, it was also evident from the early metabolic feeding studies that not all fat sources had the same impact on blood lipids, even within the same class of fat subtype; for example, marine and vegetable polyunsaturated fats reduced blood lipids, but when polyunsaturated fats underwent industrial hydrogenation, the hydrogenated PUFA increased blood lipids [5].

Different high-saturated fat containing foods, like coconut and cows milk, were also shown to differentially increased blood lipids [5]. This is an important aspect of the evidence on SFA that has received more attention recently, particularly for dairy fats. For example, a number of controlled studies have shown that when comparing the same amount of butter vs. cheese, both with the same absolute saturated fat content, butter will drive up LDL-cholesterol, while whole-milk dairy fats have a relatively neutral effect [6,7]. Historically, the foods contributing the most to dietary saturated fat content in population diets, for example the UK, were meat, butter, lard, and milk [8].

A number of points of nuance arise in this context:

- 'High' saturated fat, in terms of potential risk, is generally >18% energy;
- Dairy fat from whole-milk sources - yogurt, cheese, milk - has different effects on blood cholesterol;

However, the effect of specific foods themselves in the context of the wider diet is somewhat unclear. This may be due to meta-analyses in nutrition often be misleading. For example, a meta-analysis which found that animal protein foods had no impact on blood cholesterol, lumped in studies which compared chicken, beef, pork, fish, to vegetarian diets [9]; such divergent studies mixed in would inevitably result in a weak overall effect.

The present study investigated whether the food source of dietary protein - red meat, white meat, or plant proteins - influenced blood cholesterol levels in the context of either higher or lower dietary saturated fat content. The main hypothesis was that red meat in the context of a high saturated fat diet would increase blood cholesterol levels compared to other dietary protein sources.

The Study

Healthy participants aged between 21-65yrs were recruited, and randomly assigned to one of two parallel arms:

- Red meat/High Saturated Fat vs. Red meat/Low Saturated Fat
- White meat/High Saturated Fat vs. White meat/Low Saturated Fat
- Non-meat/High Saturated Fat vs. Non-meat/Low Saturated Fat

The High Saturated Fat [HSFA] contained 14% energy from SFA; the Low Saturated Fat [LSFA] contained 7% energy from SFA. Each diet contained 12% energy from either protein source; red meat, white meat, or non-meat. To change the SFA content of the diets, butter and dairy foods were increased to achieve the HSFA diet; the red meat and white meat diets provided around 2-3% SFA. Participants attended at a research centre to collect key study foods, received dietary counselling, and were weighed to ensure weight stability throughout the intervention.

The foods comprising the protein source were:

- Red meat diet: 11% energy from beef, 1% from pork;
- White meat diet: 8% energy from chicken, 4% from turkey;
- Non-meat diet: 12% of energy from mixed legumes, nuts, grains, and isoflavone-free soy

In both diets, the remainder of the 25% total protein content was from eggs, diary, and vegetable sources. All dietary interventions ran for 4-weeks, and were separated by a 2-7 week washout period.

Primary outcome measures were LDL cholesterol, ApoB, and LDL particle size [small, medium, or large particle concentrations], and the total:HDL cholesterol ratio. The study employed a per-protocol analysis*, restricting statistical analysis only to participants who completed the study.

*Geek Box: Per-Protocol Analysis

You may recall from a previous Deepdive that we covered something known as 'intention to treat', which is a form of analysis which includes all participants that were randomised in the study, irrespective of whether they dropped out or not. To do this, the investigators take the last value recorded for that participant forward into the analysis, as if that value represented the data point the participant finished the trial with. Intention-to-treat is recommended in any trials comparing the effects of a treatment, because if the intervention and control arm are not balanced, it may lead to bias in the results. In contrast, a 'per-protocol analysis' is an analysis which only includes the data of participants who completed the study according to which intervention they were originally allocated to. This has the potential to introduce bias, if the arms of the trial are not balanced. In the present study, 24 were excluded from analysis in the LSFA arm, while 15 were excluded in the HSFA arm; the HFSA arm thus had 11 more participants in the final analysis. Whether this influenced the results, we don't know, because the investigators did not do an intention-to-treat analysis alongside the per protocol analysis.

Results: Compared to the non-meat diet, LDL and non-HDL cholesterol [i.e., a measure of all atherogenic lipoproteins in circulation] were higher after either the red meat or white meat diets, which was statistically significantly.

ApoB measures increased on the red meat and white meat diets, compared to the non-meat diet, which was also statistically significant.

Independent of protein source, the HSFA diets resulted in significantly higher total cholesterol, LDL-cholesterol, and non-HDL cholesterol, then the LSFA diet, in particular an associated increase in ApoB concentrations.

*Geek Box: Apolipoprotein B

Known as 'ApoB', this marker has emerged as a refined measure of all atherogenic lipoproteins in circulation. Historically, LDL-cholesterol has been the focus of assessing cardiovascular risk, given this atherogenic lipoprotein has been established as causal in the process of atherosclerosis. However, there are other lipoproteins with atherogenic potential; very-low density lipoprotein [VLDL]., intermediate density lipoprotein [IDL]., and lipoprotein(a) [Lp(a)], all of which have the potential to penetrate the arteries, become trapped, and generate the processes of atherosclerosis. This pool of atherogenic lipoproteins has not historically been measured with any accuracy; 'non-HDL cholesterol' was a crude measure, taken by subtracting HDL out of the measure of total cholesterol. However, each atherogenic lipoprotein particle contains one molecule of ApoB; consequently, measuring ApoB provides a direct measure of the exact number of atherogenic lipoproteins in circulation. From 2019, the European Atherosclerosis Society have recommended a direct measure of ApoB to assess cardiovascular risk, where circumstances allow for it.

The Critical Breakdown

Pros: Diets were matched for fibre, a nutrient which could positively influence blood lipids. In addition, the soy in the non-meat diet was isoflavone-free, and soy isoflavones may also influence positively blood lipids Participants were kept weight stable, minimising any potential confounding by weight loss. Food was prepared by the investigating team, and collected by participants. Compliance was assessed through a range of measures, from urinary metabolites, to grocery checklists. Finally, this was a food-based intervention, with diets design to have relevance for the US population.

Cons: Diets were not matched for cholesterol, which could have been achieved through food-based means. The emphasis on dairy fats to alter the SFA content of diets may have influenced the magnitude of the results, given both butter and cheese were used - which would be expected to have differential effects on blood lipids - but no exact data is presented on the contribution of the respective added fats. There were high levels of dropouts in the study - 39 from both interventions combined, and thus an intention-to-treat analysis should have been conducted, in addition to the per-protocol analysis.

Key Characteristic

It is important to remember that this study was testing the food source of protein, not the food source of saturated fat. Red meat and white meat only contributed 2-3% of energy from saturated fat. The additional saturated fat in this study was provided with dairy fats; whole milk, cottage cheese, cheddar cheese, and some butter.

It is difficult to determine the source for the increase in atherogenic lipoproteins [more under ***Interesting Finding***, below], given that the SFA content in the non-meat diet was derived primarily from dairy foods, but yet the red meat and white meat diets resulted in higher blood lipids than the non-meat diet in both LSFA and HSFA diet arms.

The fat in whole milk dairy food sources is encapsulated in the milk-fat globule membrane [MFGM], which is lost in the process of refining to butter, and may exert positive influences on blood lipids [7]. An 8-week RCT compared 40g MFGM milk fat to butter oil found that, while butter increased atherogenic lipoproteins, the MFGM milk fat did not [7]. Another intervention comparing 40g dairy fat either from full-fat cheddar cheese, a mix of low-fat cheese and butter, or butter alone, found that LDL-cholesterol was significantly reduced when dairy fat was consumed entirely in the whole-food matrix, i.e., as full-fat cheddar [6].

While even the non-meat diet resulted in higher blood lipids after the HSFA diet compared to the LSFA diet, the respective composition of the dairy sources of fat added to the diet - whole milk vs. butter - could be expected to influence the magnitude of the results. While it is important to reiterate - the study was testing food source of protein, not saturated fat - it could have been interesting to have an additional arm where the higher saturated fat came from meat sources.

Interesting Finding

LDL-cholesterol and ApoB were higher from the red meat and white meat diets compared to the non-meat diet, independent of SFA content - although the magnitude of effect was greater on the HSFA diet. This is interesting because the red meat and white meat diets were designed to be lean, and both food sources only contributed 2-3% energy from SFA.

Although dietary cholesterol content was higher on these diets, this could explain a certain degree of the difference, however, from the well-established equations for blood cholesterol changes, this would only account for a certain amount of difference.

We know from metabolic ward studies that LDL-cholesterol increases by 0.02-0.04 mmol/L for each 1% increase in energy from SFA [10]; thus, the 7% increase in SFA between the low and high SFA diets alone could explain a degree of difference: for example, in this study the difference could account for 0.14-0.28mmol/L.

The fact that the magnitude of increase was broadly similar: 0.24mmol/L on the non-meat diet; 0.23mmol/L on the white meat diet, and 0.29mmol/L on the red meat diet, supports that the overall effect may simply relate to the higher SFA content.

However, rather than minimise the small differences in blood lipids on the LSFA diet, from a scientific perspective there was a difference. And that is where the question lies, because it does suggest some effect of food source that is independent of the usual dietary factors influencing blood cholesterol levels from animal source foods, namely saturated fat, and to a lesser degree, dietary cholesterol.

Relevance

The difference between a low SFA non-meat diet and high SFA red meat diet was 0.42mmol/L; if we consider that maintaining LDL-cholesterol levels <2.6mmol/L is the recommended treatment target for people at moderate risk for cardiovascular disease, this difference is clinically relevant [11].

This isn' t the food first food-based intervention to investigate foods like red meat, that are traditionally associated with adverse effects on cardiovascular health, in the context of the wider diet. The Beef in an Optimally Lean Diet [BOLD] Trial investigated the effects of daily, lean unprocessed red meat consumption [113g/d or 153g/d] against a background established healthy diet pattern, the DASH diet. However, while the intervention was emphasising daily red meat, the saturated fat content of the diet was 6%. The BOLD trial reduced LDL to the same degree as the original DASH diet, which limited red meat, and interestingly reduced ApoB to a greater degree [12].

This is in contrast to the present study, in which red and white meat increased LDL and ApoB, independent of SFA content. However, in the BOLD trial, the red meat groups still consumed significant amounts of plant proteins [legumes and nuts], grains, and vegetables, which were not prominent in the present study on the meat diets.

In addition, dietary cholesterol in the BOLD trial was 193mg, compared to 400-473mg in the present study. While dietary cholesterol does not have major impacts on blood cholesterol levels, in the context of high SFA intake, tightly controlled interventions have demonstrated an additive effect of higher dietary cholesterol intake with a high SFA intake, on blood lipids [13]. It may simply be that overall, the BOLD diet was a better diet pattern to observe reductions in blood lipids in the context of meat intake.

Ultimately, the fact that the higher SFA intake was associated with increased atherogenic lipoproteins, independent of red meat, white meat, or non-meat diets, is consistent with what we know about the relationship between SFA and blood lipids. In the context of the LSFA diet in the present study, the magnitude of difference in blood lipids between red meat, white meat, and non-meat diets was relatively small, suggesting that in the context of a low saturated fat diet, lean sources of meat may not have as deleterious impacts of blood lipids.

Application to Practice

Increasing saturated fat still resulted in increased atherogenic lipoproteins: who knew? This isn' t exactly groundbreaking news. This study somewhat tells us what we already know; that while no single food source of saturated fat is inherently an issue, increasing saturated fat content of diet results in negative impacts on well-established risk factors for CVD/CHD. It does leave us with more questions over the role of dairy saturated fatty acids specifically, but practical application of the findings remains consistent with current knowledge; that animal origin foods in the context of a low saturated fat diet do no dramatically influence cardiovascular risk factors, and that plenty of plants and fibre is always beneficial.

References

1. Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. BMJ. 1997;314(7074):112-112.
2. Ahrens E, Blankenhorn D, Tsaltas T. Effect on Human Serum Lipids of Substituting Plant for Animal Fat in Diet. Experimental Biology and Medicine. 1954;86(4):872-878.
3. Beveridge J, Connell W, Mayer G, Firstbrook J, DeWolfe M. The Effects of Certain Vegetable and Animal Fats on the Plasma Lipids of Humans. The Journal of Nutrition. 1955;56(2):311-320.
4. Kromhout D, Menotti A, Bloemberg B, Aravanis C, Blackburn H, Buzina R et al. Dietary Saturated and transFatty Acids and Cholesterol and 25-Year Mortality from Coronary Heart Disease: The Seven Countries Study. Preventive Medicine. 1995;24(3):308-315.
5. Jolliffe N. Fats, Cholesterol, and Coronary Heart Disease. Circulation. 1959;20(1):109-127.
6. Feeney E, Barron R, Dible V, Hamilton Z, Power Y, Tanner L et al. Dairy matrix effects: response to consumption of dairy fat differs when eaten within the cheese matrix—a randomized controlled trial. The American Journal of Clinical Nutrition. 2018;108(4):667-674.
7. Rosqvist F, Smedman A, Lindmark-Månsson H, Paulsson M, Petrus P, Straniero S et al. Potential role of milk fat globule membrane in modulating plasma lipoproteins, gene expression, and cholesterol metabolism in humans: a randomized study1. The American Journal of Clinical Nutrition. 2015;102(1):20-30.
8. Foster R, Lunn J. 40th Anniversary Briefing Paper: Food availability and our changing diet. Nutrition Bulletin. 2007;32(3):187-249.
9. O' Connor L, Kim J, Campbell W. Total red meat intake of $\geqslant$0.5 servings/d does not negatively influence cardiovascular disease risk factors: a systemically searched meta-analysis of randomized controlled trials. The American Journal of Clinical Nutrition. 2016;105(1):57-69.
10. Mensink R, Zock P, Kester A, Katan M. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. The American Journal of Clinical Nutrition. 2003;77(5):1146-1155.
11. ESC/EAS Task Force. 2019 Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. European Heart Journal. 2019;00:1⊠78.
12. Roussell M, Hill A, Gaugler T, West S, Vanden Heuvel J, Alaupovic P et al. Beef in an Optimal Lean Diet study: effects on lipids, lipoproteins, and apolipoproteins. The American Journal of Clinical Nutrition. 2011;95(1):9-16.
13. Fielding C, Havel R, Todd K, Yeo K, Schloetter M, Weinberg V et al. Effects of dietary cholesterol and fat saturation on plasma lipoproteins in an ethnically diverse population of healthy young men. Journal of Clinical Investigation. 1995;95(2):611-618.