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Schmidt KA, Cromer G, Burhans MS, Kuzma JN, Hagman DK, Fernando I, et al. The Impact of Diets Rich in Low-Fat or Full-Fat Dairy on Glucose Tolerance and Its Determinants : A Randomized Clinical Trial. 2020;00:1–14.

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

We know that dairy is a broad food group, and that dairy foods are generally a complex matrix of macronutrients, micronutrients, and additional bioactive food components, for example live cultures. This makes pinning down the effects of any given dairy food to one specific factor a challenge [and also largely irrelevant from a food-based perspective], and the effects are likely a composite of the whole-food matrix. Dairy produce can be differentiated along the following lines:

- Unfermented vs. fermented
- Unrefined vs. refined
- Whole-milk (aka, "full fat") vs. non-fat/low-fat
- Solid vs. liquid

Fermented dairy produce includes fermented milks (e.g., kefir, buttermilk), yogurts, and cheeses. The process of fermentation yields particular nutritional characteristics, including provision of lactic acid bacteria, higher protein content [in the case of certain yogurts], and the formation of bioactive peptides which may exert beneficial effects on blood lipids, pressure, gut immune and microbiota function.

Milk may also be subject to various refinement processes, which alter the nutritional characteristics of the end product. For example, butter is produced by separating cream from whole milk, and churning the cream until the fat separates from the remaining liquid, a process which alters the nutritional composition: compared to cheese, butter is low in calcium, higher in fat, and the process of churning removes the milk fat globule membrane [MFGM], a tri-layered membrane rich in bioactive phospholipids and proteins which encloses the milk fat (1).

Thus, 'dairy' is a broad food group with characteristics that may be important for cardio-metabolic health. For type-2 diabetes, a meta-analysis of seven prospective cohort studies indicated a 14% reduction in risk for T2DM, comparing the highest to lowest levels of total dairy product intake (2). In particular, the highest category of low-fat dairy produce has been shown to result in a 27% lower risk of T2DM (3).

These studies have also been assisted by biomarker studies*. Because the two biomarkers of dairy intake, the odd-chain fatty acids C15:0 pentadecanoic acid and C17:0 hexadecanoic acid, cannot be synthesised in the body, they provide a reliable reflection of dietary intake. In the EPIC-InterAct study, both C15:0 and C17:0 were each associated with a 21% and 33% reduction in risk for T2DM, respectively [4].

These observations have led to interest in the potentially anti-diabetic role of dairy foods. However, to date there is a lack of well-conducted randomised intervention studies examining the effects of dairy on parameters of glucose tolerance.

*Geek Box: Biomarkers of Dietary Intake

"Biomarkers" are an attractive tool for nutrition science, particular epidemiology, as biomarkers may provide useful complementary data to dietary questionnaires used in cohort studies. However, there are a number of important criteria which must be considered to determine whether a particular biomarker is useful or not. The levels of any given nutrient in the blood or in a tissue, for example adipose tissue, may be influenced not only be the actual levels of dietary intake of that nutrient, but also by factors like absorption, distribution, competition with other nutrients for transport, metabolism and excretion. For example, some nutrients may be synthesised in the body - an example of this is saturated fatty acids, which may be synthesised from carbohydrate intake. As a result, biomarkers of saturated fatty acids are generally a poor reflection of dietary intake of saturated fat. However, these saturated fatty acids are even-chain [i.e., 8, 12, 16, 18 carbon fatty acids]. The two biomarkers for dairy intake mentioned above have an odd number of carbons - 15 and 17. And these fatty acids cannot be synthesised in the body, and are only found in dairy foods. Therefore, these biomarkers are a reliable reflection of dietary intake of dairy. Another example is the omega-6 essential fatty acid linoleic acid - as an essential fat, humans require dietary intake, and therefore measuring tissue levels of linoleic acid is reliable reflection of dietary intake. Biomarkers can be classified in two broad categories: recovery biomarkers and concentration biomarkers. Recovery biomarkers have a quantitative relationship between the biomarker and dietary intake in a specific period. For example, sodium is excreted from the body to maintain sodium balance, and therefore urinary sodium [if enough collections are made over the course of a 24hr day] can be used to 'recover' data on sodium intake. A concentration biomarker has a correlation between dietary intake and the levels ['concentrations'] - of the nutrient in a sample, for example plasma, red blood cells, or adipose tissue. Most nutrient biomarkers are concentration biomarkers, such as the examples of the C15:0 and C17:0 dairy fatty acids, and linoleic acid, above.

The Study

The study was conducted as a randomised, parallel arm [each diet group ran concurrently] intervention to compare the effects of 12-weeks of the following diets:

- Full-fat dairy enriched diet [3.3 servings per day milk, yogurt, and cheese]
- Low-fat dairy enriched diet [3.3 servings per day non-fat versions of milk, yogurt, and low-fat cheese]
- Limited dairy diet [<3 servings of non-fat milk per week]

One serving was defined as 240mL of milk, 170g of yogurt, and 42.5g of cheese. The average total amount of dairy fat in the administered dairy foods was 0g/d in the limited dairy diet, 8g/d in the low-fat dairy diet, and 29g/d in the full-fat dairy diet.

All participants completed a 4-week 'wash in' diet where all dairy was limited to <3 servings per week, prior to being randomised to one of the three diet groups above. During the study, there were two 5-day periods - once during the wash-in and again in the first 3-weeks of the intervention - where all study foods were provided to participants to provide a 25% energy surplus. Participants still had to consume their dairy foods: the purpose of these experiments was to see whether dairy foods resulted in a compensatory decrease in energy from the other provided foods.

Participants were between 18-75yrs old and met the criteria for metabolic syndrome. The primary outcome was change in glucose tolerance measured by a 3-hr oral glucose tolerance test. Secondary outcomes included insulin sensitivity, beta-cell function, liver fat content, and inflammation.

Results: 24 participants were randomised to each diet group. Based on repeated 24hr dietary recalls, the major dietary changes included a significant increase in total energy intake by 555kcal in the high-fat dairy group [compared to 224kcal and 81kcal in the low-fat and limited dairy groups, respectively]. Intake of saturated fat increased significantly by 5.2% in the high-fat group, compared to no meaningful change in either the low-fat or limited dairy groups. Intake of calcium [reported as mg per 1,000kcal] increased by 401mg and 277mg in the low-fat and high-fat groups, respectively [no change in the limited dairy group].

Glucose Tolerance:

- ***OGTT:*** There was no significant difference between diets in the glucose response to the OGTT.
- ***Insulin Sensitivity Index:*** Compared to the limited dairy diet, there was a significant decrease in the ISI in the low-fat dairy group and high-fat dairy groups.
- ***Insulin Resistance:*** Compared to the limited dairy diet, there was a significant increase in IR in the low-fat dairy group and high-fat dairy groups.
- ***Fasting Insulin:*** There was a significant increase in fasting insulin in the low-fat dairy group compared to the limited dairy group.

There was no significant difference in other glucose/insulin outcomes, including HbA1c, fasting glucose, or glucose sensitivity. There was also no significant difference in liver fat or inflammatory markers.

Bodyweight increased by 1.0kg in the high-fat dairy group, compared to a 0.3kg increase in the low-fat dairy and a decrease of 0.4kg in the limited dairy group: these findings were statistically significant, albeit clinically insignificant.

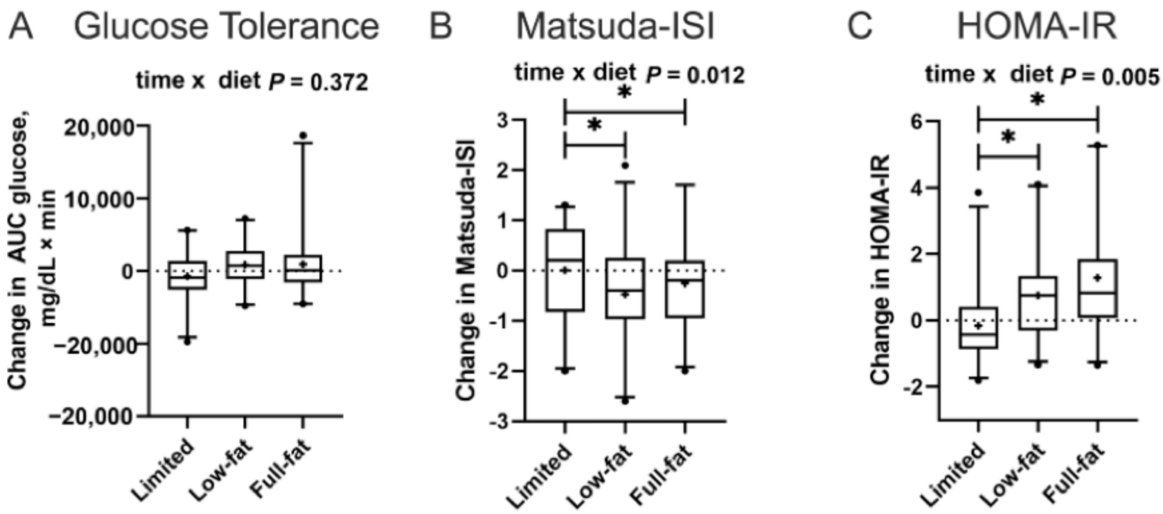
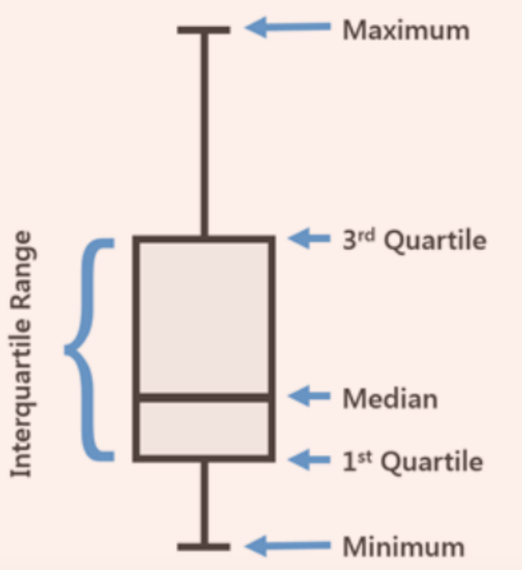


Figure *from paper illustrating the effects of the three dietary interventions on glucose tolerance, insulin sensitivity and insulin resistance, over 12-weeks. The significant effect of 'time x diet' indicates the results of a '2-Way ANOVA'. The '2-Way' indicates that the categorical exposure has two levels, i.e., 'time' [the duration of the intervention] and 'diet' [the three diet groups]. A 2-Way ANOVA tests the effects of these two categories - time and diet - on a numerical outcome ['glucose']. The data is presented as box plots*.*

*Geek Box: Interpreting Box Plots

Box plots may appear confusing, but in fact are easy - and very informative - when you know what you are looking at. Box plots are one of the best ways to display quantitative data, yet bar charts are often used - sometimes inappropriately as bar charts are more appropriate for categorical data, such as frequencies, percentages, or scales. Box plots provide substantially more information. So, let's start with the box itself: this represents the middle 50% of the data - known as the 'interquartile range', i.e., the top of the box is the 75% percentile and the bottom is the 25% percentile. Across the box you can see a horizontal line: this is the median, i.e., the middle value(s) in the data. Inside the box, you can also see a + sign: this is the mean, i.e., the average of all values in the data set. A large box, i.e., a large interquartile range indicates that there is large variability in the values in the data; a small box indicates that most values fall closely within the middle of the data, i.e., are gathered around the median. If the + sign is close to or overlapping with the horizontal median line, this indicates the mean and median are similar, i.e., the data is normally distributed and symmetrical. You'll also notice 'whiskers' extending from the top and bottom of the box, and also a 'dot' which lies beyond those whiskers. There are a number of options when plotting whiskers, but generally they depict the minimum and maximum values. Any dot beyond these whiskers indicates any outlier(s). There is a lot of data presented, which is very helpful. For example, look above at the very far right box plot [HOMA-IR], and look at the full-fat dairy group on the right of the plot: you can see the entire box is above the 0 value, indicating that the entire interquartile range of data showed an increase in HOMA-IR. Conversely in the limited dairy group, despite the maximum values and an outlier having a large increase, most values - and the mean and median - indicate a reduction in HOMA-IR.



The Critical Breakdown

Pros: The randomisation method was detailed and appropriate, including stratification of the groups by gender and level of insulin resistance [assessed at baseline visit]. All dairy foods were provided to participants by the research team, and the the dairy foods were identical in manufacture other than the fat content of the foods. The compliance with consuming study foods was very high ~98% in both dairy groups. Both an intention-to-treat analysis, where data from all participants randomised is included in analysis [even with dropouts, the last data point is brought forward], and a per-protocol analysis, where data is analysed only for those who completed the intervention, were conducted. This is a positive because if there are an uneven number of dropouts between groups, conducted a per-protocol analysis only - as many studies do - may bias the results toward a particular group. The results were similar for both analyses.

Cons: Participants were not asked to consume the exact study foods each day, but to consume all the provided foods before the next collection of study foods. This means that, for example, a participant could forget to have the cheese and milk an a number of consecutive days, and then make up for the missed intake with a day or two of 'dairy loading'. This instruction seems wishy washy, and may have influenced consistency of intake [although the reported average intakes and compliance was high overall]. The data was analysed using repeated measures ANOVA: this test models the association between a <u>numerical outcome</u> [e.g., 'blood glucose' and a <u>categorical exposure</u> [e.g., 'time']. Another type of analysis, known as regression analysis, models the association between a <u>numerical outcome</u> [e.g., 'blood glucose' and a <u>numerical exposure</u> [e.g., 'calories']. Because calorie intake increased by 224kcal and 554kcal in the low-fat and full-fat dairy groups, it would have been useful to conduct a regression analysis to see if this change influenced the results: no such analysis was undertaken.

Key Characteristic

There could be a degree of 'false negative' to the findings, based on the baseline characteristics of the participants in each diet group relative to the methods used to calculate insulin resistance and sensitivity. Insulin sensitivity was measured using the data from the OGTT, to calculate the Matsuda Insulin Sensitivity Index [5]. With this index, a score of <4.3 is indicative of insulin resistance: the baseline Matsuda ISI scores in the limited dairy, low-fat dairy, and high-fat diary diets were 2.4, 2.4, and 1.2, respectively. In effect, all participants were insulin resistant at baseline, and the actual change in scores was minimal. Similarly for insulin resistance, a score of >2.9 indicates significant insulin resistance: baseline scores in the limited dairy, low-fat dairy, and high-fat diary diets were 2.5, 3.3, and 3.0, respectively, and the actual changes were similar. Bear in mind that the primary analysis was glucose tolerance assessed by the OGTT: there was no significant difference between diet groups in this outcome. In sum, a more apt interpretation of this data may be that already insulin resistant and insulin insensitive markers got slightly worse over 12-weeks.

Interesting Finding

The "study within the study", where two separate 5-day controlled feeding periods were undertaken, is an interested aspect of this study. One 5-day period occurred during the limited dairy wash-in phase, and another 5-day period within the the first 3-weeks of the intervention. Participants were asked to consume their allotted dairy foods, and then consume the rest of the diet as desired: any unconsumed foods were weighed back by the research team to calculate total energy intake. The aim was to compare whether the dairy diet groups reduced consumption of the additional foods to compensate for extra energy intake - which was mandatory - from dairy.

This means that in the 5-day period during the intervention, there was an extra 281kcal and 463kcal per day in the low-fat dairy and high-fat dairy groups, respectively. And the result - which was an increase in energy in both dairy groups, with the greatest increase in the high-fat dairy group, was predictable. So was analysis which then found a relationship between extra energy and body weight. This is all a bit bizarre. They make no claims or form any hypothesis about the potential satiating effects of dairy foods. This is like saying "we fed the participants more, then they didn' t eat less, and bodyweight increased". Well, thanks science.

Relevance

The first thing to highlight is the difference between statistical significance and clinical relevance. While there was no significant difference in glucose tolerance measured by OGTT, there were statistically significant differences in the mathematical models used to calculate insulin sensitivity and resistance. These changes were overall minor, and occurred in the context of participants with baseline index scores indicative of high levels of insulin resistance, and low levels of insulin sensitivity.

It is possible that dairy may be protective prospectively, i.e., in otherwise healthy people before the onset of disease. This is what cohort studies generally indicate; and other interventions which have been conducted in healthy adults [6,7], or with specific supplemental dairy interventions like whey protein [8]. Thus, it it possible that the present study - in participants with metabolic syndrome - indicates that dairy intake may not reverse underlying metabolic dysfunction, and may slightly worsen glycaemic control. That is the implication we are left with pending further corroborating research. It is also important to note that reductions in T2DM risk observed in prospective cohort studies from dairy intake may be by other means than glucose/insulin parameters.

The lack of food data makes it difficult to try and draw conclusions from the data. Relevant macronutrient data is presented in the paper, with certain differences between diets but overall factors which may be expected to influence glucose tolerance outcomes - for example fibre - were relatively well matched between diets. This makes the data on actual foods consumed more important to fully contextualise the study. For example, other than the macronutrient intakes all we know of the limited dairy diet is, well, that it limited dairy.

Application to Practice

This is currently an equivocal area of research: data goes both ways, with some randomised trials demonstrating beneficial effects on glycaemic control, while others have not - including the present study. There is no need to toss the yogurt out for fear of diabetes at this point, in the context of a wider health-promoting dietary pattern. With continued interest in this area, at least we can almost hang our hat on further well-conducted interventions being published in the future to help reconcile this data.

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