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Investigating a progression of carb and saturated fat intakes

Effects of step-wise increases in dietary carbohydrate on circulating saturated fatty acids and palmitoleic acid in adults with metabolic syndrome

Introduction

Saturated fat reduction has long been a major target of dietary guidelines, although recent meta-analyses have failed to show an association with heart disease. Current recommendations in the U.S. include limiting saturated fat intake to less than 10% of total energy intake. However, a reduction in fat intake typically leads to an increase in carbohydrate intake. A consequence of overconsumption of carbohydrates is increased de novo lipogenesis (DNL). DNL is a process which involves the synthesis of fatty acids from non-lipid sources, such as carbohydrates or amino acids. Interestingly, even energy-balanced diets, and single-meal consumption of carbohydrates above the normal oxidative capacity of the body have been shown to

increase DNL. The percentage of ingested carbohydrate contributing to DNL is however quite minor in people who aren't insulin resistant and overfeeding on refined carbohydrate.

The major end-product of DNL is the saturated fat palmitic acid (denoted 16:0, referring to 16 carbons and zero double bonds), which can be desaturated within the body to form the monounsaturated fat palmitoleic acid (16:1). Higher blood levels of palmitoleic acid have been associated with an increased risk of metabolic syndrome and greater amount of inflammatory markers. Palmitoleic has mixed evidence however, also being associated with some positive biomarkers such as higher HDL and greater insulin sensitivity. Divergent impacts could be due to the effects of different lifestyle factors and different physiological conditions (such as how much of DNL is from adipose tissue versus from the liver).

This study sought to assess how incremental changes in dietary carbohydrate intake and decreases in saturated fat intake affect plasma saturated fatty acid and palmitoleic acid levels. The study was conducted in adults with metabolic syndrome under hypocaloric conditions.

Saturated fat is commonly targeted for reduction by dietary guidelines. This typically leads to an increase in carbohydrate intake, which at high levels may cause the body to create fats through de novo lipogenesis. This study investigated several levels of saturated fat and carb intake to see how they affected plasma saturated fats and palmitoleic acid.

Who and what was studied?

The study was an 18-week controlled dietary intervention in which the participants were initially fed a low-carbohydrate diet that gradually shifted to a high-carbohydrate diet over six consecutive phases (from lowest carb to highest carb: C1→C2→C3→C4→C5→C6).

Prior to beginning the six feeding interventions, the participants were instructed to follow a low-carbohydrate "run-in" diet for three weeks that mimicked the first low-carbohydrate phase, in order to initiate metabolic adaptations to carbohydrate restriction. Baseline and "run-in" nutrient intakes were determined with the help of three-day food logs.

> "The percentage of ingested carbohydrate contributing to DNL is however quite minor in those who aren't insulin resistant and overfeeding on refined carbohydrate."

All food was provided for the subjects during the 18-week intervention. Participants picked up their meals three to four times per week, and if the subjects could not travel to pick up their food, the researchers arranged for delivery in order to ensure that every subject received their food as planned. Blood testing was done at baseline, after the run-in diet, and after each phase (before transition to the next diet) to determine fatty acid composition and other blood markers.

Over the entire 21-week period (intervention and run-in), the subject's diets were designed to produce a 300 kcal deficit per day. Resting energy expenditure (REE) was estimated at baseline with indirect calorimetry and multiplied by an activity factor to estimate the total daily energy expenditure (TDEE) of the subjects. Protein was held constant at 1.8 grams per kilogram of ideal bodyweight. As carbohydrates were increased every three weeks over the six feeding phases, total fat was decreased to maintain energy intake. Thus, across the entire study, protein and calorie intake was similar. Saturated fat was also maintained, at 40% of total fat intake. In comparison, Americans only derive around 34% of their calories from any kind of fat, with around 13% coming from saturated fat.

Indirect calorimetry

Indirect calorimetry measures the production of carbon dioxide and consumption of oxygen to estimate heat production. This is then entered into an equation to estimate resting energy expenditure. Although not without error, indirect calorimetry remains the gold standard for measuring energy expenditure in laboratory settings.

Only very-low and non-caloric products such as coffee, tea, water, and diet soda were allowed to be consumed by the participants in addition to the provided foods. Beef, eggs, and dairy were the primary protein and fat sources, with higher and lower fat versions used depending on the study phase. Low-glycemic carbohydrates were emphasized throughout.

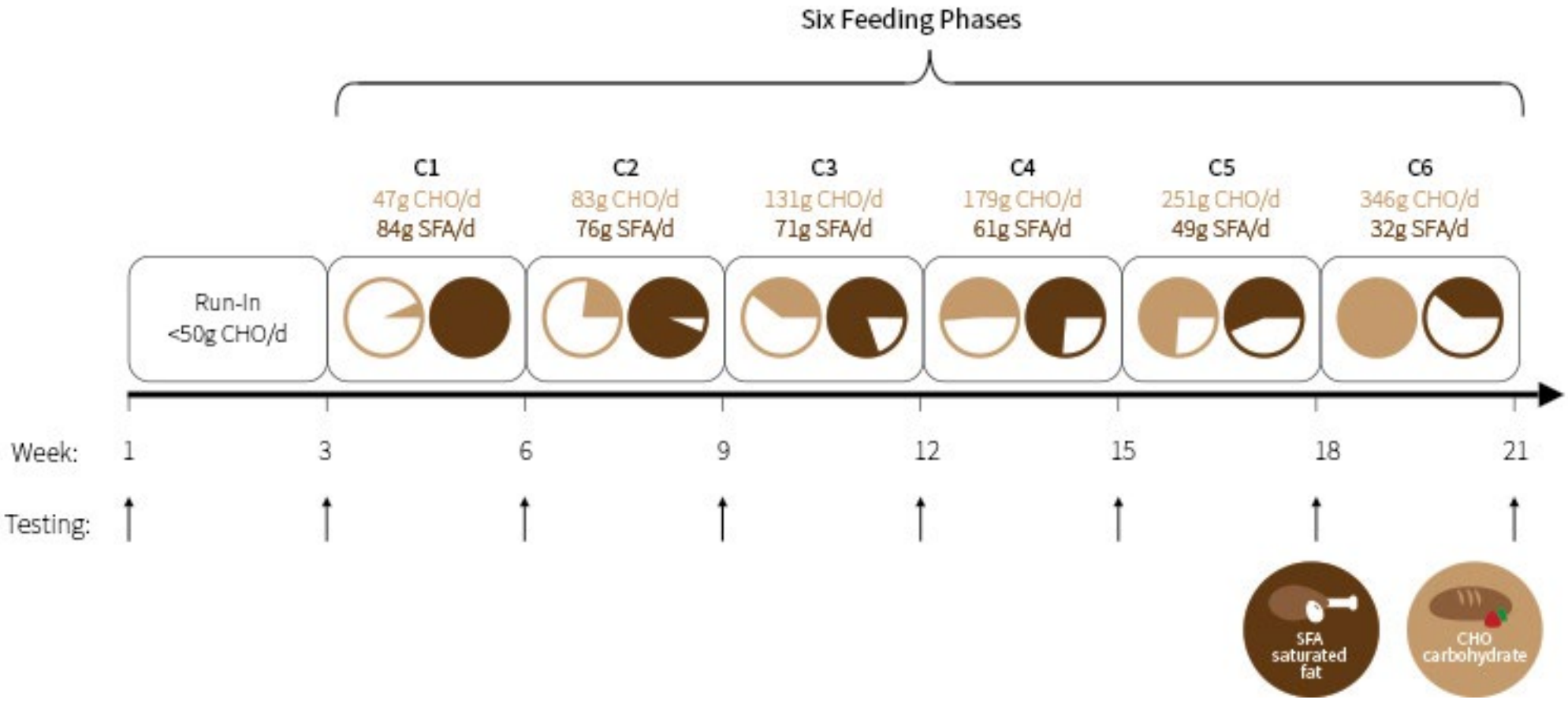
The subjects were 12 overweight and obese men and four women with metabolic syndrome, between 30 and 66 years old (average 44.9) with BMI ranging from 27-50 kg/m2 (average 37.9). Exclusion criteria included having diabetes, liver, kidney, or other metabolic or endocrine dysfunction. Participants who were physically active were asked to maintain their activity levels while sedentary people were asked not to begin an exercise program.

This study investigated the effects of various carbohydrate diets on a group of overweight and obese participants. Study participants initially ate a low-carbohydrate diet that turned into a high-carbohydrate diet over 18 weeks, in six phases.

What were the findings?

Energy intake (EI) across the feeding interventions averaged about 2,500 kcal per day and protein intake averaged about 125g per day (20% EI). As designed, protein and energy intake remained constant over the 18-week intervention. As seen in Figure 1, carbohydrate intake started at an average of 47 grams per day (7% EI) and rose to an average of 346 grams per day (55% EI). Total fat intake started at an average of 209 grams per day (73% EI) and dropped

Figure 1: Carb and saturated fat intake by study period



to an average of 80 grams per day (28% EI). The authors claim that compliance was high, based on verbal communication and inspection of returned food containers. There were no dropouts.

Both body weight and fat mass (measured by DXA) showed a significant decline from baseline to C1 (about seven kilograms and four kilograms, respectively), and continued to decline throughout the entire intervention, ultimately resulting in an average loss of about 10 kilograms of bodyweight and eight kilograms of body fat. Neither weight loss nor fat mass were significantly different between C4 and C6, suggesting that most of the change occurred in the first 12 weeks (run-in, C1, C2, & C3).

Total, LDL, and HDL cholesterol values were not significantly altered across any of the feeding phases. Triglycerides levels dropped about 22% from baseline to C1. These levels stayed constant through C5 and had a significant return to baseline values at C6.

Compared to baseline, fasting glucose & insulin, HOMA-IR (measure of insulin resistance), and systolic and diastolic blood pressure significantly decreased at C1, but were not significantly altered throughout the six feeding phases.

Despite saturated fat intake starting at 84 grams per day and decreasing to 32 grams per day, the proportion of total saturated fatty acids in blood lipids was not significantly affected. Palmitic acid (16:0), the predominant saturated fatty acid within blood lipids, significantly increased from baseline to C1 and significantly decreased from C1 to C2, C3, C4, and C5. C6 was not significantly different from C1.

Stearic acid (18:0, which is commonly found in animal fats and cocoa) was not significantly changed in cholesterol esters. But from baseline to C1, it was significantly reduced in phospholipids and also decreased in triglycerides through the intervention, ending with a significant reduction in C6 relative to C1. Phospholipid concentrations showed an oppo-

site pattern, increasing throughout the intervention and ending with a significant increase in C6 relative to C1.

There was a significant reduction in total monounsaturated fatty acid concentrations from baseline to C1 only. Similar to 18:0, as carbohydrate increased, plasma oleic acid (18:1) decreased in triglycerides, but increased in phospholipids.

Palmitoleic acid (16:1) was significantly reduced from baseline to C1 in triglycerides and cholesterol esters, and trended for an increase in phospholipid concentrations. All these markers showed increasing concentrations with increasing carbohydrate intake and ended the intervention with significantly greater concentrations of palmitoleic acid at C6 relative to C1.

There was great individual variation in palmitoleic acid concentrations during each diet phase with notable outliers. For instance, one subject had triglyceride concentrations of palmitoleic acid rise by nearly three-fold from C1 to C4 (2% to about 5.8%) and further rise from C4 to C6 (about 5.8% to 7%). However, another subject showed no changes across the entire intervention, and another showed reductions as carbohydrate intake increased.

Study participants lost body weight and fat over the 18-week intervention, with most of the changes occurring in the first 12 weeks. The blood samples researchers analyzed suggested that carbohydrate intake can influence blood levels of compounds like palmitoleic, stearic, and palmitic acid.

What does the study really tell us?

There are numerous studies showing associations between higher proportions of palmitoleic acid in blood and tissue, and adverse health outcomes such as metabolic syndrome in adults and adolescents, hypertriglyceridemia, type-2 diabetes, coronary heart disease, and prostate cancer. However, since none of these studies establish causality, it is possible that these conditions lead to high-

Lipoproteins and lipid fractions

This study looked at how much palmitoleic acid was contained in three different locations in blood plasma: triglycerides, phospholipids, and cholesterol esters. Lipoproteins shuttle lipids (such as fatty acids and cholesterol) around the body. Phospholipids form the outer shell of lipoproteins, while cholesterol esters and triglycerides make up the majority of the core.

So the "phospholipid fraction" refers to the fats that are contained in the phospholipids, with the same reasoning for "triglyceride fraction" and "cholesterol ester fraction". Sometimes these different fractions respond the same way to diet, and sometimes they don't. Hence it's important to measure all of them.

er proportions of palmitoleic acid (for example, palmitoleic acid may be the body's attempt at a protective response to what is being eaten) rather than vice-versa. With the mixed associations shown in studies, it is hard to know for sure what the exact health effects of palmitoleic acid are.

It is also difficult to quantify the amount of palmitoleic acid needed to increase the risk of these endpoints, as few studies have done so. In the Physicians Health Study, one standard deviation increase in plasma phospholipid palmitoleic acid concentrations was associated with a significant 17% higher risk of heart failure even after adjustment for BMI, alcohol consumption, smoking, exercise, and plasma omega-3 levels.

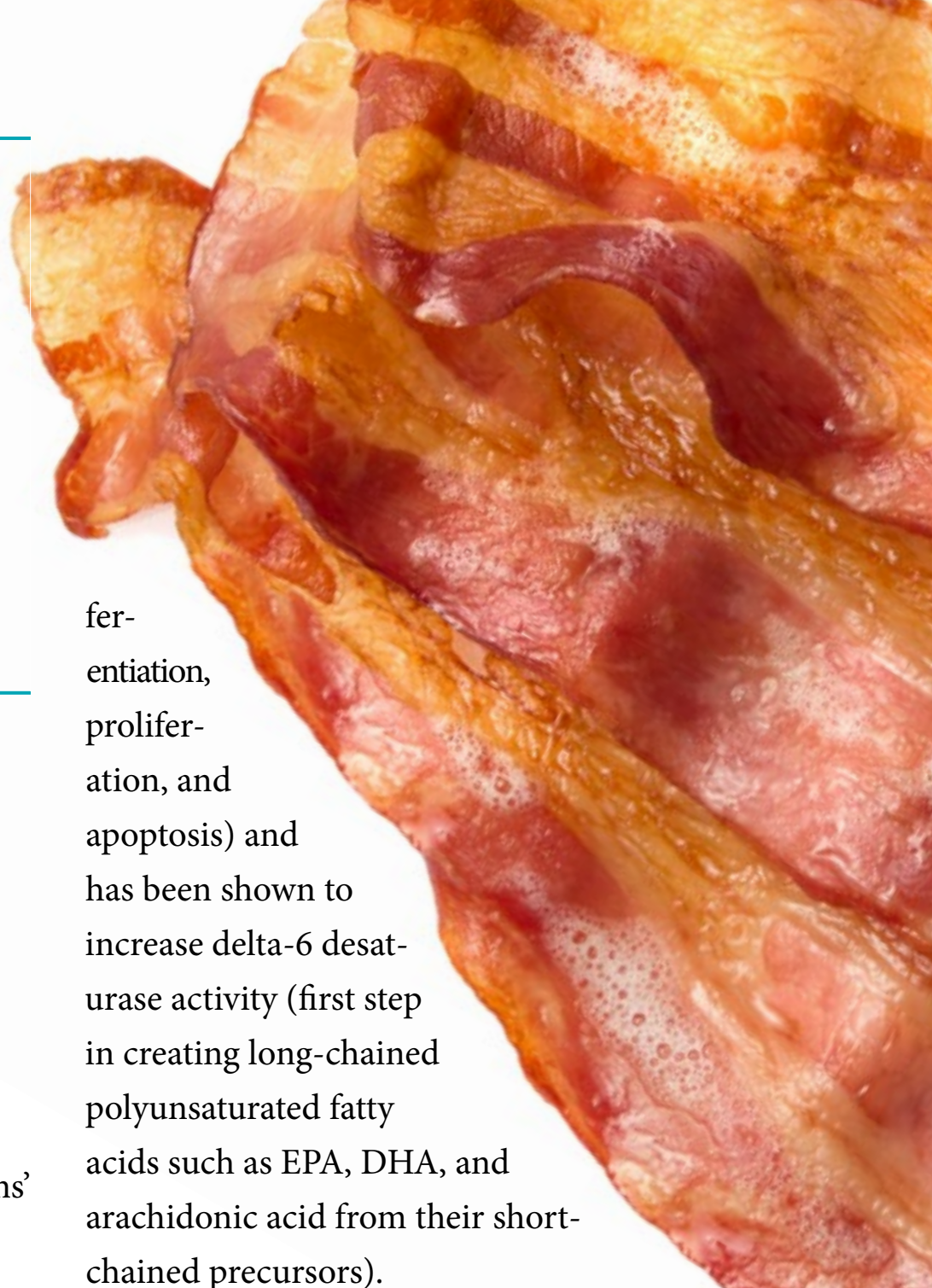
> "With the mixed associations shown in studies, it is hard to know for sure what the exact health effects of palmitoleic acid are."

In the study under review, baseline daily intake of carbohydrate and fat averaged 333 grams and 130 grams, respectively. During the first phase of the intervention, carbohydrate intake dropped to an average of 47 grams, while fat intake rose to an average of 209 grams. It was during this time that the most significant changes in blood lipid fatty acid concentrations occurred, including a major reductions in palmitoleic acid levels. Additionally, this was when significant improvements in insulin sensitivity, blood pressure, and plasma triglyceride levels were observed. However, this was also when the most significant reductions in weight and fat mass were observed, making the causative factor difficult to isolate. And there was no weight loss matched control group to account for weight loss effects. Between the lower palmitoleic acid concentrations, the weight and fat loss, and the reduction in carbohydrate intake, we cannot say which came first and which led to which.

On the other hand, by the end of the intervention, when carbohydrate intake was similar to baseline intake (346 grams vs. 333 grams) plasma palmitoleic acid levels returned to levels similar to those observed at baseline despite significantly lower weight and fat mass, strongly suggesting that it was carbohydrate intake that influenced plasma palmitoleic acid levels.

The authors also repeated the entire experiment backwards in five additional subjects (from high to low carbohydrate intake) and found that plasma palmitoleic acid responded in the exact opposite pattern as the main study group, which supports the idea that carbohydrate intake influences palmitoleic acid concentrations. Even so, the overall diets were hypocaloric, and we cannot conclude how carbohydrate intake would influence palmitoleic acid levels under eucaloric or hypercaloric contexts.

This study provides evidence to suggest carbohydrate intake influences palmitoleic acid levels. Although evidence is mixed, high levels of palmitoleic acid in the blood are associated with metabolic syndrome, type 2 diabetes, coronary heart disease, and other health problems. In this study, participants experienced a drop in palmitoleic acid levels when they were eating low-carb meals in the first phase of the study.



The big picture

With 18 full weeks' worth of food provided for the participants, this study provided a well-controlled environment in which to study the effects of diet on palmitoleic acid. Yet despite the findings from this study, the relative risk from various palmitoleic acid concentrations in the blood remains to be determined. In the previously mentioned Physicians' Health Study, the highest quartile had an average palmitoleic acid level of only 0.50%, whereas in the current study, even when phospholipid palmitoleic acid concentrations were at their lowest during the low carbohydrate phase, absolute concentrations averaged 0.61%, putting these participants above the vast majority of the Physicians Health Study subjects.

Other blood lipid changes add further complexity to the implications of this study. For instance, increasing carbohydrate intake led to greater phospholipid oleic acid concentration, which in contrast to palmitoleic acid, has been shown to attenuate the pro-inflammatory and cytotoxic effects of excessive saturated fatty acid incorporation. Myristic acid, which showed a reduction with carbohydrate restriction, plays a physiologically critical role in de novo ceramide synthesis (necessary for regulating cell differentiation, proliferation, and apoptosis) and has been shown to increase delta-6 desaturase activity (first step in creating long-chained polyunsaturated fatty acids such as EPA, DHA, and arachidonic acid from their short-chained precursors).

The applicability of this study to real-life situations is uncertain. There were only 16 participants, with widely varying BMIs, each using a particular dietary composition for a limited period of time. The effect of carbs on blood lipids was confounded by the weight loss that was designed into the study, without a weight loss control group that would help to isolate the effects of carbs. Also, a variety of different outcomes were measured. So while palmitoleic acid was emphasized in the title and study discussion, other important outcomes had different results.

For example, outside of C1, cholesterol and blood pressure didn't change regardless of diet. The subjects in this study already had metabolic syndrome,

so changes in things like blood pressure and triglycerides may be more important than changes in bound plasma fatty acids, since some of these fatty acids are linked to metabolic syndrome (which they already have) while blood pressure may have a more direct impact on their health. Also, circulating free fatty acids, which are linked to metabolic and heart health, were not assessed.

> “A given topic [...] can be explored in a variety of different ways, and the results can be interpreted by the study authors in different ways as well.”

While the total proportion of plasma saturated fats didn't differ in any of the diet phases, different individual plasma fatty acids can have different effects. Palmitic acid, the predominant saturated fatty acid which was noted in the paper to be a predictor of metabolic syndrome and heart disease, was actually lower in phospholipids (but not the other two lipid fractions) from C2-C5 than it was during the low carb C1 or high-carb C6 periods. This finding was not explained, nor were changes in stearic acid and oleic acid. So while a variety of fatty acids were measured and reported, palmitoleic acid was the only one focused on in the discussion. Unfortunately it was also the only focused on in many news stories with inaccurate headlines such as “Heart disease and diabetes risk linked to carbs, not fat, study finds”.

It must be noted that this study was funded by the Dairy Research Institute, The Beef Checkoff program, the Egg Nutrition Center, and the Robert C. And Veronica Atkins Foundation. The funding sources did not have a say in designing the study or writing the manuscript. However, these organizations are quite clearly interested in the research on saturated fatty acids, thus the variety of studies funded by them. The primary investigators are also noted low-carb researchers. This also doesn't mean the study is biased, but it is one thing to keep in mind when interpreting the study findings. A given topic (here, the effect of carbohydrate intake on plasma saturated fatty acids), can be explored in a variety of different ways, and the results can be interpreted by the study authors in different ways as well. It's important to look at the broader context of literature and the nitty-gritty study details rather than just take the author's word for it.

Other plasma fatty acids, such as palmitic, myristic, and oleic acid, may be important for evaluating the health effects of different carbohydrate and fat intakes. Although measured, these were not a focus of the study. Nor were more direct predictors of heart and metabolic health, such as blood pressure. The study was funded by dairy, beef, and low-carbohydrate organizations.

Frequently Asked Questions

What else influences plasma palmitoleic acid levels?

The current study lends support to the idea that palmitoleic acid concentration in the plasma is more reliant on carbohydrate intake than fat intake. However, the study was conducted under hypocaloric conditions, and previous research has suggested that dietary intake of palmitoleic acid (which is rich in a few select foods such as macadamia nuts) does significantly influence plasma concentrations during weight maintenance. Alcohol has also been suggested to reduce palmitoleic acid concentrations, with one study reporting significantly lower levels in people consuming more than 100mL of ethanol consumption per week (seven regular 12-ounce beers) compared to people consuming less. This study also found palmitoleic acid concentrations to be independent of smoking status.

How do various biomarkers of fatty acids in the body differ?

Biomarkers of fatty acid composition differ from dietary intake, in that biomarkers reflect both the intake and the utilization of the fatty acids. Because not everyone is similar in how we absorb, transport, and metabolize nutrients, biomarkers allow us to look beyond simple dietary intake and focus on the physiological consequences of consuming certain substances. Moreover, biomarkers can provide a long-term picture of dietary intake.

Due to the essential nature of fatty acids in cell structure, assessment can involve numerous body tissues in addition to blood and urine (e.g. hair, nails, skin, breath, saliva, feces). However, measuring blood plasma is the most common method. Serum triglycerides reflect dietary intakes over the past hours to days, whereas cholesterol esters and phospholipids reflect daily intakes. Only body fat stores (adipose tissue) tend to reflect long-term dietary fat consumption (e.g. years), and even this measure can be inaccurate in people who have experienced cycles of fat loss and gain.

How strongly is palmitoleic acid associated with heart disease, when compared to other biomarkers?

Although statistically significant, the strength of the relationships

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between palmitoleic acid and health parameters is low to moderate. For instance, in one study of over 3200 Chinese adults, palmitoleic acid concentrations could only explain about 37% of the variance in triglyceride levels and 14% of the variance in HDL-cholesterol levels.

It should also be kept in mind that fatty acid levels in any biomarker represent a proportion and not an absolute measure. Thus, greater integration of certain fatty acids into the biomarker can reduce the percentage of other fatty acids without their absolute amount changing. All of the aforementioned studies demonstrating associations between fatty acids and health outcomes were based on percentages, making it difficult to draw conclusions as these are not quantifiable values. One person could have double the amount of palmitoleic acid in serum as another person and still have similar percentages if they also have double the amount of blood lipid.

There is also evidence of seasonal variations in fatty acid profiles. One early study showed greater proportions of saturated fatty acids in the adipose tissue of the legs and arms during summer compared to winter. This difference was attributable to a reduction of palmitoleic and oleic acid levels, with a simultaneous increase in palmitic, myristic, and stearic acid levels. Although these changes were in adipose tissue and not serum biomarkers, it raises the question of whether the current study could have been influenced by seasonal changes as its six month duration, by necessity, spanned more than one season. Since subtle changes in plasma fatty acid levels were tracked over increments of time, it would be difficult to differentiate what changes were at least partly a result of the season.

What dietary sources have a lot of palmitoleic acid in them?

According to the USDA nutrient database, roasted chicken skin from the leg and thigh contains the greatest amount of palmitoleic acid with 2.8 grams per 100 grams of food. Beef fat follows with about 1.9 grams, then turkey skin with 1.34-1.5 grams, and finally butter at 0.96 grams. Poultry skins contain the most palmitoleic acid on average, followed by beef fat and butter. Macadamia oil is a rich source, containing 19% palmitoleic acid.

Keep in mind that palmitoleic acid is different than trans-palmitoleic acid. The latter comes from very limited sources, mostly red meat and dairy from grass-fed cows, and is not synthesized by the body.

Trans-
palmitoleic
acid in plasma lipids and adi-
pose tissue has been repeatedly associated with better metabolic outcomes, as shown in this paper by ERD reviewer Stephan Guyenet, Ph.D.

Are there benefits to palmitoleic acid from diet? In plasma? Elsewhere?

A very recent study published in December of 2014 found that feeding mice 300 milligrams of pure palmitoleic acid per kilogram of bodyweight daily, in addition to their normal diets for ten days significantly increased glucose uptake in fat tissue through increased expression of glucose-uptake transporter 4 (GLUT4; necessary for insulin-stimulated glucose uptake into tissues). This was despite no changes in plasma fatty acid levels.

Earlier studies have also found palmitoleic acid to enhance glucose uptake and insulin sensitivity of skeletal muscle, and reduce liver fat buildup. The authors of this study suggest that palmitoleic acid may act as a major signaling lipid produced from fat tissue for communication with distant organs. In obese sheep, infusion of palmitoleic acid twice daily for 28 days preserved insulin sensitivity before beginning an obesogenic diet, possibly through a reduction of intramuscular fat.

It appears that the benefits of palmitoleic acid revolve
around insu-
lin-mediated glucose disposal into
both muscle and fat tissue. This raises an interesting contradiction, with the studies demonstrating associations between palmitoleic fatty acid levels in the blood and some adverse health outcomes such as diabetes. Like certain cholesterol markers, palmitoleic acid may be more of an indicator that something might be physiologically wrong rather than a cause. DNL is one possible cause of increased palmitoleic acid levels, and very high levels may be a marker that something is increasing DNL to dangerous amounts (such as prolonged overeating of carbohydrate, or worsening glucose tolerance from uncontrolled diabetes, both of which can disrupt carbohydrate metabolism). Suggesting that palmitoleic acid is 100% detrimental does not seem accurate given the complexity of evidence on the topic.

What should I know?

This study suggests that the presence of certain fatty acids in blood lipids appears to depend more on carbohydrate than fat intake under hypocaloric conditions in overweight and obese people with metabolic syndrome. There were minor – but uniform – changes in a few select fatty acids, such as

myristic acid, oleic acid, and palmitoleic acid, but no significant changes in total saturated and monounsaturated fatty acid concentrations.

There was also inter-individual variance in the palmitoleic concentration response to carbohydrate intake, which is important given the small sample size. While most subjects showed a positive association, others stayed relatively unchanged and some showed an inverse association. Moreover, there was greater variance as carbohydrate intake increased. The absolute palmitoleic concentration varied between about 2-4% in plasma triglycerides when carbohydrate intake was lowest during C1, but varied between about 2-7% during the high-carbohydrate C6 phase.

Still, the implications of changes in plasma palmitoleic acid levels have yet to be determined. Many studies demonstrate associations between adverse health outcomes and increased palmitoleic acid levels, but reverse causality cannot be ruled out, nor differing impacts of palmitoleic acid in different contexts. We also do not know what influence many other dietary, lifestyle, and environmental factors have.

Rather than having obvious health implications for differing carb levels, this study serves as additional evidence for those eating low-carb higher saturated fat diets (and losing weight) who are apprehensive about impacts on their plasma fatty acids. As is the case with cholesterol, what you eat does not translate directly to what is floating around in your blood. However, the lack of correlation between dietary saturated fat and plasma saturated fat was already shown by a previous paper from the same research group (albeit only the triglyceride fraction was studied).

It's also important to know what this study does not show: it doesn't show that DNL happens at major or dangerous rates when eating moderate carb levels, it doesn't show that increasing levels of carb intake increased overall plasma saturated fat, and it doesn't prove that low-carb diets are superior to moderate carb diets for heart or metabolic health. While weight loss decreased as carbs were added, that may very well be due to increased water weight or changes in compliance.

The authors conclude that the increased proportions of palmitoleic acid concentrations may signal impaired carbohydrate metabolism, yet in vitro and animal studies have suggested that palmitoleic acid is insulin-sensitizing. It seems prudent not to draw health-based conclusions from this study. Rather, the conclusion appears to be that consumption of carbohydrates can have an impact on plasma fatty acid proportions in overweight and obese individuals under hypocaloric conditions. Whatever health implications this may lead to will require further testing to illuminate. ◆

The health implications of this study are unclear. The lack of impact of dietary saturated fat on plasma saturated fatty acids was already shown in previous studies. This study did show an effect of carbohydrate on palmitoleic acid levels, but the relative importance of that is unknown.