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TABLE OF

CONTENTS

Bard JM, Drouet L, Lairon D, Cazaubiel M, Marmonier C, Ninio E, Bal Dit Sollier C, Martin JC, Boyer C, Bobin-Dubigeon C; INNOVALAIT Study group. Effect of milk fat on LDL cholesterol and other cardiovascular risk markers in healthy humans: the INNOVALAIT project. Eur J Clin Nutr. 2020 Feb;74(2):285-296.

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

The broad and complex food group that is dairy means that there is no shortage of research angles that can be taken to investigate the role of dairy foods, and their composition, on human health. In a recent Deepdive, we covered the most recent meta-analysis of cohort studies that focused specifically on dairy foods and atherosclerotic cardiovascular disease [ASCVD]. This analysis, consistent with other nutritional epidemiology [1,2], found food-based differences in associated risk, e.g., whole-fat milk associated with higher ASCVD risk, while cheese was associated with lower risk.

The epidemiology of dairy foods confirms a now well-established concept for nutrition research; that the food matrix is a highly relevant determinant of health effects beyond what nutritional content alone may explain [3,4]. The food group dairy may be stratified by fat content, fat composition, fermentation, refinement ("churning"), creating a highly heterogenous food group, which implies heterogenous influences on cardio-metabolic health.

Indeed, there is an accumulated body of evidence demonstrating these differences. For example, Nestel *et al*. [5] compared the effects of consuming 40g cheese or 40g butter for 4-weeks, showing that butter led to significant increases in total and LDL-cholesterol compared to cheese. These differences suggest that the effects of the same amount of total dairy fat differ relative to other characteristics of that dairy fat [see figure, below].

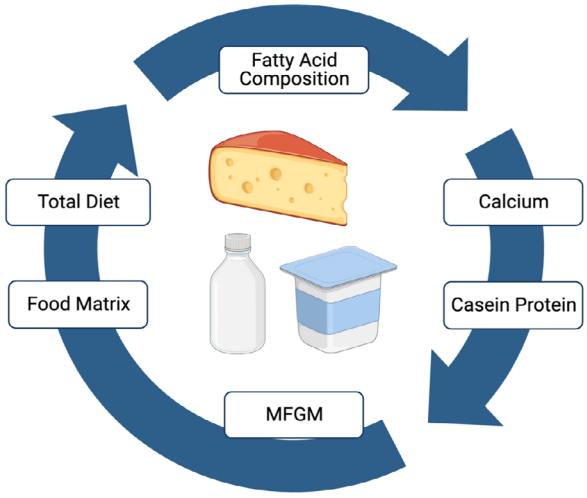


***Figure** illustrating the range of factors which may mediate differences in the impacts of different food sources of dairy fat on cardio-metabolic responses. 'MGFM' = milk-fat globule membrane, an encapsulation within which dairy fat is contained prior to churning/refinement [e.g., present in cheese but not in butter].*

To date, most studies have compared cheese to butter, with or without additional protein and/or calcium [5–8], and whether milk fits into this picture of different effects is less well established. The fatty acid composition of milk has been known to vary by season [9], which may also influence effects of milk fat. The present study investigated the effects of milks from different seasons compared to vegetable fat on cardio-metabolic risk factors.

The Study

The study was a randomised, double-blind intervention trial investigating the effects of vegetable fats compared to dairy fats in participants with mildly elevated blood cholesterol levels. The study compared four different diet groups:

- **Vegetable Fat Diet [VF]**: This served as the control/comparison diet to the dairy fat diets.
- **Spring Milk Fat Diet [SM]**: Diet based on dairy fat from spring milking.
- **Winter Milk Fat Diet [WM]**: Diet based on dairy fat from winter milking.
- **Winter Milk Fat + Calcium Diet [WM-Ca]**: Diet based on dairy fat from winter milking with added calcium.

Participants were randomised to one diet, and all diet groups ran parallel to each other. Participants were blind to their diet group allocation, as study foods were unlabelled. The investigators also remained blinded to the diet allocation of participants. The total duration of the study was 8-weeks.

Each diet aimed for ~38% of energy from total fat, with 20% of that fat caming from pre-prepared study foods that were provided to participants, and of which 12-14% was saturated fats. The study foods were a dairy dessert (or vegetable fat equivalent); cheese (or vegetable fat equivalent); fat spread (butter or vegetable fat equivalent). These foods were consumed daily.

A mixed vegetable oil [rapeseed, olive, sunflower, grape seed] made up the balance of total fat, and this was the only additional fat participants were allowed to consume. Dietary intake was assessed at baseline and participants were guided by study dietitians to achieve maintenance level energy intake. 3-day food diaries were completed every 2-weeks for the duration of the study.

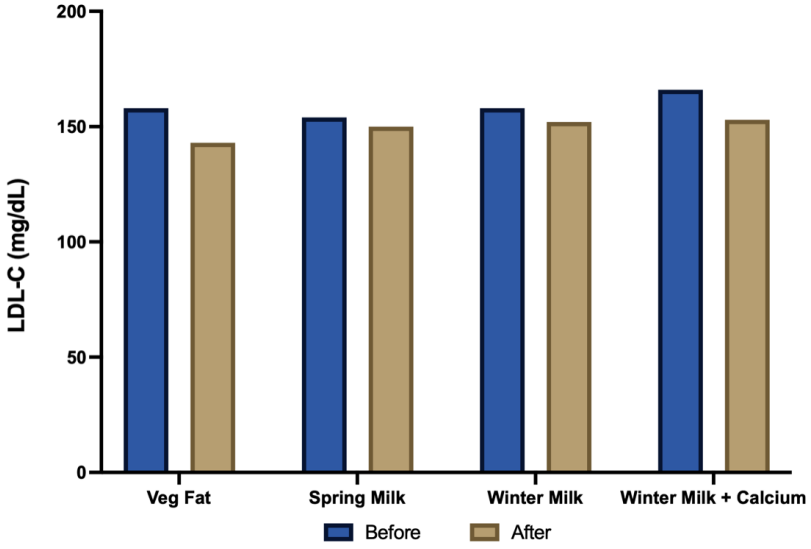
Blood samples were taken after 4-weeks and at the end of the study. The primary outcome was change in LDL-C levels; secondary outcomes included other blood lipid markers like total cholesterol [TC] and ApoB***** [see ***Geek Box** for further details], blood glucose and insulin.

*Geek Box: Apolipoprotein-B

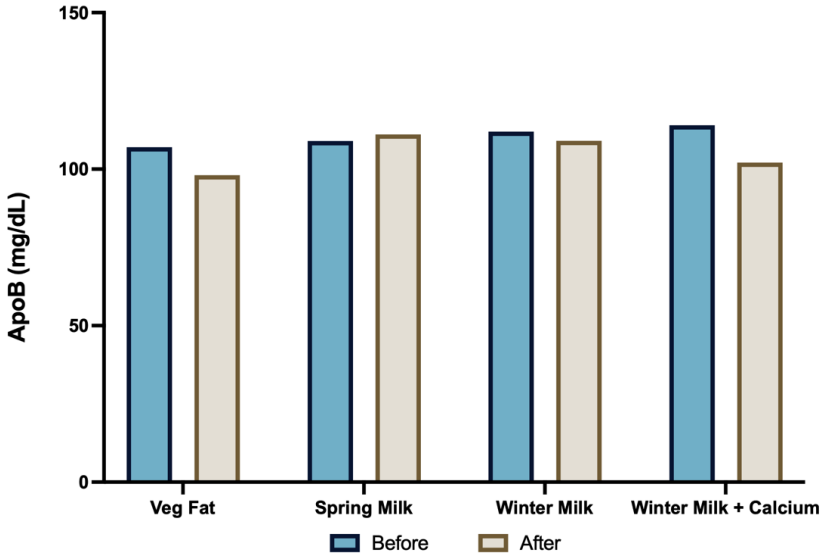
Known as 'ApoB', this marker has emerged as a refined measure of all atherogenic lipoproteins in circulation. Historically, LDL-cholesterol has been the focus of assessing cardiovascular risk, given this atherogenic lipoprotein has been established as causal in the process of atherosclerosis. However, there are other lipoproteins with atherogenic potential; chylomicron remnants ['remnants' are smaller particles compared to their parent compound, are rich in triglycerides and cholesterol, and capable of penetrating the arterial wall], very-low density lipoprotein [VLDL] and VLDL remnants, and intermediate density lipoprotein [IDL]. All of these particles 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 carries its fat and cholesterol in a protein wrapper called apolipoprotein-B ['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.

Results: A total of 154 participants completed the study; 38 in the VF, 42 in the SM, 39 in the WM, and 35 in the WM-Ca groups. There were no significant differences in total energy and macronutrient intakes between groups. Achieved total dietary fat was ~35% of daily energy intake.

***Primary Outcome – Effect of Diets on LDL-C*:** After 8-weeks, LDL-C decreased by 14mg/dL on the VF diet, 4mg/dL on the SM diet, 6mg/dL on the WM diet, and 13mg/dL on the WM-Ca diet; these differences were not statistically significantly different between groups. The **figure** below illustrates the differences in LDL-C in each diet during the study.



***Secondary Outcomes – Effect of Diets on TC and ApoB*:** After 8-weeks, TC decreased by 23mg/dL on both the VF and WM-Ca diets. Further, ApoB decreased by 9mg/dL on the VF diet, 3mg/dL on the WM diet, and 12mg/dL on the WM-Ca diet. ApoB increased by 2mg/dL on the SM diet. Thus, the VF and WM-Ca diets were significantly different compared to the SM diet. The **figure** below illustrates the differences in ApoB in each diet during the study.



***Secondary Outcome – Effect of Diets on Glucose/Insulin*:** After 8-weeks, there were no significant differences in plasma glucose or insulin levels between any of the diet groups.

***Secondary Outcome – Effect of Diets on Inflammatory/Oxidative Stress Markers*:** After 8-weeks, there were no significant differences in inflammation or oxidative stress levels between any of the diet groups.

The Critical Breakdown

Pros: The authors clearly stated the aim and objective of the study. The study had a strong design; the study was managed by a third-party trial manager, who conducted randomisation, and randomisation was stratified by sex, baseline LDL-C, and BMI, to ensure balance of these factors between diet assignments. Both participants and investigators were blind to the diet allocation. Intention-to-treat analysis was also conducted to ensure that dropouts did not influence the statistical analyses outcomes. The primary and secondary outcomes were clearly stated. The participants all exhibited moderately elevated cholesterol [>130mg/dL or 3.3mmol/L] at baseline, thus representative of a broad section of the general population. Repeated assessments of diet were conducted, and participants were counselled to maintain energy intake; the loss of bodyweight of <1% from baseline suggests that participants were mostly in a state of energy balance for the 8-week duration of the study. The study made laudable efforts to control the dietary fat content of each diet, providing participants with specifically formulated study foods tailored to individual energy requirements.

Cons: No clear hypothesis was stated. Some of the reporting is at least bizarre, at worst sloppy; the data for age, smoking status, and sex stratifications between groups was "data not shown". This isn't data you *don't* show; usually "data not shown" are exploratory or tangential data to the study, not important covariates like age. So, we don't know what the average age of participants in each diet group were; based on the inclusion criteria, they are anywhere between 18–70yr! Similarly, participants were counselled to maintain their diets other than the fat sources, however, carbohydrate and fibre intake increased by ~10% and 5.6g/d, respectively, across all groups. We don't know why this occurred or what foods contributed to this increase, but total carbohydrate and fibre are certainly factors which could influence blood lipids. Indeed, we have no data on the background diets other than macronutrient contents. Despite clearly stating their primary and secondary outcomes, they ignore reporting on the primary outcome in the results, emphasising "statistically significant" secondary outcomes, before announcing in their discussion that their main result was that LDL did not differ between diets. It is also unclear why blood samples were taken halfway into the study; had the comparisons been between week 0 vs. week 8, would the effects have been different?

Key Characteristic

The key characteristic of the present study is the comparison to an unsaturated fat control, rather than a within-class comparison of one dairy fat food against another, e.g., milk to butter or cheese. This is because, while these comparisons are important, we know from the totality of evidence that unsaturated fats, polyunsaturated fats in particular, have a greater effect on lowering blood cholesterol markers compared to saturated fats [10,11].

By incorporating a vegetable fat control diet in this study, therefore, it allowed for three potentially important factors to be compared: vegetable fat vs. dairy fat vs. diary fat + calcium. This allowed for several findings to be evident. The first is that the SM [spring milk fat] and WM [winter milk fat] diets displayed negligible differences across all outcomes, suggesting that whatever fatty acid differences in the composition of milk from each season existed were ultimately clinically insignificant.

A recent secondary analysis of an RCT comparing 3.3 servings per day of full-fat dairy or low-fat dairy, with a mix of milk, yogurt, and cheese, to a limited dairy diet, found no significant differences between diets on LDL-C [12]. The present study adds to this by suggesting that milk fats alone, without further considering specific foods, exert largely neutral effects on blood cholesterol levels.

Interesting Finding

Ok, so we know that milk fat alone, irrespective of whether from spring or winter, had a largely neutral effect on blood lipids in this study. And we know that, compared to the milk fat without calcium enrichment, the vegetable fat diet led to reductions in TC, LDL-C, and ApoB.

This is where things get interesting for this study, because the milk fat group with additional calcium showed the same magnitude of reductions in TC, LDL-C, and ApoB, as the VF diet group. This is interesting because it adds to previous evidence that calcium may mediate the effects of dairy fats on blood lipids, potentially through the formation of calcium "soaps" in the digestive tract that inhibit fat absorption [8,13].

For example, Jakobsen *et al*. [8] compared diets with differing in protein [15% vs. 23%] and calcium [500mg vs. 1,800mg] content, and found that faecal fat excretion was 2.5-fold higher in the high calcium [and lower protein] diet group. This suggests that postprandial effects of dairy foods on lipid metabolism are influenced by calcium, rather than protein, and that calcium acts to inhibit fat absorption, thus resulting in more fat excreted.

Recently, a study in pigs [which are the best approximate animal model for the human gastrointestinal system], showed that calcium inhibited specifically the absorption of saturated fatty acids, leading to their excretion bound in calcium "soaps" [13]. Interestingly, there was no change in fatty acid excretion when calcium levels were increased in diets containing olive oil or soybean oil.

Thus, while we know that unsaturated fats decrease blood cholesterol levels through, primarily, upregulated LDL-receptor clearance of cholesterol from the blood, it may be that the effect of dairy foods on blood lipid lipids is primarily mediated by calcium.

Relevance

We have a fairly congruent body of evidence that coalesces to a similar conclusion; that when consideration is given to saturated fats and CVD risk, it is important to differentiate between food sources of saturated fat [1,2,14].

The one dairy food, however, that is not quite consistent is whole [or "full-fat"] milk, which is inconsistently associated with CVD outcomes, and may relate to whether it is fermented or not [15], in addition to the total background saturated fat content of the diet [16] Different effects on blood lipids comparing milk to butter and cheese have also been shown, with milk increasing LDL-C to a similar magnitude as butter [17].

However, the present study suggests that the effects of milk fat may be mediated by enrichment with calcium of foods high in milk fat. This builds on a body of prior evidence suggesting that both the food matrix itself, and the calcium content of the diet, are important mediating factors of the effect of dairy fats [6,8].

In a previous Deepdive, we covered a study that compared diets matched for total fat, dairy fat [~40g], casein protein, and calcium, but where the dairy fat was consumed in different structural food matrices, e.g., full-fat cheddar vs. reduced fat cheddar and butter vs. butter, casein protein, and supplemental calcium.

While all diets led to reductions in LDL-C, the order of effect directly reflected the amount of fat consumed directly from the cheese matrix, such that the dairy fat entirely consumed as full-fat cheese led to greatest reductions in LDL-C. This suggested that the effect of the food was independent of the fat content [which was matched between interventions], reflecting an overall synergistic effect of the mix of dairy fat, casein, and calcium, within the whole-food matrix.

The present study adds to this evidence by suggesting a particularly beneficial effect of calcium in shifting the effect of milk fat toward an overall reduction in atherogenic lipoproteins that are in the causal pathway of atherosclerosis development.

Application to Practice

Recall from our very recent Deepdive that epidemiological research suggests excessive calcium supplementation may in fact *increase* CVD risk through increasing coronary artery calcification, but that these effects are not observed with dietary calcium, which is associated with lower CVD risk.

This is important additional context, because in this study the calcium enrichment was added to dairy foods. However, the total calcium levels of these foods is only something that could be achieved with a food manufacturer producing a product, as was the case in the present study. Overall calcium added to these foods amounted to 2,066mg/d! That is a lot of calcium.

The point to bear in mind is that for all the emphasis on calcium in the present study, caution is still required when considering supplementation. Given that calcium is generally high in foods like cheese, and most of the research for benefits of specific dairy foods continually isolates cheese both in epidemiology [1,18] and in interventions [5–7,17,19], for those that do consume dairy this is the most evidence-based, food-based advice to emerge from the study of this food group.

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