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Retterstøl K, Svendsen M, Narverud I, Holven KB. Effect of low carbohydrate high fat diet on LDL cholesterol and gene expression in normal-weight, young adults: A randomized controlled study. Atherosclerosis. 2018;279(April):52–61.

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

Over the past 5-10yrs we have seen an acceleration of nutritional evangelism in public health discourse, as competing dietary factions have duelled over the primacy of their respective beliefs as to what constitutes "the optimal" diet for human health.

In the UK in particular, the campaigning of the low-carb, high-fat [LCHF] movement since around 2013 has been a cacophony of sophistry, delusion, and bad science. Campaigners such as Zoe Harcombe and Aseem Malhotra called for an overhaul of current dietary guidelines to remove the emphasis on reducing saturated fat, and to promote a low-carbohydrate diet in public health nutrition [1,2,3].

The public-facing driver of this advocacy in the UK was a organisation of quacks known as the 'Public Health Collaboration'. Typically targeting enthusiastic GPs with seductive words like "nutrition" and "lifestyle", in 2017 they produced a document with the highly sophisticated, if only slightly reductionist, title: *'Eat Fat, Cut The Carbs and Avoid Snacking To Reverse Obesity and Type 2 Diabetes'*, in which the following claim was advanced:

"Evidence from multiple randomised controlled trials have revealed that a higher fat, lower carbohydrate diet is superior to a low-fat diet for weight loss and cardiovascular disease risk reduction."

The former claim - the difference between low-carb and low-fat diets for weight loss - is about as interesting a talking point as Boris Johnson's favourite Christmas jumper. The latter claim, however, is rather like painting the side of a bus announcing "we send the EU £350 million a week": a fabrication based on the self-selected alternative facts of a deluded few. My, things are salty this morning.

A major contention of the UK LCHF movement has been that dietary guidelines regarding saturated fat were introduced without evidence from randomised controlled trials [this is false], that a shift was needed away from LDL-cholesterol as a marker of cardiovascular health [this is laughable], and that an ad libitum low-carb and high-fat diet should be encouraged.

The study we look at today examined a LCHF diet in a controlled trial on cardiovascular risk factors.

The Study

39 participants [32 women, 7 men] were randomised to either a low-carbohydrate, high-fat [LCHF] Atkins Diet with <20g/d carbohydrate and ad libitum [i.e., as much as desired] fat and protein intake, or a control diet. Participants were young [average age 25yrs], average body mass index of 21, and healthy in all baseline markers, including blood pressure, blood cholesterol levels, blood glucose levels, etc.

The study was a parallel-arm intervention [both diet groups ran concurrently] conducted for 3-weeks. The LCHF diet was self-selected, and participants in the intervention group were informed that they could eat unlimited amounts of meat, poultry, fish, seafood, eggs, and vegetable oils. The control group continued with their habitual diet. Dietary intake was quantified prior to the study by a 4-day weighed food record. A further 3-day weighed food record was taken during the LCHF intervention diet.

The primary outcome measure was change in LDL-C within-group [i.e., from baseline to end of intervention] and between-group [i.e., the change in the intervention group compared to the change in the control group]. Secondary outcomes included other blood lipid markers, i.e., non-HDL cholesterol and ApoB* and genes regulating lipid metabolism*. Data for blood cholesterol is reported in mmol/L except for ApoB, which is mg/dL.

*Geek Box: Lipoprotein Measures & Pathways of Cholesterol Regulation

There are a number of relevant measures for blood lipids which are useful in the overall risk equation. The most important historically is LDL-C, as this is the lipoprotein which is causal in the initiation of atherosclerosis and the most abundant atherogenic lipoprotein in circulation. However, LDL-C is not the only lipoprotein class that has atherogenic potential. In fact, all lipoproteins of <70-nanometers in diameter are capable of penetrating into the arteries: this includes smaller VLDL, IDL, and Lp(a) in addition to LDL-C. Previously, the best way of accounting for all circulating atherogenic lipoproteins in circulation was the calculate 'non-HDL-C', which was a crude measure derived from subtracting the measured level for HDL from the total cholesterol value. This remains useful, particularly for prospective cohort studies where it provides an inexpensive means of calculating a score which may be more predictive than LDL-C alone. However, each atherogenic lipoprotein particle contains one molecule of Apolipoprotein-B; 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. So each of these measures is valuable, it depends on the context as to which may be more informative for risk assessment [individual vs. population, etc.]. Now, circulating cholesterol is influenced by a number of relevant gene pathways. The most important of these is the LDL-receptor, the discovery of which won Joseph Goldstein and Michael Brown the Nobel Prize in 1985. The LDLR is the main receptor which provides cells with cholesterol, which it uptakes from lipoproteins like LDL transporting cholesterol to body tissues. Thus, the LDLR is responsible for clearing cholesterol from the circulation. All of the effective drugs for reducing cardiovascular disease - statins, ezetimibe, and PCSK9-inhibitors - act by ultimately upregulating the LDLR expression and activity, clearing and reducing cholesterol levels. The PCSK9 gene is critical in this process, as PCSK9 negatively regulates circulating LDL levels by mediating the degradation of the LDLR. In simple terms, high PCSK9 activity reduces expression of the LDLR, inhibiting the uptake of cholesterol and resulting in elevated cholesterol levels. PCSK9-inhibitor drugs act by doing exactly that: inhibiting PCSK9 expression, resulting in upregulated LDLR activity and cholesterol clearance. Finally, the SREBP gene pathway influences cholesterol metabolism by sensing the levels of cholesterol within cells, and SREBP proteins regulate multiple pathways that influence cholesterol metabolism: the HmG-CoA-reductase enzyme [which statins inhibit], the LDLR, and also HDL-receptors. Decreasing cholesterol within cells results in increased cholesterol uptake, mediated by SREBP. There are, however, limits to this because cells will prevent accumulating excess cholesterol, meaning that an overproduction of cholesterol from diet may not be compensated by just increasing cellular uptake. In fact, the most well-established dietary regulator of blood cholesterol levels - the ratio of saturated to polyunsaturated fats - is due to oppositional effects, i.e., polyunsaturated fats positively influence cholesterol clearance, while saturated fats inhibit cholesterol uptake.

Results: The habitual control diet contained 17% protein, 30% fat [11% saturated], 45% carbohydrate, and 261mg dietary cholesterol; the LCHF diet contained 25% protein, 70% fat [29% saturated], 3% carbohydrate, and 1,072mg dietary cholesterol. Reported carbohydrate intake in the LCHF diet averaged ~16g/d.

Blood Lipids:

- **LDL-C:** increased from 2.2mmol/L [85mg/dL] to 3.1mmol/L [120mg/dL] in the LCHF group, compared to no change in the control group. The average increase in LDL-C was 44%, however, significant inter-individual differences were noted, with a minimum increase of 5% in one participant up to 107% in anther [see Figure, below].
- **HDL-C:** increased from 1.6mmol/L [61mg/dL] to 1.9mmol/L [73mg/dL] in the LCHF group, compared to no change in the control group.
- **Non-HDL-C:** increased from 2.5mmo/L [96mg/dL] to 3.4mmol/L [131mg/dL] in the LCHF group, compared to no significant change in the control group.
- **ApoB:** increased from 2.5mmo/L [96mg/dL] to 3.4mmol/L [131mg/dL] in the LCHF group, compared to no significant change in the control group.
- **Free fatty acids:** increased from 0.4mmol/L [1.5mg/dL] to 0.8mmol/L [30mg/dL] in the LCHF group, compared to no significant change in the control group.

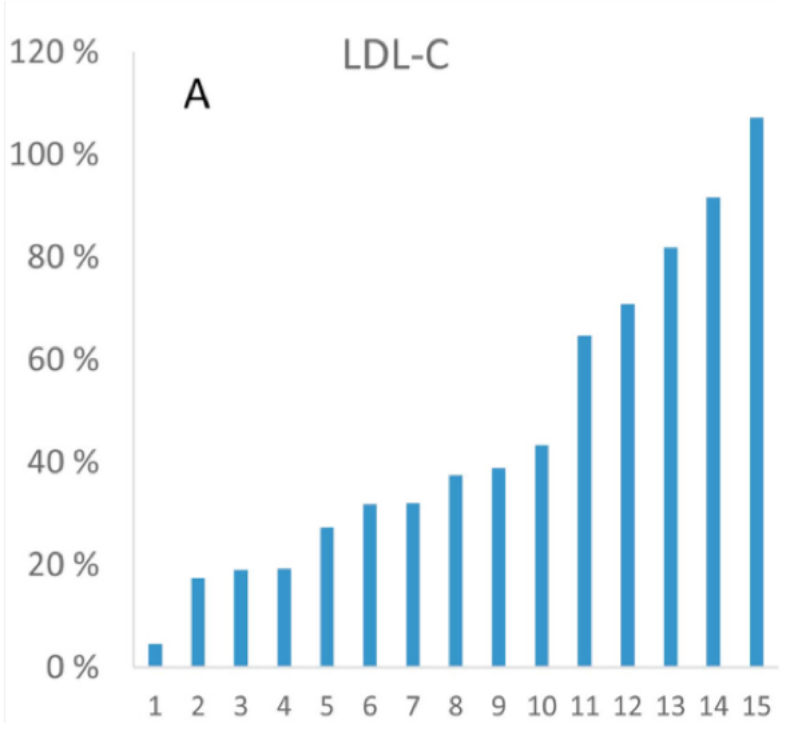

Figure *from paper illustrating the individual responses for LDL-C in 15 participants randomised to an LCHF diet for 3-weeks. The range of response [from 1 to 15, left to right] was 5-107%, with an average increase of 44%.*

Lipid-related Genes:

- **LDL-Receptor:** Expression of the LDL-R gene decreased by 33% following the LCHF diet, which was borderline statistically significant, and there was no significant difference between groups.
- **SREBP:** Expression of the SREBP gene increased by 16% following the LCHF diet, and there was a significant difference between groups after the intervention.
- **PCSK9:** There was no significant difference between groups in circulating PCSK9, however, this decreased significantly within-group [i.e., between baseline value and end of intervention in each group].

There were no significant differences in blood pressure, glycaemic markers, or other lipid markers, e.g., triglycerides.

The Critical Breakdown

Pros: Extensive analyses were conducted on blood markers and genetic regulators of lipid metabolism, providing detailed insight into the effects of the diet. The intervention diet appeared to be a 'true' LCHF diet, as often advocates for such diets [correctly] point out that many studies use diets that are not that low in carbohydrate or high in fat: in this study fat intake - as measured by weighed food record - was 70% fat and 3% carbohydrate. Participants were young and otherwise completely healthy, thus the effects of the diet were not modified by related cardio-metabolic factors. Weighed food records were used to assess diet at baseline, and during the intervention in the LCHF group. The self-selecting nature of the LCHF diet may have greater generalisability to such diets in free-living contexts.

Cons: The study was quite imbalanced between women and men, and there are well-established sex differences in cholesterol responses to diet [men tend to have greater responses compared to premenopausal women] [4]. While weighed food records are the most accurate means of assessing diet in free-living humans, there is always the caveat in free-living studies that diet may have varied from the measured intakes. It would have been useful to have data on actual foods consumed, particularly sources of dietary fat. While 3-weeks was sufficient to detect differences between diets, it is also a very short-term study. Given the known individual variation in blood cholesterol responses to diet, it could have been useful to assess participants for the ApoE genotype, which is common in European populations and may account for variability. The population was a Northern European, and the extent of the variability and responsiveness to diet may not be generalisable to other ethnic groups.

Key Characteristic

The dietary intervention was a 'true' LCHF intervention. To be fair to advocates of carbohydrate-restricted diets, they have a point when highlighting that many intervention trials labelled 'low-carb' may have an average of ~30% carbohydrate. Such advocates have defined a true very-low carbohydrate high-fat diet as one containing 20-50g/d carbohydrate, or <10% of energy [5]. The present study achieved an average carbohydrate intake of 16g/d, with a range of 9-20g, thus definitely satisfying the criteria of a very-low carb diet. In addition, the fact that saturated fat averaged 29% of total energy likely reflects the food choices mandated for Atkins-diet style low-carb diets, i.e., animal meats and fats. This level of intake is important, as certain recent studies have purported to find that "high" saturated fat intakes of up to 18% energy have no effect on LDL-C: scrutiny of the data revealed that participants reduced total energy intake by ~500kcal/d, and the gram intake of saturated fat did not change significantly [i.e., the percentage increase reflected the change in other dietary variables] [6]. In the present study, energy intake averaged 2,062kcal/d in the LCHF group, with 29% saturated fat: this constitutes around 66g/d saturated fat. These levels of intake correlate over the long-term with a strong association between blood cholesterol level and coronary heart disease mortality [7].

Interesting Finding

The variability in the LDL-C response to the LCHF diet. The variability in blood cholesterol responses to diet is well-established, with hyper-responders and minimal responders evident in the literature [8]. So, this finding isn' t necessarily new - what makes it interesting is the related factors which may explain the difference. First, the average intake of saturated fat was at consistently high, yet the LDL-C response varied. This could be due to genetic differences, for example the ApoE polymorphism may account for up to 10% of variation in LDL-C in populations [9]. In addition, with 11% polyunsaturated fat and 24% monounsaturated fat in the diet, it is highly probably that the sources and types of these unsaturated fats modulated the LDL-C response [10]. As we have no data on actual dietary intake, we may only speculate in this regard. Further, although this finding was not statistically significant, the 33% decrease in LDL-receptor activity is the most biologically plausible mechanistic explanation for the increase in LDL-C in response to saturated fat intake [11]. None of this entirely explains the inter-individual variation observed in the present study, however. But it is also important to realise that the variation was the context of an average increase in LDL-C of 44%: only one participant could be classified as a minimal responder [number 1 on the X-axis, above].

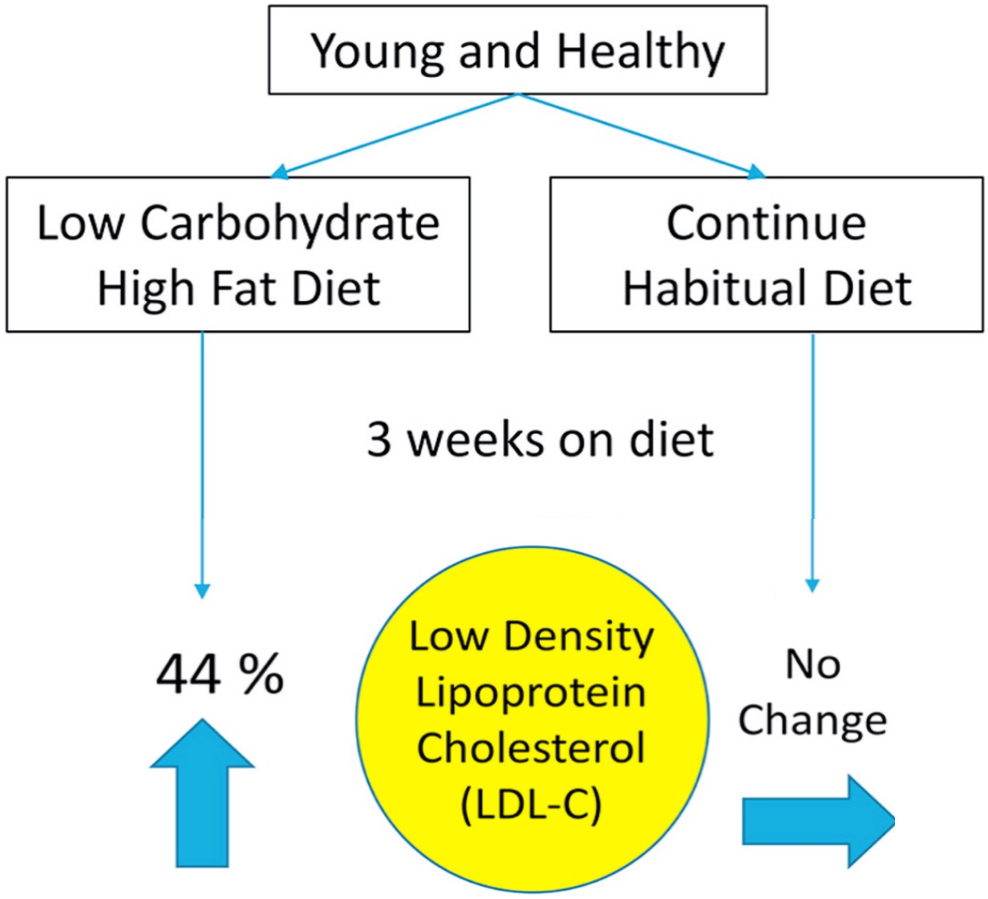

Figure *from paper illustrating the individual responses for LDL-C in 15 participants randomised to an LCHF diet for 3-weeks. The range of response [from 1 to 15, left to right] was 5-107%, with an average increase of 44%.*

Relevance

The popularity of high fat diets hasn' t gone away: in fact, they' ve become more extreme with increasing popularity of outlier diets like the 'Carnivore' diet. All of these LCHF iterations have two core beliefs in common:

1. LDL-C Denialism, and;
2. Embrace of high saturated fat intake.

As you' ve read about a thousand times here, nonetheless we shall repeat: LDL-C is causal in atherosclerosis [12,13].

Moving on, the trial diet - a self-selected ad lib LCHF diet emphasising what Zoe Harcombe calls "omnipresent natural fats" - potentially reflects the composition of diet when people undertake low-carb 'Atkins' style diets in the real world. While the study demonstrates that certain individuals may not have a pronounced cholesterol-raising response to the diet, a scrutiny of the individually presented data suggests all bar one participant experienced at least a 20% increase in LDL-C.

It could be tempting to reach for the "but look, HDL increased and that is protective so hey that' s good right" narrative routinely touted from the low-carb movement. Except, this doesn' t hold the weight it once may have: interventions directly targeting increasing HDL as a means of lowering CVD risk have been unsuccessful to date, and an emerging hypothesis from a dietary perspective is that the concomitant increase in HDL alongside LDL may be a compensatory effect to LDL increases in response to diet [14].

While this study is only a 3-week intervention, the composition of the diet and the effect of causal risk factors for CVD needs to be considered in the context of evidence from long-term prospective cohort studies. A consistent pattern of findings across different populations indicates the replacement of carbohydrate with animal fat and proteins increases risk of all-cause and cardiovascular mortality [15]. A significant factor underscoring these associations is increased consumption of saturated animal fat in a lower-carbohydrate dietary pattern [16,17].

This is not reflective of lower carbohydrate per se: LC diets which emphasis plant fats and proteins are associated with diametrically opposed effects on all-cause and cardiovascular mortality [17]. It is difficult to extrapolate a 3-week study to long-term effects, but the causal role of LDL-C in atherosclerosis taken together with the effects of diet on LDL-C provides strong biological plausibility which would explain the adverse cardiovascular effects of animal-based low-carb diets consistently observed in prospective studies.

Ultimately, this study was one of the first to test a true LCHF diet high in saturated fat and low in fibre/carbohydrates generally, on cardiovascular risk factors. The findings are consistent with a wider literature, including over 390 metabolic ward studies on the impacts of macronutrients on blood lipid levels [10]. It is important to reiterate the effective role LC diets have in glycaemic control and diabetes management, which is possible without concomitant adverse effects on blood lipids when saturated fat intakes are <8% [18].

Application to Practice

Unfortunately, low-carbohydrate diets are synonymous with wider cholesterol-denialism and heart disease revisionism. This results in a diet which throws polyunsaturated fats out the window as "toxic", thinks fibre research is a "conspiracy", and pours the butter on the bacon. It doesn' t have to be this way, evident in a number of diabetes interventions using LC, but low saturated fat, diets. However, given the continued popularity low-carb diets of the Atkins, Paleo, and Carnivore varieties, this study should serve as pause for caution in terms of cardiovascular health.

References

1. Malhotra A. Saturated fat is not the major issue. BMJ. 2013;347(oct22 1):f6340-f6340.
2. Malhotra A, Redberg R, Meier P. Saturated fat does not clog the arteries: coronary heart disease is a chronic inflammatory condition, the risk of which can be effectively reduced from healthy lifestyle interventions. British Journal of Sports Medicine. 2017;51(15):1111-1112.
3. Harcombe Z. US dietary guidelines: is saturated fat a nutrient of concern?. British Journal of Sports Medicine. 2018;:bjsports-2018-099420.
4. Weggemans R, Zock P, Urgert U, Katan M. Differences between men and women in the response of serum cholesterol to dietary changes. European Journal of Clinical Investigation. 1999;29(10):827-834.
5. Feinman R, Pogozelski W, Astrup A, Bernstein R, Fine E, Westman E et al. Dietary carbohydrate restriction as the first approach in diabetes management: Critical review and evidence base. Nutrition. 2015;31(1):1-13.
6. Shih C, Hauser M, Aronica L, Rigdon J, Gardner C. Changes in blood lipid concentrations associated with changes in intake of dietary saturated fat in the context of a healthy low-carbohydrate weight-loss diet: a secondary analysis of the Diet Intervention Examining The Factors Interacting with Treatment Success (DIETFITS) trial. The American Journal of Clinical Nutrition. 2019;109(2):433-441.
7. 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.
8. Cox C, Mann J, Sutherland W, Ball M. Individual variation in plasma cholesterol response to dietary saturated fat. BMJ. 1995;311(7015):1260-1264.
9. Griffin B, Walker C, Jebb S, Moore C, Frost G, Goff L et al. APOE4 Genotype Exerts Greater Benefit in Lowering Plasma Cholesterol and Apolipoprotein B than Wild Type (E3/E3), after Replacement of Dietary Saturated Fats with Low Glycaemic Index Carbohydrates. Nutrients. 2018;10(10):1524.
10. 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.
11. Mustad VA, Etherton TD, Cooper AD, Mastro AM, Pearson TA, Jonnalagadda SS, Kris-Etherton PM. Reducing saturated fat intake is associated with increased levels of LDL receptors on mononuclear cells in healthy men and women. J Lipid Res. 1997 Mar;38(3):459-68.
12. Ference B, Ginsberg H, Graham I, Ray K, Packard C, Bruckert E et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. European Heart Journal. 2017;38(32):2459-2472.
13. Borén J, Chapman M, Krauss R, Packard C, Bentzon J, Binder C et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. European Heart Journal. 2020;41(24):2313-2330.

References

14. Griffin BA, Lovegrove JA. Butter Increases High-Density Lipoprotein Functional Capacity: Is This Compensation for Its Adverse Effect on Serum Low-Density Lipoprotein Cholesterol? J Nutr. 2018 Jul 1;148(7):1069-1070.

15. Noto H, Goto A, Tsujimoto T, Noda M. Low-carbohydrate diets and all-cause mortality: a systematic review and meta-analysis of observational studies. PLoS One. 2013;8(1):e55030.

16. Nilsson LM, Winkvist A, Eliasson M, Jansson JH, Hallmans G, Johansson I, Lindahl B, Lenner P, Van Guelpen B. Low-carbohydrate, high-protein score and mortality in a northern Swedish population-based cohort. Eur J Clin Nutr. 2012 Jun;66(6):694-700.

17. Li S, Flint A, Pai JK, Forman JP, Hu FB, Willett WC, Rexrode KM, Mukamal KJ, Rimm EB. Low carbohydrate diet from plant or animal sources and mortality among myocardial infarction survivors. J Am Heart Assoc. 2014 Sep 22;3(5):e001169.

18. Tay J, Luscombe-Marsh ND, Thompson CH, Noakes M, Buckley JD, Wittert GA, et al. A very low-carbohydrate, low-saturated fat diet for type 2 diabetes management: A randomized trial. Diabetes Care. 2014;37(11):2909–18.