ERD

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Contributors

Researchers

Margaret Wertheim
M.S., RD

Alex Leaf
M.S(c)

Courtney Silverthorn
Ph.D.

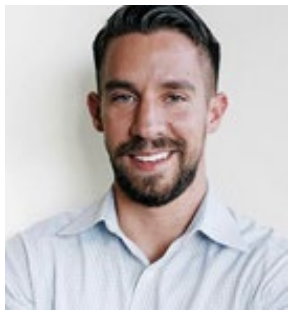
Zach Bohannan
M.S.

Anders Nedergaard
Ph.D.

Jeff Rothschild
M.Sc., RD

Editors

Gregory Lopez
Pharm.D.



Pablo Sanchez Soria
Ph.D.

Kamal Patel
M.B.A., M.P.H.,
Ph.D(c)

Reviewers

Arya Sharma
Ph.D., M.D.

Natalie Muth
M.D., M.P.H., RD

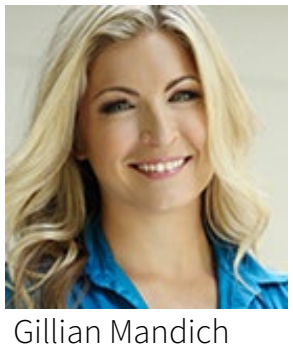
Stephan Guyenet
Ph.D.

Sarah Ballantyne
Ph.D.

Katherine Rizzone
M.D.

Spencer Nadolsky
D.O.

Mark Kern
Ph.D., RD



Gillian Mandich
Ph.D(c)

Adel Moussa
Ph.D(c)

From the Editor

Have you ever been depressed?

If so (or even if not), you probably know of the somewhat simplistic "Low serotonin theory of depression" and how it's increasingly fallen out of favor. And you may also know that trials of SSRIs have been criticized for publication bias. So in a nutshell, while some people are definitely helped by SSRIs (and even pulled away from the brink of very dark situations), both the science and evidence is weaker than was thought a couple decades ago.

Nutrition theories have similar issues. The brain is so complex that not even the most well-trained cognition researcher knows what the hell is really happening. But the rest of the body is also complex. The difference is that few people pretend to know exactly how the brain works, while everyone and their mom (and brother, and grandma) has an opinion on nutrition issues.

Not that these opinions hold no water. People do know how they acutely feel when eating different foods. Tummy upset, energy levels, and whatnot. People don't, however, know how different organs and tissues are impacted by the foods they eat, and it's quite rare to do strict personal experiments on foods and nutrients and then get some kind of testing done.

So in the absence of obvious health effects from specific foods and nutrients, coupled with too many studies for any one researcher to read, there have been many competing nutrition hypotheses throughout the years. And many of these hypotheses have fallen. Dietary cholesterol and heart disease, the evils of even moderately high salt intake, and so on.

But that's how science goes. It's not the changing of hypotheses that's holding up progress (as many lay people would think), rather it's the behind the scenes action. If a big study showed something, were there other ones that showed the opposite but didn't get much attention? Who funded which study, and might that have impacted the study design?

And now back to depression.

The truth is that (don't you hate it when people say "the truth is"? as if they know some truth that you had never considered) these theories are made by humans to explain complex phenomena that are deeply, deeply interrelated. Babies aren't usually born depressed, or at least I suspect not. They shoot out, get cooed over, and eventually get fed breast milk or formula. The path from there becomes way less dichotomous. Stress, relationships, medications, germs, and so on -- they all go in discrete buckets to us humans, but that doesn't mean germs in your gut can't be part of the reason you're stressed or depressed.

That's why interdisciplinary research is so crazy important. If you're a nutrition geek and only read about bodybuilding nutrition, you're not just missing out on some really fascinating stuff, you might be hurting your health. The brain may be complex, the gut might only be in nascent stages of research, and the gut-brain axis even more so. But that's part of the fun. If you like research, the era of internet-enabled health exploration is quite an exciting time to live in. Drink deep, and drink well.

Kamal Patel, *Editor-in-Chief*

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Not-so-safe supplements

Studies have shown that supplement buyers generally trust the supplements they buy. That might not be the safest assumption, as dietary supplements that are presumed helpful or neutral may sometimes cause serious side effects, as quantified by this study.

Sugar Wars, Episode 2: "Fructose Strikes Back"

Few food components have been demonized as much as fructose in the past decade. With fructose being presumed guilty in metabolic syndrome and heart disease, this systematic review sheds light on it’s actual impact on blood lipids.

Tea time means only tea for optimal EGCG absorption

Many people drink green tea for health, and some take green tea or EGCG supplements in an attempt to shed extra fat. While these topics have been researched at length, there hasn’t been as much research on timing. This study looks at EGCG absorption with and without food.

The study that didn't end the low-fat/low-carb "wars"

A recent metabolic ward study set the low-carb world on fire, and produced many inaccurate media headlines disparaging low-carb diets. We cover the study and its implications, detail by detail.

Gluten-intolerant? There’s a pill for that

Some people are lactose intolerant, but still drink milk thanks to the availability of lactase enzymes. That setup isn't yet possible for those who don't handle gluten well. This study examines the efficacy of a promising enzymatic adjunct to a gluten-free diet.

All up in your krill

The story on krill oil thus far has been fairly simplistic: it’s better than fish oil and more expensive. But there’s a reason why you can't draw conclusions based off few studies, and successful results in one condition don’t apply to other conditions. This trial gives some of the first pieces of evidence for possible negative metabolic effects of krill oil.

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Beet out your competition with dietary nitrate!

Beets have shown promise for solo exercise, but what about for longer activity of typical team sports? This study examines the effects of one week of dietary nitrate supplementation on exercise performance and cognitive function during a repeated sprint test protocol designed to reflect work and recovery patterns that typically occur during team sport play.

Got milk (fat globule membrane)?

Butter and milk don't have the same impact on heart disease, and their fat structures may help explain why. This study investigate whether the effects of milk fat on plasma lipids and cardiometabolic risk markers are modulated by the milk fat globule membrane content.

Vitamin (K)cardiovascular health?

Results are in from the first long-term trial of vitamin K2 for cardiovascular health. This double-blind randomised clinical trial looks at the effect of of Menaquinone-7 supplementation on arterial stiffness in healthy postmenopausal women.

I get by with a little help from my friends: probiotics and depression

Mix a few beneficial probiotic strains, take daily, lower your chances of depression? The objective of this Dutch study was to determine the effects of a probiotic supplement on cognitive reactivity to sad mood, as well as symptoms of depression and anxiety in non-depressed, healthy adults.

Does BCAA+Arginine prevent fatigue during exercise?

Nervous system fatigue can limit exercise duration, and this supplement combo might help. The purpose of this study is to investigate the effect of combined supplementation of branched-chain amino acids (BCAA) and arginine on intermittent sprint performance in simulated handball games on 2 consecutive days.

Not-so-safe supplements

Emergency Department Visits for Adverse Events Related to Dietary Supplements

Introduction

Dietary supplements are sometimes erroneously perceived as inherently healthy. And because of the way many supplements are advertised, it's easy to overlook that improper administration can lead to adverse outcomes.

The classification of a supplement is defined in the United States Dietary Supplement Health and Education Act of 1994 (DSHEA) as a vitamin, mineral, herb or botanical, amino acid, and any concentrate, metabolite, constituent, or extract of these substances. In the U.S., the Food and Drug Administration (FDA) is the governing body that oversees the regulation of dietary supplements. If a supplement has been reported to be causing serious adverse events or reactions, the FDA has the authority to pull it from the market. However, no safety testing or FDA approval is required before a company can market their supplement. The lack of oversight authority given to the FDA has even drawn the attention of late night talk shows hosts like John Oliver, who humorously covered the issue in this YouTube video.

Many adults are using one or more supplements to address illnesses or symptoms, and to maintain or improve health. Half of all U.S. adults have reported using at least one supplement in the past 30 days. Twelve percent of college students have reported taking five or more supplements a week. Now, more than ever, there are seemingly endless options to choose from. The number of supplement products currently available on the market is thought to be in excess of 55,000. Compare that to the mere 4,000 available in 1994, when DSHEA was passed.

Furthermore, confidence in the safety and efficacy of these supplements is very high despite the lack of rigorous oversight by the FDA. A survey conducted by the trade association, Council for Responsible Nutrition, found that "85% of American adults … are confident in the safety, quality and effectiveness of dietary supplements." An independent survey has echoed these results, finding that 67.2% of respondents felt extremely or somewhat confident in supplement efficacy and 70.8% felt extremely or somewhat confident about their safety.

While the majority of Americans trust in their supplements, more than one-third have not told their physician about using them. There are numerous documented drug-supplement interactions ranging from the mild to the severe. The herb St. John's Wort is thought to be able to reduce symptoms in people with mild to moderate depression. But this 'natural' supplement also has 200 documented major drug interactions, including some with common depression medication. However, no good data currently exists to document how common adverse events related to dietary supplements may be. The authors of the present study have used surveillance data to try and fill this knowledge gap.

Due to DSHEA, supplements remain largely unregulated by the FDA. But dietary supplements are becoming ever more popular, as about half of U.S. adults report using one or more in the past 30 days. Trust in the safety and efficacy of these supplements also remains high. The authors of this study aimed to investigate how many annual adverse events are caused by improper supplement usage.

Who and what was studied?

The researchers looked at 10 years of data (2004-2013) to estimate the adverse events associated with dietary supplements in the United States from 63 different hospitals. The selection of these hospitals was meant to be nationally representative and included locations that had 24-hour emergency departments. Trained patient record abstractors reviewed the reports from each hospital to identify cases where supplements had been implicated as the likely source of the adverse event. These abstractors have been trained to analyze and compile medical information contained in patient records.

Cases were scanned for emergency room visits where the treating clinician had explicitly ascribed dietary supplements as the root cause of the medical issue. This included herbal or complementary nutritional products such as botanicals, microbial additives, and amino acids, in addition to micronutrients like vitamins and minerals. Products that may typically be classified as food were excluded, like energy drinks and herbal tea beverages. Topical herbal items and homeopathic products were included in the analysis even though they do not fall under the regulatory definition of dietary supplements.

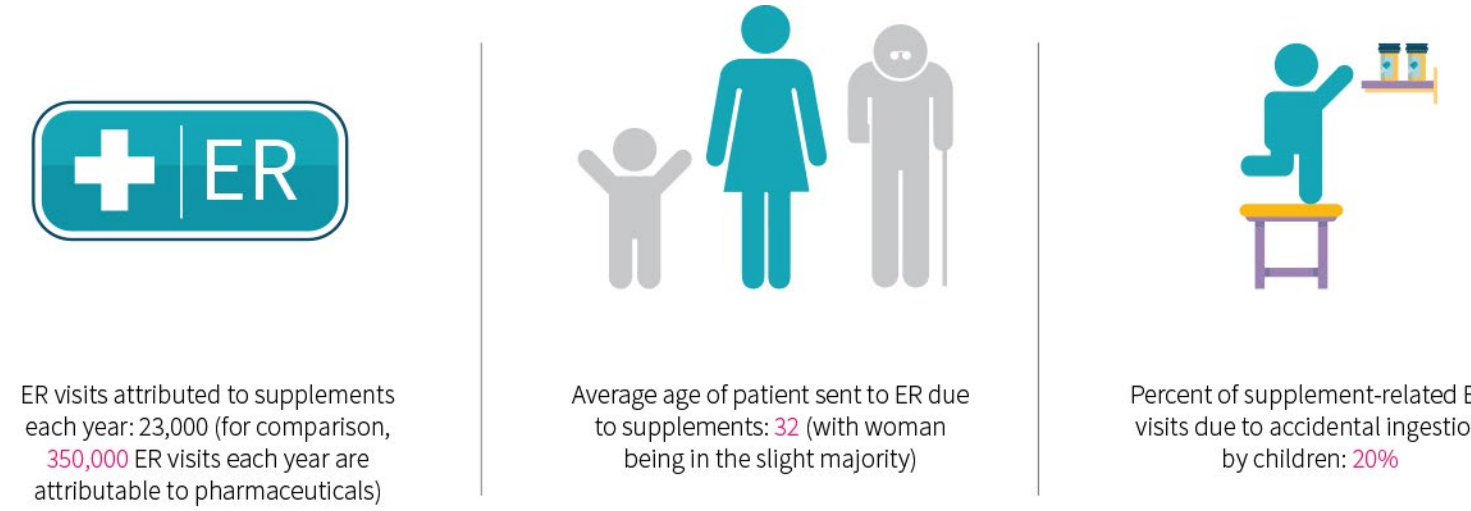
Adverse events were classified as anything causing adverse or allergic reactions, excess doses, unsupervised ingestion by children, or other events like choking. Due to the non-standard death registration practices among different hospitals, cases involving a mortality were not included, as were any cases involving intentional self-harm, drug abuse, therapeutic failures, nonadherence, and withdrawal.

Researchers examined patient records from 2004 to 2013 from 63 different hospitals. Cases where the treating clinician had identified a supplement as the cause of the medical emergency were extracted from the dataset. However, deaths associated with or caused by supplements were not included, as hospitals differ in their practice of registering mortalities.

What were the findings?

Some of the major findings are summarized in Figure 1. Over 3,600 cases were identified within the predetermined 10-year period. The researchers extrapolated from these data that the U.S. experienced an average

Figure 1: Supplement safety by the numbers

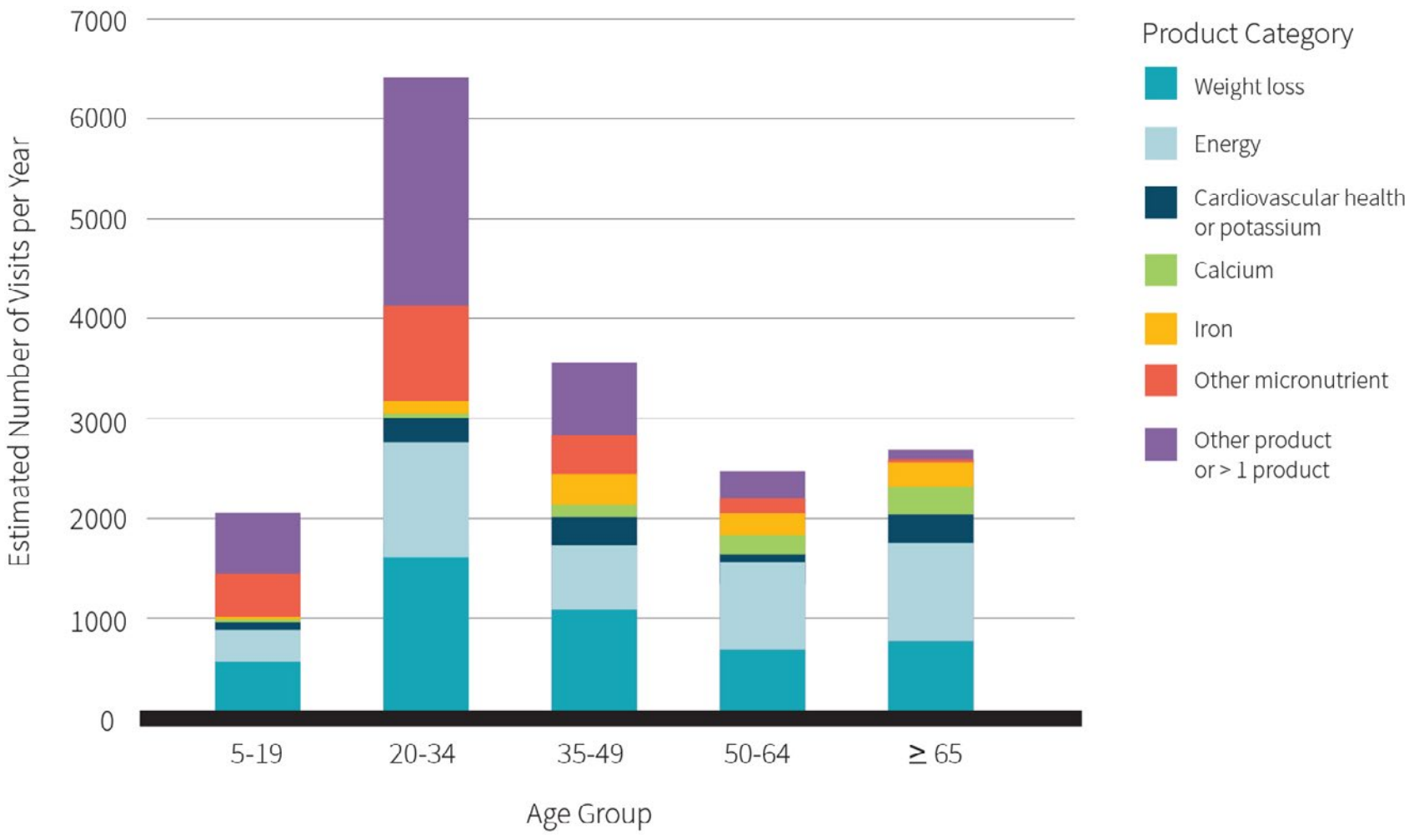


of 23,000 supplement-related emergency department visits per year, with estimates ranging from 18,600 to 27,400. Of these 23,000 emergency room visits, it was calculated that about 2,150 (9.4%) of these result in hospitalization. About 88% of these ER visits were attributed to a single supplement, as opposed to interactions or mixtures of multiple supplements. The average age of patients treated for supplement-related adverse events was 32 years, and the majority of these cases were female.

Figure 2 shows age and supplement category related results. About a quarter of ER visits involved people between the ages of 20 to 34, but people older than 65 years old were more likely to have a visit that resulted in hospitalization. Of patients above 65 admitted to the ER, 16% had to be hospitalized. Surprisingly, one-fifth of supplement-related ER visits were due to accidental ingestion by children. When the data covering unsupervised ingestion of dietary supplements by children was not included, the researchers found that the majority of ER visits (65.9%) were due to herbal or complementary nutritional products. The top five products in this category included the following: weight loss (25.5%), energy (10.0%), sexual enhancement (3.4%), cardiovascular health (3.1%), and sleep, sedation, or anxiolysis (i.e. anti-anxiety) (2.9%). Multivitamins or unspecified vitamin products were the biggest contributors to ER visits under the micronutrient product category.

ER visits also varied according to gender and age. Weight loss and micronutrient supplements disproportionately landed females in the ER, while sexual enhancement and bodybuilding products largely affected males. Among patients younger than four years old and adults over 65, micronutrients were the number one cause of emergency department visits. This is in contrast to the other age groups, where herbal and complementary nutritional products were the biggest contributor. In people ages five to 34, weight loss products or energy products were implicated in more than 50% of ER visits. Weight loss products mostly affected

Figure 2: Summary of which types of supplements lead to ER visits by age



Source: Geller AI et al. N Engl J Med. 2015 Oct.

patients from 20 to 34 years of age, while the micronutrients iron, calcium, and potassium mostly affected those older than 65.

About 23,000 people go to the ER for supplement-related visits every year. The biggest contributors to this are herbal or complementary nutritional products like weight loss and energy supplements, which largely affect people between the ages of five to 34. Females are more likely than males to end up in the ER due to adverse supplement reactions. Those over the age of 65 are most at risk for an ER visit due to micronutrient supplements such as iron, calcium, and potassium.

What does the study really tell us?

While 23,000 annual supplement-related emergency visits may sound high, this is less than 5% of pharmaceutical product-related ER visits. However, these ER admittance rates do not line up with the marketing that has promoted dietary supplements as fundamentally healthy. That is, the general public overwhelmingly perceives these products to be safe and effective, but the present data does not support this notion (ERD readers excluded. We think you are all ahead of the curve on this one).

However, it should also be noted that overall incidences of supplement-related ER visits have remained constant over time. No significant changes were detected between 2004 and 2013 when accounting for population increases. The only increase that occurred was ER visits associated with micronutrient supplements, which jumped 42.5%, from 3,212 to 4,578 cases in this same time frame.

Unlike their highly regulated pharmaceutical counterparts, there are no legal requirements for dietary supplements to identify any potential adverse effects or major drug interactions on their packaging. The lack of adequate warning labels may be a contributing factor to why histories of dietary supplement usage are rarely obtained by clinicians. This can be due to a combination of clinicians not asking proper patient screening questions and to a lack of disclosure by the patient.

Proprietary Blends

The FDA has established labeling standards dictating what must appear on a supplement's packaging. Manufacturers must list out each ingredient, and are required to display the amount or percentage of daily value of those ingredients.

A proprietary blend falls under a slightly different set of regulations. Blends are a unique mixture of ingredients that are typically developed by the manufacturer. The FDA requires that all ingredients of a proprietary blend be listed on the label in descending order according to predominance of weight. While the amount of the blend as a whole must be listed, the amount of each ingredient included in the blend does not.

Blends are used to help prevent the competition from knowing what the specific formulation is. But it can also hide the fact that very little of an active ingredient may be in the bottle. So while a proven performance enhancing ingredient like creatine may be listed in a proprietary blend, it could be well below what is considered to be an effective dose.

Given that there is a tendency to underreport supplement usage, the researchers have noted that their calculations of emergency department visits attributed to supplement-related adverse events are probably an underestimation. A further limitation was the relatively small sample of hospitals used. But this method of data collection is likely to yield more accurate results over voluntary reporting despite the fact that voluntary reporting would have likely allowed for a larger sample population.

While 23,000 annual supplement-related emergency visits may not be a large contributor to ER visits in the larger scheme of things, it does provide a counter-narrative to the marketing that often portrays supplements as always health promoting. Supplements are not required to come with labels warning of adverse events or potential drug interactions, which can be a contributing factor to supplement-related ER visits.

The big picture

The supplement industry is the wild west of nutrition. By and large, DSHEA has hampered the ability of the FDA to adequately regulate supplements. If you have ever taken a supplement that makes a health claim, you may have encountered this statement on the label: "These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease." While all ingredients must be declared on the label, there is little oversight to ensure that these ingredients are present in the supplement, at the doses that are advertised on the packaging. Under DSHEA, there is no requirement for companies to provide any data to the FDA showing that their supplement is safe and effective, unless they are introducing a new or novel ingredient. It falls on the FDA to show that a supplement is unsafe before any action can be taken.

In light of this lack of regulatory oversight, if you are currently taking or thinking about adding a supplement to your diet, be sure to notify your doctor. Supplements can interact with prescription medication or could exacerbate certain medical conditions. Warfarin (Coumadin) is a good example. It is a blood-thinning medication that can be prescribed to people at risk of forming blood clots. To ensure that the medication works properly, these patients are usually placed on a low vitamin K diet, as vitamin K plays an essential role in forming blood clots. If these patients do not disclose that they are taking a multivitamin with vitamin K, multivitamins being one of the most commonly used supplements, they could be putting themselves at risk for developing unwanted clots.

Currently, the supplement industry is partially policed by itself. Companies that market and sell supplement products do not have to show the FDA data of safety or efficacy in the same fashion that pharmaceutical companies do. The FDA can step in when a supplement has been shown to cause harm and pull it from the market. It is important to discuss all supplements you may be taking with your doctor to avoid unpleasant or dangerous interactions. Be sure to tell them even if they do not ask during your screening.

Frequently asked questions

Is there any way to ensure that I'm purchasing a quality supplement??

There are companies out there that do supply third-party certifications to supplement manufacturers. These companies will verify that the supplements listed on the ingredient list are present in the concentrations claimed. There are four major companies that provide these certifications, which are shown in Figure 3: NSF International, Informed Choice, Consumer Lab, and U.S. Pharmacopeia. With the exception of Consumer

Lab, all of these third-party certifiers print their seal on the products they have screened.

The testing process often involves looking at the purity, strength, and bioavailability of the product. Good manufacturing practices, which help to provide systems that track proper design, monitoring, and control of the manufacturing process and facilities, are also frequently taken into account. Many employ continuous random testing in order for a given supplement to remain certified. It is very important to note that these companies do not test for efficacy. That is to say, these certifications do not ensure that any health claims made about the supplement are truthful.

What should I know?

While 23,000 dietary-supplement related ER visits may not seem like a lot when compared to something like the 610,000 deaths caused by heart disease every year in the U.S., it is something that can be easily prevented with education and awareness. Although supplement related deaths were not included in the ER visit projection, which could lead to an underestimation, it is also possible that emergency department physicians may have incorrectly ascribed certain signs and symptoms to supplements, which could consequently lead to overestimation. Essentially, the 23,000 annual ER visits should be viewed as a very rough estimation.

If you are currently taking or planning to introduce a supplement to your diet, be sure that you are consuming the recommended dose for that product and consult your doctor before hand. Supplements are not automatically beneficial for health, no matter what the marketing says. Treat dietary supplements the way you would treat medication, with caution and respect for their ability to both help and harm your health. ◆

Figure 3: Third-party supplement certifications

Sugar Wars, Episode 2: “Fructose Strikes Back”

[*Effect of Fructose on Established Lipid Targets: A Systematic Review and Meta-Analysis of Controlled Feeding Trials*]

Introduction

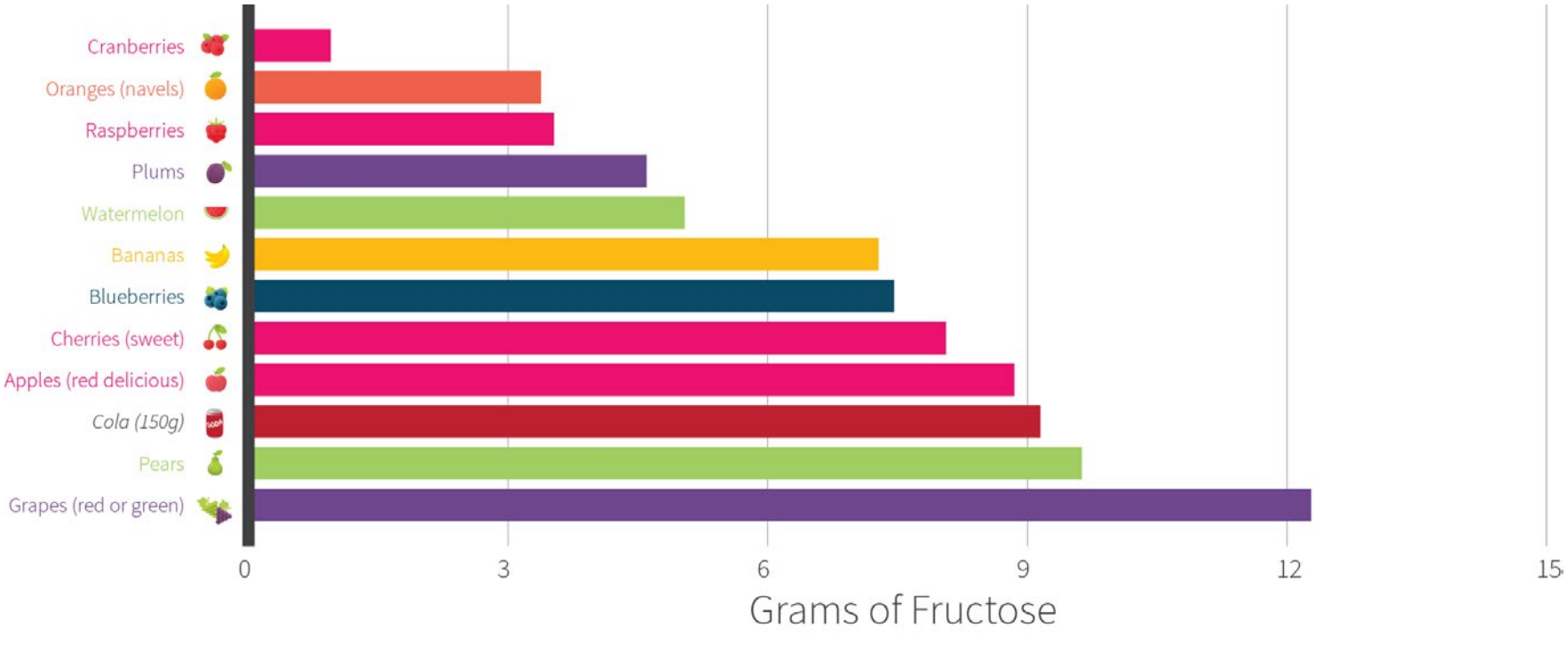
Low-carb diets, saturated fat, and fructose: these constitute the holy trinity of hotly-debated topics in both the scientific literature and popular media. Luckily for us, when these disputes arise we often see an uptick in research as scientists try to fill in any knowledge gaps. In fact, 23 (39%) of the 59 trials included in the current meta-analysis on fructose were published within the past 15 years.

The deliberation over fructose has centered around what its metabolic effects may be, like its impact on diabetes risk or its role in the obesity epidemic. Dr. Robert Lustig has been a leading vocal proponent of the fructose hypothesis, which contends that fructose plays a dominant role in the high rates of obesity, metabolic syndrome, type 2 diabetes, cardiovascular heart disease, non-alcoholic fatty liver disease, cancer, and poor lipid profiles. Dr. Lustig has also proposed fructose as a main mechanism in his "unifying hypothesis of metabolic syndrome" and has drawn parallels between the negative health outcomes of chronic alcohol and fructose consumption. His hypothesis has resonated with many. Dr. Lustig's popular YouTube talk, Sugar: The Bitter Truth, has been viewed nearly six million times.

The concern over fructose has been echoed in public health guidelines provided by the American Heart Association (AHA) and the Canadian Diabetes Association (CDA). The AHA has recommended limiting added sugars to 100 calories a day for women (about 34 grams) and 150 for men (about 51 grams), which is about 5% of daily calories. Their consensus statement also concluded that there were data indicating fructose intakes greater than 50-100 grams per day may elevate triglyceride levels. For reference (and as depicted in Figure 1), 50 grams of fructose would equate to about two 12 ounce cans of cola, 3.5 *large* red delicious apples, or seven cups of blueberries. The CDA has called for added sugars to make up no more than 10% of daily calories (50 grams on a 2,000 calorie diet) and that added fructose consumption above 60 grams a day may moderately increase triglycerides in people with type 2 diabetes. The CDA is careful to note that consuming naturally occurring fructose from fruit has not shown evidence of harm.

The fructose hypothesis has been contested by many scientists, including some of the authors of the current paper. In the present review, Dr. John Sievenpiper and his team examine the effects of fructose on lipid targets,

Figure 1: Fructose content of 1 cup (~150 g) of fruits (and cola as a reference)



Source: USDA National Nutrient Database for Standard Reference Release 27

such as HDL, LDL, and triglycerides, for cardiovascular disease and metabolic syndrome. Two previous reviews on the effect of fructose on lipid profiles have been conducted. A 2008 review by Livesey and Taylor indicated a ≥100 grams per day threshold, above which triglyceride levels were adversely affected. However, the review contained data from trials of both healthy and unhealthy participants, which may confound some of the findings. A second 2009 review conducted by Sievenpiper et al. identified that a fructose intake greater than 60 grams per day in people with diabetes caused triglyceride levels to rise. Since then, 13 additional controlled fructose feeding trials have been conducted. The current review updates and expands on Sievenpiper's previous paper.

Dr. Robert Lustig has proposed that fructose plays a primary role in causing obesity, type 2 diabetes, poor lipid profiles, and cardiovascular heart disease. The American Heart Association and the Canadian Diabetes Association have responded to these worries by proposing upper daily intakes of fructose. Many scientists disagree with the fructose hypothesis, including some of the authors of this review. This study aims to examine the effect that fructose may have on lipid profiles. Fifty-nine controlled feeding trials were examined for this analysis.

Who and what was studied?

A systematic review and meta-analysis is a different type of study than the kind you usually read about in ERD. In these papers, no new studies have been conducted. Instead, the literature has been thoroughly examined for all papers pertaining to a particular topic. In this case, the researchers were looking for two types of controlled feeding trials: trials where calories were kept constant (isocaloric) but included a portion of dietary carbohydrates swapped out for fructose, and trials where calories from fructose were added to the diet (hypercaloric). These hypercaloric trials were not specifically overfeeding trials, but rather studies where a fructose supplement was added to a participant's standard diet to create caloric excess.

The results from all these trials are then standardized so comparisons can be made between studies. Looking at all available data makes it easier to recognize trends and identify where the weight of the evidence may lie. The purpose of this meta-analysis was to determine the effects of fructose on five lipid levels in people who were healthy or had diseases. These lipid targets included:

- Low-density lipoprotein (LDL) – A lipoprotein is a molecule that carries cholesterol through your bloodstream. Lipoproteins like LDL promote the formation of plaques in the arteries. LDLs carry cholesterol particles from the liver to the rest of the body.
- Apolipoprotein B (Apo B) – The primary structural protein of lipoprotein particles such as LDL that have been implicated in the progression of heart disease.
- Non-high-density lipoprotein (Non-HDL-C) – Non-HDL-C is your HDL cholesterol number subtracted from your total cholesterol. It can be used as a marker for heart disease risk.
- High-density lipoprotein (HDL) – Carries cholesterol from the body to the liver. High levels of HDL are associated with lower cardiovascular disease risk.
- Triglycerides – A type of fat found in the blood. High levels are associated with cardiovascular disease risk.

The research team identified 59 controlled trials that used a crossover or parallel study design. In a crossover trial, all participants receive both treatments at different periods and act as their own control group. These 59 trials included 51 isocaloric trials, eight hypercaloric

Randomized trial quality

In this review, the authors evaluated the quality of included trials with the Heyland Methodological Quality Score (MQS). These evaluations are commonly used to help root out the most rigorously conducted studies. There are many different scales used to assess studies (GRADE, PEDro) but they all attempt to do the same thing: evaluate sources of bias that can be introduced through the study's design, execution, and analysis. The score each individual paper receives can help researchers determine what the quality threshold will be for inclusion into a meta-analysis.

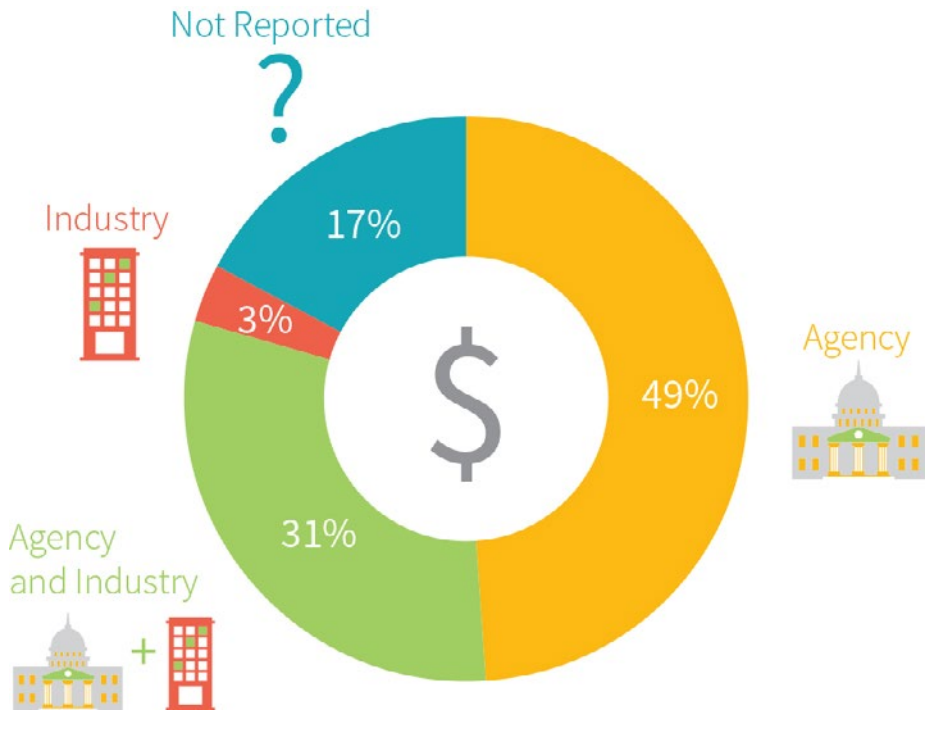
With MQS, studies are judged in nine criteria. Only studies that receive an eight or higher on the 13-point scale are considered to be high in methodological quality. By excluding lower quality trials, like those that do not blind their participants, researchers can avoid overestimating the benefits of an intervention, which tends to occur in poorly-controlled trials.

trials, and had a combined total of 1,068 participants. Trials were excluded if they had a follow-up of fewer than seven days, gave fructose intravenously, did not have a control diet, or reported end-points unsuitable for the analysis. All included trials were reviewed by four researchers to assess methodological quality using the Methodological Quality Score (MQS) system. Trials with a score of eight or higher are considered to be of high methodological quality. The average MQS score of included trials was 6.78.

When conducting their analysis, the researchers separated participants by health status. For each of the five lipid targets assessed, a subgroup analysis was performed for the following groups: participants with diabetes, those with insulin resistance or hypertriglyceridemia, and healthy individuals. By conducting subgroup analyses, the authors were able to eliminate some of the confounding variables present in the 2008 fructose review by Livesey and Taylor. However, due to the limited number of hypercaloric trials, subgroup analysis was not performed for those studies. A combined overall result was also given for each lipid target.

An interesting aside is that the authors reported the funding source of all included trials (summarized in Figure 2), something not typically seen in a meta-analysis. Of the 59 trials, 29 were funded by an agency (government, university, or non-profit), 18 were funded by both agency and industry, two were funded by industry, and 10 did not report their funding source. The mixed agency/industry-funded studies had the highest average MQS score (7.6), followed by agency (7.4), not reported (6.6), and industry (5.5).

Figure 2: Where the funding came from for the studies in this meta-analysis



This paper updates a previous meta-analysis by including 13 additional controlled feeding trials. The authors sought to determine the effects of fructose on lipids in both healthy and unhealthy individuals. Fifty-nine trials including 1,068 participants were assessed. Healthy and unhealthy individuals from isocaloric feeding trials were assessed separately to avoid confounding variables.

What were the findings?

The main findings are summarized in Figure 3. Among the isocaloric trial comparisons, in which part of the dietary carbohydrates were swapped for fructose, no significant changes on any lipid target were seen. In the hypercaloric trials, where fructose was added to the diet to create a caloric excess, an increase in apo B and triglycerides was observed. Fructose could possess the unique ability to modestly raise apo B and triglycerides when eaten in a hypercaloric state, but there is a caveat to this possibility. If fructose has an effect independent of total calories, it should have been observed in the isocaloric comparisons. Because no effect of fructose was observed in the isocaloric trials, it seems likely that most of the increase in apo B and triglycerides seen in the hypercaloric studies was due to the excess calories, as opposed to the fructose.

In the 2008 and 2009 meta-analyses discussed earlier, the researchers had found that fructose was able to increase fasting triglycerides at a dose of 60 grams a day in people with diabetes and 100 grams a day in people with mixed health statuses. The meta-analysis under review was not able to replicate those earlier findings. This is an important discovery, as the AHA and CDA practice guidelines cited one or both of the older meta-analyses as evidence used to help set their daily sugar intake recommendations.

One unexplained finding was that there seemed to be an inconsistent effect on some lipids depending on what form the fructose was delivered in: solid, liquid, or mixed. This is not the first time the form of fructose has led to a curious finding. In a systematic review and meta-analysis of the effects of fructose on body weight in controlled feeding studies, fructose delivered in solid and fluid form had a weight-decreasing effect that differed statistically compared to fructose delivered

Figure 3: Fructose's effects on blood lipids

	ISOCALORIC STUDIES			HYPERCALORIC STUDIES
	People with insulin resistance, diabetes, or high triglycerides	Healthy population	Total population	Total population
HDL-C	—	—	—	—
LDL-C	—	—	—	—
Non-HDL-C	—	—	—	—
Apo B	—	—	—	↑
Triglycerides	—	—	—	↑

in mixed form, which had weight-increasing effects. Furthermore, when looking into prospective cohort studies that examine the relationship between fructose and diabetes, liquids like sugar-sweetened beverages and fruit drinks are correlated with increased risk of diabetes (except not 100% fruit juice, another curiosity) while solid foods like cakes, cookies and fruit are not correlated with increased risk. The researchers have noted that these findings are likely due to the trials being underpowered, differences seen in study populations, and possibly due the observational nature of some studies.

No effect on lipid targets were seen in the isocaloric trials, but apo B and triglycerides were elevated in hypercaloric trials. The dose-response curve for fructose intake on triglycerides established in previous research was not able to be replicated in this analysis. The new data presented may alter future clinical practice guidelines published by health organizations like the AHA and CDA. It is likely that the apo B and triglyceride increases seen in the hypercaloric studies were due to excess calories and not necessarily because of the fructose itself.

What does the study really tell us?

It is important to note the limitations of the evidence when attempting to extrapolate these results to larger populations. The long-term effects of fructose consumption may not yet be fully understood, especially as the average follow-up period for all trials was three weeks. Fructose dosing was also very high in these studies, as the median dose was 96.8 grams per day, way beyond the 95th percentile of American standard intake.

We discussed the issue of non-real world doses of fructose in trials in the ERD #9 Volume 2 article, "Fructose: the sweet truth". Ultra-high fructose intakes in clinical trials may have limited applicability to real-world relevance. But even these high fructose doses were unable to elicit a negative effect in the isocaloric trials, giving credence to the hypothesis that it is the excess calories and not the fructose itself that may be most detrimental. The overall evidence quality was also modest, as 51% of the trials had an MQS of less than eight.

Based on the data analyzed, there does seem to be moderate evidence-quality controlled feeding trials suggesting that when other carbohydrates are replaced by fructose on an energy balanced diet, blood lipids are not likely to change in a way that promotes cardiovascular disease. This same moderate evidence base has indicated that when fructose is consumed to the point of creating a positive caloric balance it may adversely affect some lipids. However, these effects may be due to the excess calories themselves, rather than the fructose.

The short duration of trials, moderate methodological quality, high fructose dose typically administered, and dissimilarities of study populations compared leaves some questions about the effects of fructose. These questions can be answered by future trials that are larger in sample size, longer in duration, of higher methodological quality, and use appropriate "real world" fructose doses. Such trials could greatly increase our understanding of the metabolic effects of fructose and guide our public health policy.

The evidence assessed in this study was of moderate methodological quality. Limitations included short trial duration, unrealistically high fructose dose, and the comparison of dissimilar study participants. Based on the data analyzed, there is moderate evidence that isocaloric fructose consumption does not harm lipid targets while overconsumption may. Negative effects of excess fructose could be due to the extra calories themselves and not the fructose.

The big picture

There has been a concerted effort in the past few years to try and elucidate the role fructose plays in our health. The study we just examined is one part of the puzzle in an ever-expanding line of literature. There are six main areas where scientists have produced systematic reviews and meta-analyses examining the effects of fructose on health markers, many of which have been conducted by Dr. Sievenpiper. They are:

- Blood pressure
- Glycemic control
- Lipids
- Body weight
- Uricemia (Gout)
- Non-Alcoholic Fatty Liver Disease (NAFLD)

To give you a picture of where the weight of the evidence on fructose currently stands, the findings from the most recent reviews are briefly summarized below.

Blood pressure

Two reviews of prospective cohort studies and one of controlled feeding trials have been conducted for blood pressure. The first cohort review looked at the association between fructose-containing sugar-sweetened beverages (SSBs) and the risk of hypertension. The researchers found that one or more SSB per day was associated with a 12% higher risk. The second cohort review looked at total fructose intake but found no association between fructose intake and hypertension risk. Cohort studies are not without their limitations, though they are useful in finding correlations. Luckily, the last review was of controlled feeding trials. When fructose replaced a portion of carbohydrates in an isocaloric diet, significant improvements were seen in diastolic pressure (when the heart relaxes to refill with blood) and mean arterial pressure (average blood pressure) but not systolic pressure (when the heart contracts). The hypercaloric trials saw no overall effect on mean arterial pressure.

Glycemic control

Glycemic control is very important for people with diabetes for maintaining long-term health. Historically, fructose has been suggested to play a role in helping people with diabetes control their blood sugar due to its low glycemic index. A 2012 review of controlled feeding trials examined the effect of fructose on glycemic control in individuals with diabetes. The researchers found that when fructose replaced other carbohydrates under energy balanced diets, participants saw approximately a 0.53% reduction in HbA1c, a measure of average glucose levels over two to three months. It may not seem like much, but a 0.53% reduction in HbA1c is considered clinically significant by the US Food and Drug Administration. Fasting glucose and insulin were not affected.

Lipids

Apart from the study under review, there is one additional review that specifically looked at the effect fructose had on post-meal triglycerides. Isocaloric exchange of carbohydrates for fructose resulted in no significant triglyceride increases for otherwise healthy individuals and participants with diabetes, but researchers did see increases in participants with obesity. When fructose was supplemented hypercalorically,

> “The difference in weight loss could have been partially due to malabsorption of fructose.”

triglycerides did increase. This effect was also observed in the study under review. However, the excessively high dose (about 175 grams per day) could be a confounding variable.

Body weight

Perhaps the most debated area of the fructose hypothesis is its role in weight gain. Two reviews, one by Te Morenga et al. and the other by Sievenpiper et al., found that diets providing similar calories but different fructose intakes did not appear to affect weight gain. Surprisingly, the Sievenpiper review found that a subgroup of participants who were overweight or obese saw significant weight loss on the higher fructose diets. However, this finding became insignificant after a sensitivity analysis. The difference in weight loss could have been partially due to malabsorption of fructose. Participants may not have been fully absorbed the calories from fructose, excreting them instead. It is also possible that fructose may have a higher thermic effect over other carbohydrates like glucose, leading to a slight but insignificant weight loss advantage. Within the hypercaloric fructose arm, there was significant weight gain when given high daily fructose doses (104 to 250 grams a day). In essence, fructose doesn't seem to have any special weight-increasing effects beyond the calories it contains.

Uricemia

When uric acid accumulates in the blood, it can lead to gout, a painful inflammation of the joints. Among the isocaloric trials of fructose reviewed, no effect was seen in uric acid levels. The hypercaloric fructose intake did significantly raise uric acid though. The clinical and practical applications of this remain unclear, as the fructose doses were very high (213 to 219 grams a day).

NAFLD

Non-alcoholic fatty liver disease is a condition characterized by a buildup of excess fat in the liver, affecting 10 to 20% of Americans. NAFLD can progress to cirrhosis, causing permanent liver damage. Both reviews in this area came to similar conclusions: isocaloric exchange of fructose did not induce NAFLD in healthy participants. Fructose overfeeding did negatively affect some markers of liver health, but that was confounded by excessive energy intake, and the overall level of evidence was not robust.

These summaries may help shed some light on the state of the fructose hypothesis. The common theme seen among all these analyses was that negative health effects were not observed until fructose was administered in caloric excess. The overall quality of evidence was consistently rated as poor or moderate. Common limitations included small sample sizes and trials of short duration. Nearly all the authors called for longer and larger trials.

The past five years have produced a flurry of systematic reviews and meta-analyses as scientists try to understand the health implications of fructose consumption. The current evidence indicates that the negative health effects of fructose may be due to the excess calories they can provide in a diet, rather than to the fructose. The call for for better, longer trials was a uniform message across all papers.

Frequently asked questions

Some of the authors have taken money from the food industry. Isn't that a conflict of interest that could bias their interpretation of the data?

At the bottom of the reviewed meta-analysis is a robust conflict of interest disclosure statement. Coming in at over 1,300 words, many of the authors list just about every source of funding they have ever received. Some of them have worked for or received money from large players in the food industry, including Coca-Cola. Dr. Sievenpiper even disclosed that his wife is an employee of Unilever Canada. Lengthy disclosures like this one

are not standard. Typically, the conflict of interest section is used to reveal any financial ties or relationships that may be potential sources of bias of the authors. Dr. Sievenpiper has stated that his super-disclosures were influenced by his mentors, Dr. Vladimir V.V. Vuksan and Dr. David J.A. Jenkins, who highly encouraged full transparency.

You may be worried about the influence of industry on the findings of this paper. It is true that papers published by researchers with ties to industry deserve more scrutiny, but there are some important items to note in this paper's case. The review was not funded by industry, but rather by grants from the Canadian Institutes of Health and the Calorie Control Council. None of the sponsors of this trial had a role in its design or conduct. Additionally, all 16 authors had "access to the study data and reviewed and approved the final manuscript." Lastly, the trial was pre-registered at ClinicalTrials.gov. By registering the study methodology and primary outcome measures before conducting a trial, the researchers have fewer degrees of freedom to change endpoints as the study progressed. Simply put, trial registration makes it easy to see if any drastic changes have been made between the time of registration and publication that could raise any red flags.

What should I know?

Fructose in the diet does not appear to be an issue for lipid targets as long as it is not consumed to the point where a caloric surplus is created. The available evidence suggests that you may see an increase in your apo B and triglyceride levels when you over consume fructose, although this may not be a unique trait to fructose and could be caused by the excess calories themselves. However, naturally occurring fructose from fruit consumption has currently shown no evidence of harm. Increased fruit (and vegetable) intake has long been associated with improved health. Fruit is also packed with fiber, flavonols, anthocyanins, micronutrients, and antioxidants that the vast majority of SSBs lack.

Because the evidence quality is modest, setting strict upper limits on fructose intake may be difficult, based on the current evidence. However, limiting liquid sources of calories from SSBs and fruit juices can be an easy method for reducing overall calorie intake. ◆

> "[...] many of the authors list just about every source of funding they have ever received. Some of them have worked for or received money from large players in the food industry, including Coca-Cola."



Tea time means only tea for optimal EGCG absorption

Food Inhibits the Oral Bioavailability of the Major Green Tea Antioxidant Epigallocatechin Gallate in Humans





Introduction

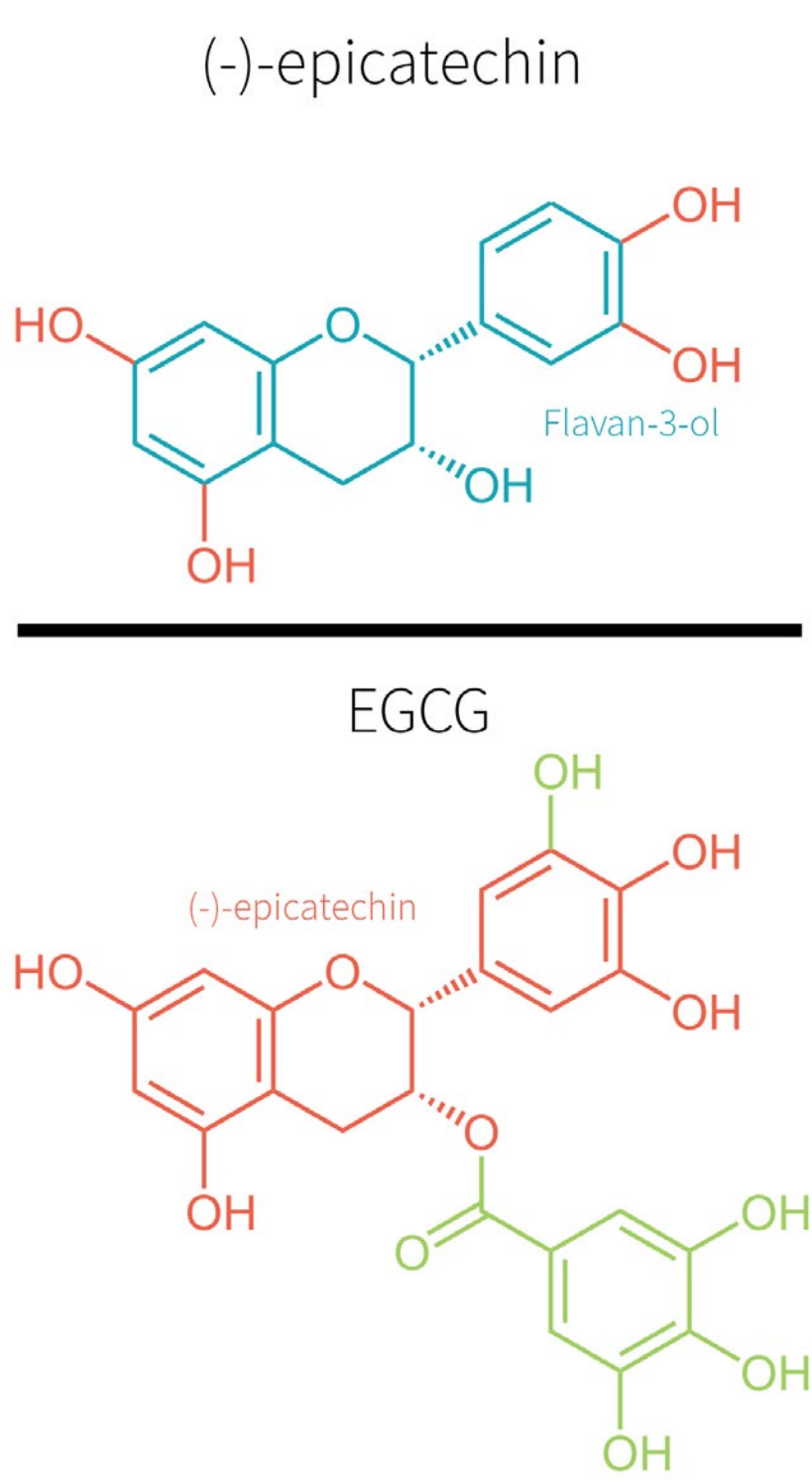
Epigallocatechin gallate (EGCG) or epigallocatechin-3-gallate (structure shown in Figure 1) is a catechin best known for being in various teas. Specifically, it's one of the most abundant flavonoids present in the leaves from Camellia sinensis. The US Department of Agriculture reports that EGCG is found at concentrations of 42.45 mg/100 g of leaves in white tea and at 70.20 mg/100 g in green tea.

EGCG, green tea and green tea extracts have been claimed to have antidiabetic, anticarcinogenic, weight loss and anti-inflammatory properties. However, when studied closer, the evidence becomes flimsy for some of these claims for a couple of reasons. One reason is that the biochemical components in green tea are different from those in extracts, which is again different from pure EGCG. In particular, green tea also contains three other catechins whose effects may be overlapping, as well as caffeine and theanine that each in their own right carries some biological activity. Thus, assigning biological activity to EGCG from studies using green tea or extracts is difficult. Furthermore, while EGCG and green tea extracts have shown promising results in several types of *in vitro* experiments, they have sometimes translated poorly into human *in vivo* studies. As of now, the FDA does not condone health claims associated with EGCG or green tea.

Some of the discrepancies reported can possibly be attributed to varying bioavailability with different types of administration, especially as oral bioavailability appears to be quite poor in rodents, which does not appear to be the case for humans. Thus, while EGCG has been associated with many types of effects, the evidence is far from solid.

The purpose of the present study was to examine how well EGCG is absorbed in healthy humans when ingested alone, with a breakfast, or in a strawberry sorbet, and thus essentially to find out whether EGCG has better bioavailability when consumed with water and on empty stomach or when ingested with food.

Figure 1: Flavans, catechins, and EGCG



Green tea and one of its active components, EGCG, have a lot of health claims associated with them, some more well-supported than others. However, one confounding factor in studying EGCG's effects is its absorption from the gut and whether it depends on food consumption or not. The purpose of this study was to examine this question in healthy humans.

Who and what was studied?

In this study, four subjects (three men and one woman) ingested EGCG under three different conditions on three different occasions. They were from 18 to 64 years old, healthy, and either of normal weight or just a little overweight.

Why so few subjects? It was a mechanistic study, with subjects essentially acting as their own controls by visiting the clinic three times and ingesting 500 mg of EGCG on three different occasions in three different ways in the morning after an overnight fast. The participants either took two 250 mg EGCG capsules with water on an empty stomach, the same capsules along with a serving of 50 grams of Kellogg's Special K breakfast cereal in 200 mL of full cream milk, or 200 grams of a specially-prepared strawberry sorbet with 500 mg EGCG infused into it.

This last condition was to test whether or not embedding EGCG in a low acid food could enhance absorption. However, the researchers who made the sorbet thought that much of the EGCG would be destroyed in making the sorbet. So they also measured levels after making it, in order to ensure proper dosing. That's how they came up with the 200 gram number.

On each visit, blood was drawn before the EGCG ingestion. After 30 min, 1 h, 2 h, 3 h, 5 h and 8 h, EGCG levels were measured to see how well EGCG was absorbed in each of the three conditions.

Four healthy study participants ingested 500 mg EGCG on three occasions and in three ways: either just with water, with milk and cereal, or in a strawberry sorbet. Their EGCG blood levels were measured for up to 8 hours after ingestion in order to compare absorption under the three conditions.

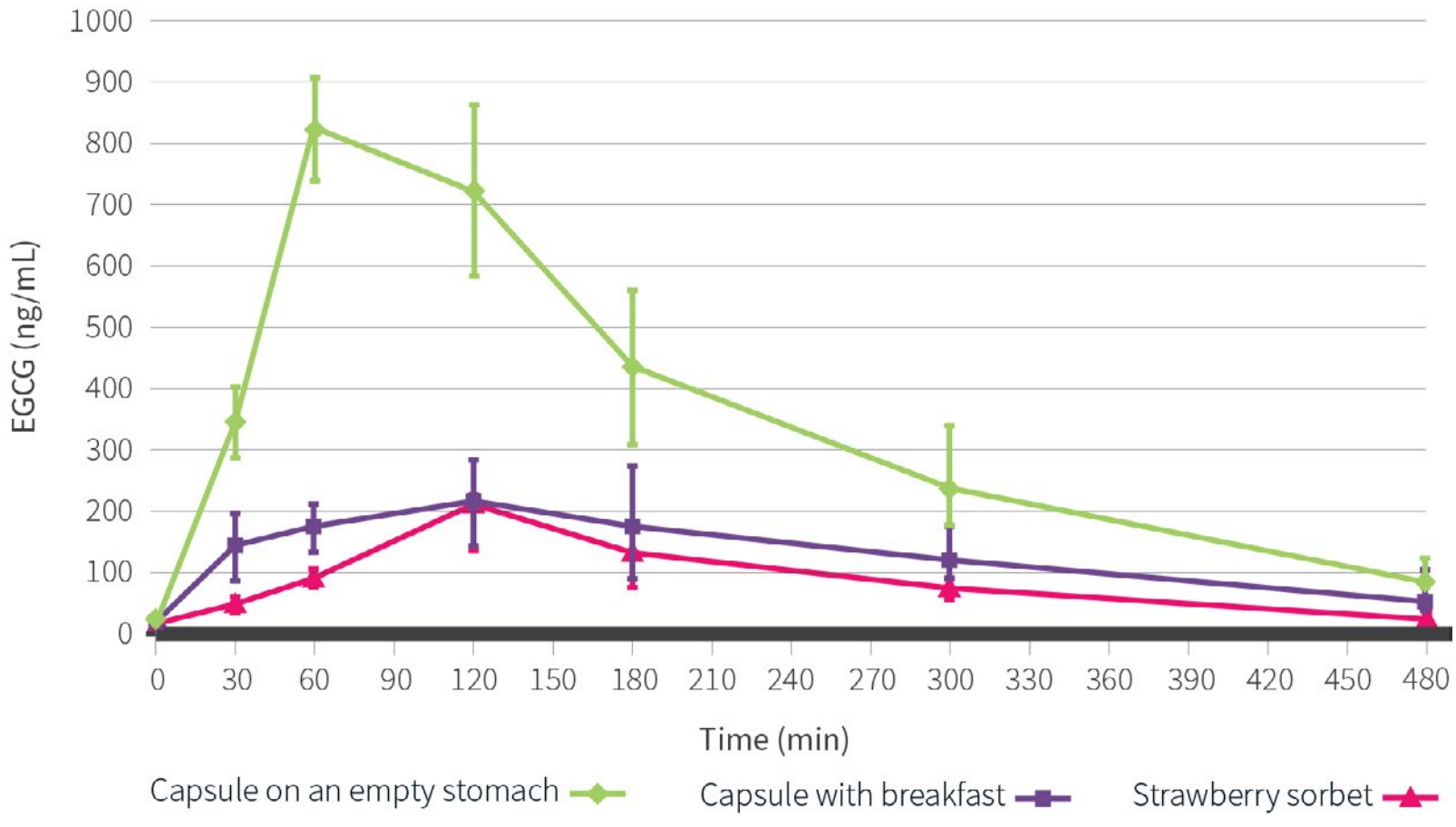
What were the findings?

In case you're hoping to make your own EGCG-infused strawberry sorbet at home, you'll be happy to hear that 97% of the EGCG that was originally added survived preparation and being frozen at -20 oC for a week. This means that EGCG did not appear to be degraded in the preparation of their strawberry sorbet.

In terms of EGCG absorption, taking it on an empty stomach with water alone came out clearly ahead. Ingesting EGCG capsules just with water led to noticeably higher plasma concentrations than either ingesting them with a breakfast cereal or a strawberry sorbet. Maximum plasma levels were around four times as high in the water-only group as the other two groups. And the total amount absorbed in the water-only empty stomach group was 3 times as high as the cereal group and four times as high as the sorbet group. Also, the

> "Green tea and one of its active components, EGCG, have a lot of health claims associated with them, some more well-supported than others."

Figure 2: Plasma EGCG levels for the three groups



peak plasma EGCG levels (shown in Figure 2) were reached at 60 minutes post-ingestion in the water-only group, whereas it was reached 120 minutes post-ingestion in both the cereal and sorbet conditions, indicating a slower uptake.

Absorption of 500 mg EGCG is much better on an empty stomach than if it's taken with food.

What does the study really tell us?

The study very clearly tells us that the absorption of EGCG is much better when ingested on an empty stomach. About 3-4 times the EGCG is absorbed when taken on an empty stomach as opposed to being taken with the kinds of food used in this study. The results also tells us that EGCG concentration in the blood peaks sooner when taken on an empty stomach.

Also, the study found that EGCG is not degraded in the preparation of the strawberry sorbet, which is slightly acidic and was frozen, which could theoretically have influenced degradation rates. Combined with previous reports showing that 30 minutes of boiling only leads to a minor 12.4% loss of EGCG, this indicates that EGCG is actually quite stable during normal food preparation conditions.

However, there are a few things this study doesn't tell us. There's a possibility that different populations may absorb EGCG differently. Keep in mind that this study was done in relatively healthy people who were of pretty normal weight. Whether food would affect EGCG absorption to a similar degree in people with gastrointestinal issues or people with higher BMIs is an open question. One other question which this study does not address is whether similar plasma concentrations can be obtained simply by upping the dose of EGCG when it is ingested with food.

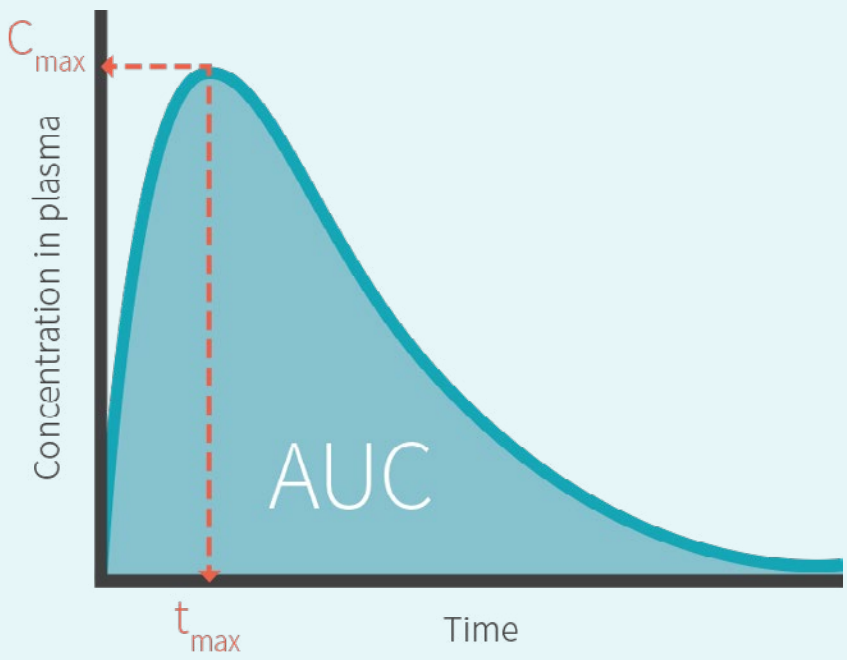
Finally, this study doesn't tell us much about the details of which macro- and micronutrients may impact EGCG absorption the most. We don't know which components of the food administered along with the dose of EGCG had the greatest impact on absorption.

Pharmacokinetics 101

We described how EGCG was absorbed into the bloodstream in simple language in "What were the findings?" However, if you want to be able to read some papers on your own, knowing the technical terms for some of these things may be useful, and may either impress or bore your friends at cocktail parties to boot!

The study of how chemicals are absorbed, distributed, metabolized, and ultimately excreted is called pharmacokinetics. Some pharmacokinetic measurements that were made in this study for EGCG were its tmax, which is the time it takes for it to reach its peak concentration in the blood, and Cmax, which is the maximum concentration in the blood that was reached. The half-life, or t1/2, was also measured. In it's simplest definition, it's the time it takes for the compound to reach half of its concentration in the blood. One other pharmacokinetic parameter that was measured is the area under the curve over 8 hours (AUC0–8). The AUC is simply the area under the blood concentration curve over a period of time, and is typically a very practical indicator of absorption and disposition for compounds absorbed orally. A simple visualization of some parameters is shown in Figure 3.

Figure 3: Some basic pharmacokinetic parameters



EGCG is better-absorbed on an empty stomach, at least in healthy people of normal weight. This study doesn't tell us much about whether taking more EGCG with food is safe or effective in raising blood levels to similar amounts seen here. The authors also found that EGCG does not degrade much when put into a low-acid sorbet.

The big picture

The findings reported here are in agreement with a previous study in which food was shown to interfere with absorption of EGCG from a green tea extract ingested with food. However, this is the first study to research how coingestion of food with pure EGCG influences ECGC absorption. The results found here are similar to those of another dosing study of pure EGCG in healthy volunteers when given on an empty stomach.

Since it seems fairly likely that food impacts EGCG absorption, this is a factor that should be considered when looking at research concerning EGCG and green tea. Back in ERD #8, we covered a clinical trial examining green tea's effects on fat loss. The trial found no overall effect of green tea supplementation containing a daily dose of about 540 mg of EGCG for 12 weeks. However, the participants were instructed to take the supplement between breakfast and lunch, lunch and dinner, and more than two hours after dinner. It is possible that the food affected EGCG absorption, which could have impacted the results.

Unfortunately, the current study only examined EGCG's absorption under two conditions: on a completely empty stomach and when taken concurrently with a meal. We don't yet know if absorption would be impacted if EGCG were taken between meals. This seems like an important question that should be addressed in the future in order to better interpret and design future clinical trials on green tea and EGCG.

The results found here are in line with previous research on EGCG absorption from both the pure supplement and green tea. If food affects EGCG absorption, this has consequences for interpreting past clinical trials on EGCG and green tea as well as the design for future ones.

Frequently asked questions

So, how did they make the EGCG sorbet?

The method is described in full here. The ingredients were strawberries, caster sugar, strawberry-flavored whey protein isolate, EGCG, and a delicious dash of carboxyl methyl cellulose.

What does the body of evidence say about green tea's health benefits?

A good source to turn to to look at what the sum of evidence says about an issue are Cochrane reviews. Cochrane currently has reviews about green tea's effects on weight loss, cancer prevention, and cardiovascular disease prevention.

For weight loss, the Cochrane authors conclude that there's little if any effect, and there seems to be no effect for weight loss maintenance, either. For cardiovascular disease prevention, the authors state that green tea seems to positively impact many risk markers, but that quality long-term trials looking at impact the development of actual disease itself are lacking. For cancer prevention, they say that the data is mostly observational and it conflicts - for some cancers some of the time, there seems to be a connection with green tea intake, but sometimes not. And these are primarily correlations only, since the data reviewed were not from interventional trials.

You can also of course check out the nitty gritty details of all the research on Examine.com's page on green tea catechins.

How safe is green tea and EGCG and how much tea should I drink?

The Cochrane review mentioned above for cancer prevention states that the "desirable green tea intake is 3 to 5 cups per day (up to 1200 ml/day), providing a minimum of 250 mg/day catechins." This and other reviews state that adverse events seen in the trials they examined are either mild to moderate, or not likely to be attributed to green tea.

Another study giving a single dose of green tea catechin mixture of up to 1200 mg to healthy volunteers on an empty stomach reported that the maximum dose was well tolerated.

However, there have been several case reports of liver injury in people taking 700-2000 mg of green tea concentrate (or roughly over 14 cups of brewed green tea), and people with pre-existing liver conditions may be at higher risk, so some caution is warranted. Green tea catechins also affect the activity of many of the cytochrome P450 enzymes that metabolize drugs, so drug interactions with green tea and EGCG are possible.

What should I know?

EGCG, one of the main active compounds in green tea, is absorbed much better on an empty stomach versus when it is taken with food. This could possibly confound clinical trial results examining EGCG and green tea extracts, since when and how these supplements are taken can vary between trials. ◆

The study that didn't end the low-fat/low-carb diet "wars"

Calorie for Calorie, Dietary Fat Restriction Results in More Body Fat Loss than Carbohydrate Restriction in People with Obesity

Introduction

Some of you may have already come across our blog post about this recent blockbuster of a paper published by Dr. Kevin Hall et al. If you have, stick around for our extended F.A.Q. section where we tackle the numerous questions brought up about the study. If you haven't read the blog, let's dive right into the trial analysis.

For some, the central dogma behind the hypothetical superiority of low-carb diets for fat loss is the insulin hypothesis of obesity. Part of this hypothesis states that by restricting carbohydrates you will see a stepwise decrease in insulin secretions. Because insulin plays a part in the regulation of fat storage, it has been theorized that the less insulin secreted the more free fatty acids will be released from adipose stores leading to increased fat oxidation and rapid fat loss. These assumptions have led to the idea that low-carb diets will induce greater fat loss over a low-fat diet even when calories are held constant. Gary Taubes, an advocate of the low-carb approach, posited the following in his latest book, Why We Get Fat (p. 144-47):

"…any time we try to diet by any of the conventional [low-fat] methods, and any time we decide to "eat healthy" as it's currently defined, we will remove the most fattening carbohydrates from the diet and some portion of total carbohydrates as well. And if we lose fat, this will almost assuredly be the reason why…This is something that even researchers who run clinical trials testing the effectiveness of different diets rarely recognize."

Simply put, Taubes suggests that by reducing both carbs and fat in low-fat diets it is possible that reductions in carbohydrate intake could be responsible for any fat loss seen. Taubes is correct in that researchers who run diet trials often alter the amount of fat and carbohydrate participates eat, making it impossible to determine if restricting one will lead to greater fat loss over the other. Previous studies on low-fat and low-carb diets have changed multiple variables simultaneously. So even though they end up comparing low-fat and low-carb, they do not specifically reduce one macronutrient or the other from a baseline diet without changing other variables. In the present study, Dr. Hall and his team set out to eliminate that confounding variable by subtracting either fat or carbs from the diet without changing anything else. This was done under tightly controlled conditions, to determine if indeed there is a metabolic fat loss advantage to going low-carb.

> "[…] this was not a free living low-fat vs. low-carb study where researchers educate groups of volunteers and let them eat self-directed low-fat or low-carb diets in their own homes to see how they fare."

One important concept to understand before reading through this breakdown is that the study was not looking at the real-world efficacy of diet interventions. That is to say, this was not a free living low-fat vs. low-carb study where researchers educate groups of volunteers and let them eat self-directed low-fat or low-carb diets in their own homes to see how they fare. The investigators designed this intervention to examine some specific mechanisms of weight loss discussed in the sections below.

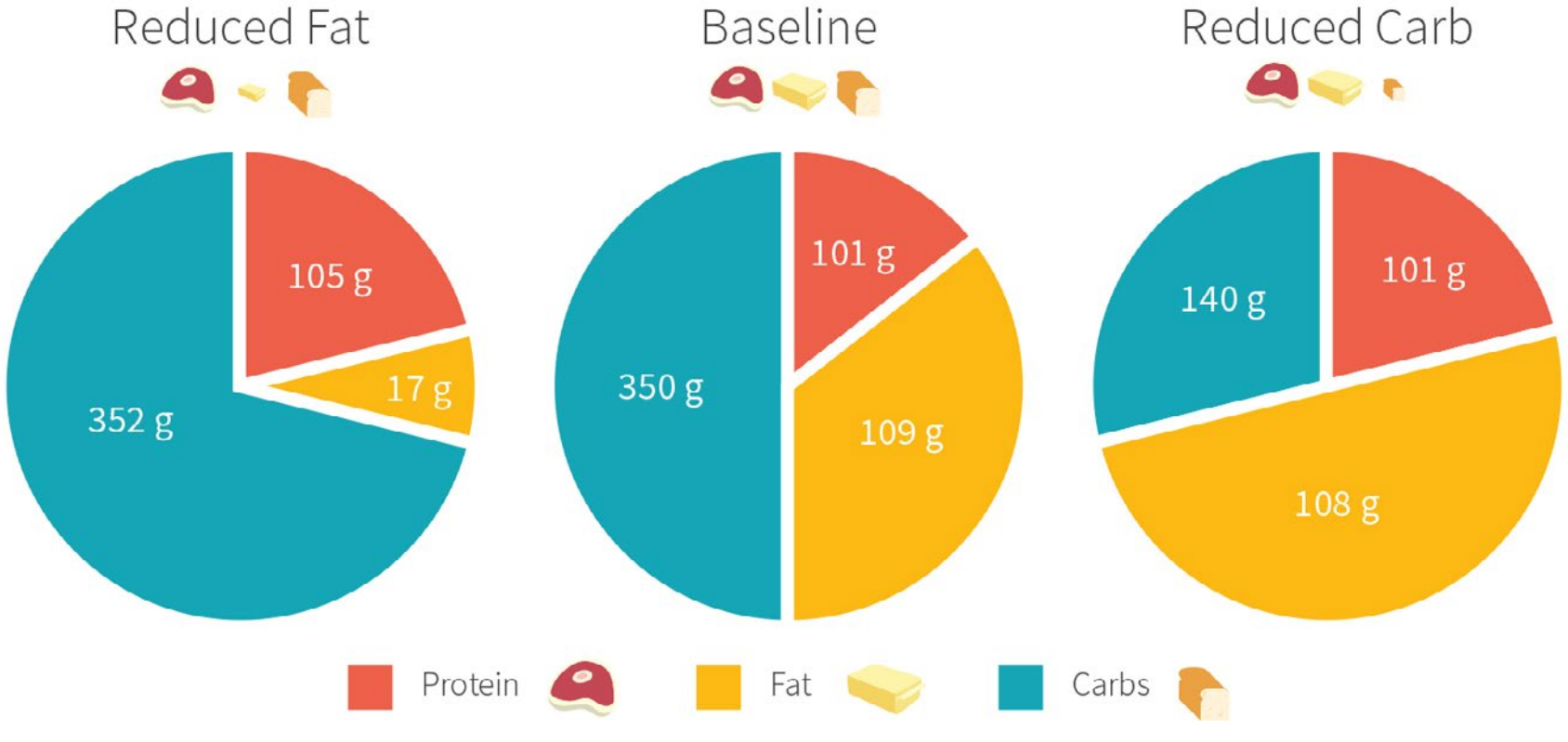
One version of the insulin hypothesis states that in order to lose body fat you must restrict carbs to bring down insulin, high levels of which will prevent fat loss. Dr. Hall's study has been designed to test this hypothesis to see if reduced-carb diets confer a fat loss advantage over reduced-fat diets when calorie intake is strictly controlled.

Who and what was studied?

Nine women and ten men with obesity were recruited for this randomized, controlled, cross-over metabolic ward study. A cross-over trial is when all patients receive both treatments at different periods, essentially acting as their own control group. Metabolic ward studies are where trial participants are strictly monitored to measure energy expenditure and energy intake. These ward studies are considered the gold standard in diet trials as free-living studies often rely on far less accurate self-reported data. Patients included were required to have been weight stable for the past 6 months and were screened to ensure they were otherwise healthy (i.e. free from diabetes, chronic illness, eating disorders, etc …). The purpose of the trial was to determine if a reduction of carbohydrates in the diet would confer a fat loss advantage above and beyond a reduction in energy intake. To test this hypothesis, Dr. Hall's research team reduced equal caloric amounts of carbs and fats in the restricted fat and restricted carb groups to determine its effects on energy expenditure, nutrient oxidation, fat loss, and bodyweight. The reduced-carb group saw a 30% caloric reduction from carbs alone and the reduced-fat group saw a 30% caloric reduction from fat alone.

Subjects underwent 5 days eating a baseline diet followed by 6 days eating one of the two calorie-restricted diets. The macro balances of each diet are shown in Figure 1. The baseline diet was 2,740 calories (50% carbohydrate, 35% fat, 15% protein) and the restricted calorie diets were both 1,918 calories. The restricted fat

Figure 1: Macronutrient contents of the diets as a percent of total calories



group cut out 828 calories of fat and the restricted carb group cut out 840 calories of carbohydrates. Protein intake was kept constant throughout. Of particular note was that sugar consumption did not decrease in the reduced-fat group compared to baseline. In fact, it went up from 152 grams/day to 170 grams/day. It was important to keep sugar intake up as to not cause any decreases in insulin secretion, which would have confounded the study results.

All the volunteers were crossed-over from one diet to the other, as they went through a 2 to 4-week washout period between the restricted fat and restricted carb diets. Food intake was meticulously monitored. All subjects were confined to the metabolic ward for the entirety of the study and were made aware of how critical it was to consume all food provided to them. Even when subjects were visiting with friends and family, they sat in a common area under the observation of research staff to ensure no food was being passed off. Daily exercise was also required. Sixty minutes of treadmill walking at a self-selected fixed pace was required everyday that patients were in the metabolic ward.

Multiple measurements were taken over the course of this trial including cholesterol, appetite hormones, insulin, cortisol, and body fat percentage. Though a dual-energy X-ray absorptiometry (DXA) scanner was employed to assess body fat, this method is not sensitive enough to pick up the small changes in body fat loss that occurred over the duration of this trial. To get a more sensitive measurement, the changes in body fatness were determined using net fat balance by indirect calorimetry while residing in a metabolic chamber, in combination with measures of nitrogen loss in urine. Essentially the difference between dietary fat intake and net fat oxidation (fat oxidation minus de novo lipogenesis) were used to measure overall fat mass loss. Although this method cannot tell us where the fat is being lost from, a sensible prediction would be that most would come from adipose tissue. However, it

“It was important to keep sugar intake up as to not cause any decreases in insulin secretion, which would have confounded the study results.”

is possible that some fat could be lost from the liver or muscles, which would also be beneficial.

A mathematical model of human metabolism was employed to predict trial outcomes and to help extrapolate the 6-day results. Data from the participant's results were plugged into this model to predict how they would continue to lose weight over the course of 6 months. Dr. Hall's model has undergone some extensive validation and has been shown to be a fairly accurate predictor of weight gain and loss in adults 18 years of age and older. His research at the National Institute of Health has been used to create the Body Weight Planner, which you can explore on their website. A brief instructional video can be found here.

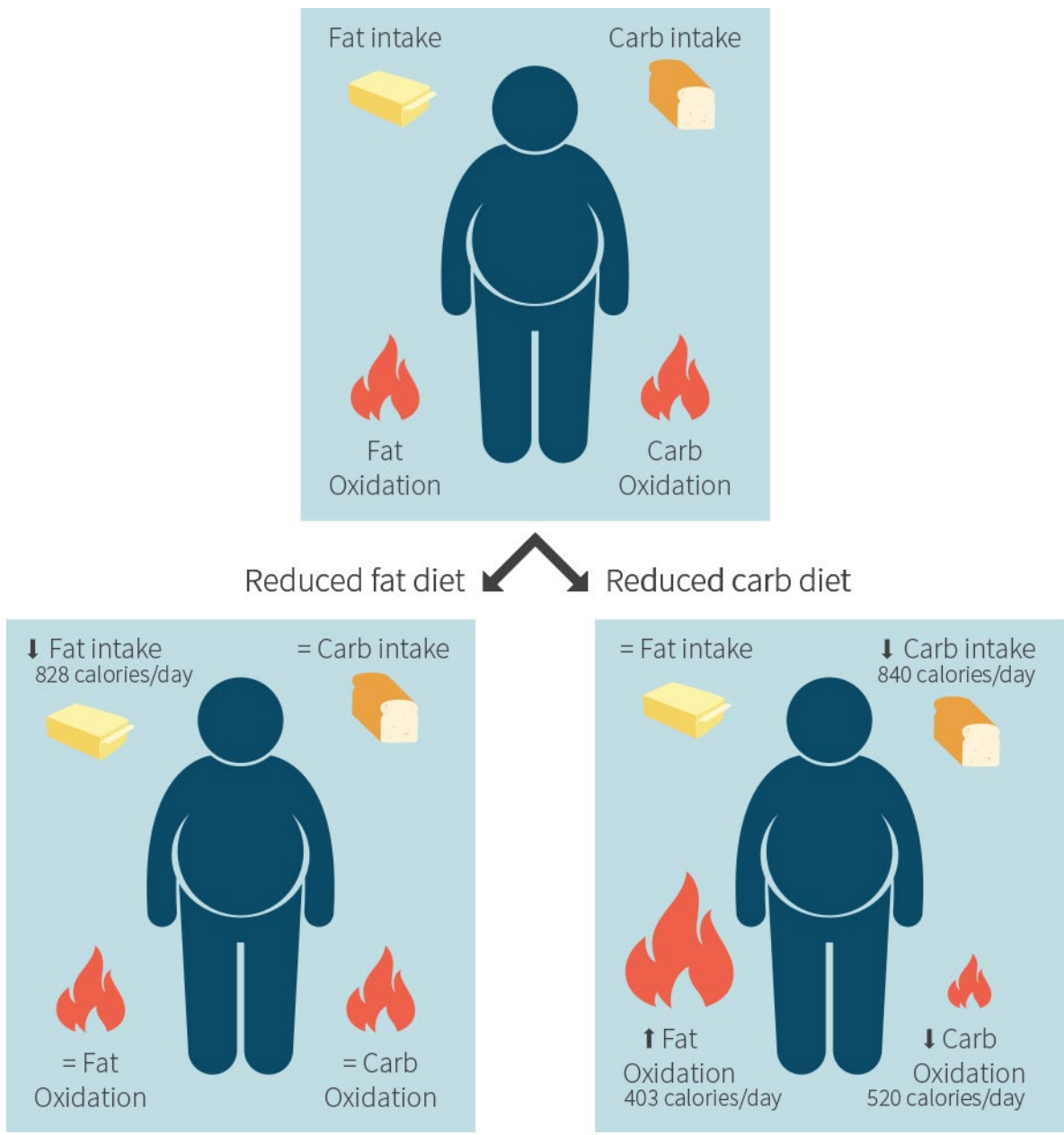
19 healthy subjects with obesity were randomized and crossed-over into both a restricted fat and restricted carb diet under strict observation in a metabolic ward. Indirect calorimetry was used to assess fat mass loss over both 6-day periods participants were on restricted diets, and a mathematical model was used to predict how much weight would be lost over 6 months.

What were the findings?

The results are summarized in Figure 2. As may have been expected, the reduced-carb group shifted to primarily oxidizing fat as fuel, and reached a steady state after about 4 days. The reduced-fat group consistently burned carbs as their main fuel source throughout the trial and saw little reduction in fat oxidation. An interesting caveat that popped up was that protein oxidation was increased in the reduced carbohydrate group, indicating that the higher carb intake of the reduced-fat group may have a slight protein sparing effect. Some may worry that this increased protein oxidation equates to muscle tissue being broken down. However, this may not necessarily be the case as the protein oxidation could be coming from the amino acids in the diet. As most reduced-carbohydrate diets are typically paired with an increased protein intake, it is unlikely that any muscle wasting would occur.

One interesting finding was that the reduced-fat group did not experience a major shift in fat or carb oxida-

Figure 2: Summary of the study and results



Adapted from: Hall, KD et al. Cell Metab. 2015 Sep.

tion the way the reduced-carb group did. Within the reduced-carb group, fat oxidation went up 403 calories (~45g) per day and carb oxidation went down 520 calories (~130g). This shift to primarily utilizing fat as energy is a known effect of low-carb diets. One might speculate that a high carb diet would see an equally dramatic shift towards burning carbs as the primary fuel, but the reduced-fat group saw fat oxidation decreased by only 31 calories (~3.4g) per day and carb oxidation increase by 44 calories (~11g). It seems that when faced with a large reduction in dietary fat intake the body keeps trucking along, burning fat and carbs at approximately the same levels.

Overall, the reduced-fat diet lead to a fat mass loss of ~463 g and the reduced-carb diet saw a fat reduction of ~245 g. The difference in these numbers can possibly be explained by the stored glycogen the reduced-carb group would have burned off in the first 2 to 4 days of the 6-day diet period, after which the fat mass loss would more closely match that of the reduced-fat group. The fat loss seen in the reduced-fat group occurred even though no significant changes in 24-hour insulin secretion were seen. By contrast, the reduced-carb group saw a 22% reduction in 24-hour insulin secretion. This finding clearly demonstrates that a reduction in dietary carbohydrate and insulin is not necessary for losing fat mass.

Figure 3 depicts the results from when the subjects' data was plugged into the human metabolism model. It predicted that the reduced-fat diet would see about 3 kg (6.6 lbs.) greater fat loss after 6 months, a 40% difference in fat loss. Of course, this was assuming that participants would adhere 100% to the diet. Real world diet studies tend to show us that compliance starts to dwindle after about the 6-month mark. Additional simulations were run to see what would happen if carbs were dropped even lower in the reduced-carb group with subtracted carb calories being swapped out for fat to keep total calories constant. The model predicted that the very low-carbohydrate diet (<50g/day) would experience comparable weight loss to the very low-fat diet, minimizing the 3 kg (6.6 lbs.) difference seen in the original prediction.

Small caveats also included the significant reduction in sleeping metabolic rate and total energy expenditure

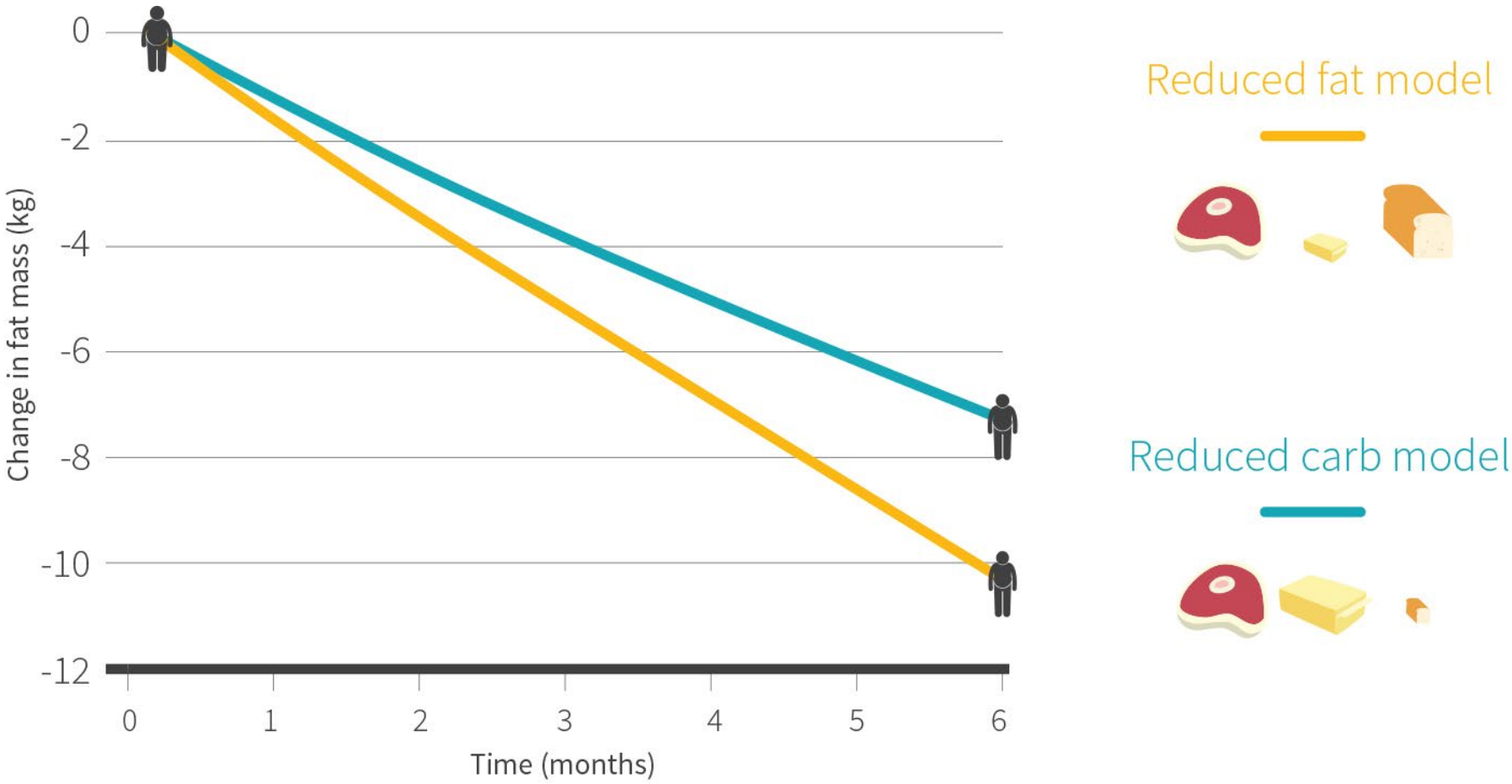
How Glycogen Affects Weight Loss

Within this 6-day trial we saw the reduced-fat group lose more fat mass than the reduced-carbohydrate group. But this is not necessarily because the fat restricted diet provides a significant fat burning metabolic advantage. The most likely explanation for why restricted-fat came out on top was that the reduced carb group was burning through their glycogen stores in the first few days of the trial.

The human body can hold about 2,000 calories worth of glycogen in the skeletal muscles and liver. When the reduced-carb participants were switched from their baseline diet of 350g carbohydrate down to 140g, they began to use up their glycogen stores as their bodies started to adapt to preferentially burning fat. Because the body was utilizing these glycogen calories it was not using fat calories. Once the glycogen stores had been depleted by about day 4, the reduced-carb dieters then reached a steady-state of fat burning.

Even though the mathematical model predicted an advantage to reduced-fat dieting in the long run, the utilization of these glycogen stores by the reduced-carb group are likely a significant contributor to why the carb restricted diet only experienced about half the fat loss in this 6-day window.

Figure 3: Mathematical modelling prediction of diets 6 months out



seen in the reduced-carb diet that was not seen in the reduced-fat diet. This is suggestive of some more subtle metabolic changes that occur due to the effect of certain dietary macronutrient compositions.

The reduced-fat diet group lost more fat mass than then reduced carbohydrate group. The reduced-fat group did not see any significant decreases in insulin production, demonstrating that reducing insulin levels is not necessary for losing fat mass. The mathematical model of human metabolism predicted an advantage to the low-fat diet over the course of 6 months, but the differences were minimal and all but disappeared when a very low-fat diet was modeled against a very low-carb diet.

What does the study really tell us?

This study lends more credence to the theory of energy balance, otherwise known as "calories in, calories out" (CICO). A common interpretation of CICO is that there should be few if any differences between diets of equal calories on fat loss or energy expenditure. This study shows us that while that strict interpretation of CICO is not 100% correct, it is pretty darn close. While the CICO model holds approximately correct over most of the macro spectrum, the mathematical model predicted that it does start to break down a little bit when looking at macronutrient extremes. As we saw in Dr. Hall's 6-month model prediction, the reduced-fat group had a slight advantage over the reduced-carb group. These small differences are about the extent to which you may see any difference between diets. And as noted earlier, that advantage all but disappeared when very low-carb was compared to very low-fat diets.

While the study was incredibly rigorous in its design and execution, the sample size was small. Only 17 of the 19 recruited individuals completed the entire study. By metabolic ward study standards, 17 is actually a pretty large sample size and provided and enough participants to ensure small differences in fat loss could

be detected. However, because of the small sample size it may be difficult to extrapolate these results to the general population. One should also note that the participants in this study were relatively healthy, so the results here may not extend to people with health issues. People with other health issues may also be on various medications that could alter metabolism, but such people were excluded from this study. These factors make any generalizations from this study to such populations very challenging.

While a calorie might not be exactly a calorie, it is pretty close in terms of its effects on metabolism during periods of weight loss. Small shifts can occur depending on the macronutrient composition, but the end results on equally caloric low-carb and low-fat diets are not strikingly different. Due to the small sample size and the type of patients recruited to this study, extrapolation of the results is limited.

The big picture

The practical implications we can take away from this study are very limited, but we can surmise that a reduction in insulin secretion brought about through low-carb dieting does not seem to confer any metabolic advantage for fat loss. In a way, this is both good and bad news. The bad news is that a low-carbohydrate diet does not appear to possess any super fat-blasting properties which, had that been proven true, would have been great news to dieters everywhere. If this paper had shown a significant advantage to low-carb dieting it very likely would have been a game changer in how we approach the treatment of obesity and weight loss research. The good news is that, because a low-carb is not necessary for fat loss, more eating styles are available to those trying to lose weight. If you are not someone who likes low-carb dieting, higher or moderate carbs diets are a perfectly viable option for weight loss.

That isn't to suggest that low-carb diets should not be employed if that is your preference. The higher protein intake that is often paired with low-carb diets can help to increase satiety, causing you to feel less hungry. Many may find a reduced-carb diet easier to adhere to than a reduced-fat diet. People who are insulin resistant, a condition commonly found among those with pre-diabetes or type 2 diabetes, can often experience better

"If this paper had shown a significant advantage to low-carb dieting it very likely would have been a game changer in how we approach the treatment of obesity and weight loss research."

results on a low-carbohydrate eating plan. In a real-world setting, adherence is king. Even if low-carb diets had the ability to melt fat off your body, if you are not able to stick with the diet it will not be an asset for your long-term weight loss goals.

The fact that low-carb diets do not confer a superhuman ability to lose fat mass is a little disappointing. A diet that did possess such properties would be a most welcome finding. However, this study does reinforce the fact that most any diet, be it Mediterranean, DASH, paleo, or vegetarian, can all work quite effectively for weight loss. It all comes down to personal preference and the ability to stick with the diet in the long term.

Frequently asked questions – XXL edition

The present study was full of intricacies and nuances. In other words, it was ripe for misinterpretation by the popular media. You may have seen flashy headlines declaring the superiority of low-fat diets and lamenting the death of low-carb. With so much hyperbole surrounding this study in the news and blogosphere, we're bringing you an XXL edition of the F.A.Q. in order to bust some common myths, misconceptions, and criticisms surrounding this trial.

A 6-day study is not long enough to get into ketosis or to become fat adapted.

A lot of people have been commenting on the short duration of this study. Many argue that it takes up to 1 month to become properly "fat-adapted" or that the carb content in the reduced-carb diet was not low-carb enough to induce a state of ketosis. This misconception about fat-adaptation likely stems from those who have gone low-carb and felt hazy or foggy, commonly known as the "low-carb flu", for 2 to 4 weeks. While it may take some time to feel normal again on a low-carb diet, the body's energy systems actually make the transition from preferentially burning carbs to preferentially burning fat rather quickly. Within the reduced-carb group of this study, it took about 4 days before they had reached maximum fat oxidation and we began to see a leveling off. This observation is corroborated by other trials that show the same quick fuel transition. Once the fat oxidation plateau has been achieved, it remains very constant over the following weeks. Hence, 6 days would have been sufficient time for subjects to achieve maximum fat oxidation on the reduced-carb diet.

On the criticism that the reduced-carb diet was not ketogenic, they are correct if you are defining ketogenic as 50 grams of carbs a day or fewer. But if the argument is that being in a ketogenic state confers bonus fat burning abilities, you'd think there might be at least some suggestion of a dose-response curve as carbs in the diet decrease. This means we should be able to see fat loss increase as carbs in the diet decreased. No such dose response was observed in this trial. The mathematical model employed also indicated that a very low-carb diet would have similar fat loss results to a very low-fat diet.

Currently, no metabolic ward study of a ketogenic vs non-ketogenic diet exists, where calories and protein are held constant. However, there have been non-metabolic ward studies indicating no metabolic advantage to ketogenic diets. Dr. Hall has just completed (but not yet published) an 8-week metabolic ward study that will hopefully shed some more light into this area of research.

Nothing can be gained from this study because it does not represent real world conditions.

This study was not about which diet leads to better results under real-world conditions. There are many other studies out there that have attempted to address that question, but as mentioned before, a successful diet comes down to adherence. The authors were very upfront in what this trial was designed to study and its real-world applications. The research team planned this

study to look at specific mechanisms of fat loss, primarily testing if a reduction in insulin is necessary to lose body fat.

Dr. Hall does have a study in the works that will be looking into some more real-world diet issues. His future trial will be examining some of the changes in metabolism and the brain that may lead to weight loss, plateau, and regain.

The authors even stated the following in their discussion:
"Translation of our results to real-world weight-loss diets for treatment of obesity is limited…. We did not address whether it would be easier to adhere to a reduced-fat or a reduced-carbohydrate diet under free-living conditions. Since diet adherence is likely the most important determinant of body fat loss, we suspect that previously observed differences in weight loss and body fat change during outpatient diet interventions were primarily due to differences in overall calorie intake rather than any metabolic advantage of a low-carbohydrate diet."

Why were obese but metabolically healthy people selected? Wouldn't having obese people who were metabolically unhealthy have made more sense?
It is possible that a future study like this may be performed in those with obesity and metabolic syndrome, but the additional factors that come with metabolic dysfunction complicate the results of the study. For example, someone with type 2 diabetes operates under a different metabolic paradigm than someone without it due to insulin resistance and potentially decreased pancreatic function. Furthermore, many type 2 diabetics may be taking medications that alter their metabolism which adds more confounding variables to the mix when trying to draw conclusions.

Why were left-handed people excluded from the trial?
If you look at the exclusion criteria for this study, you will indeed find that those who were left-handed were not allowed to participate. While this may seem odd at first, it was implemented because neuroimaging was used on all participants, most likely to be used in future publications. Those who are right-handed tend to perform tasks in either the right or left side of the brain, whereas left-handers tend to split that task evenly across the brain. If you are using neuroimaging to look at a specific part of the brain, this difference in brain hemisphere usage in right and left handers can throw off your results.

What about the hiccups in the study where people receive incorrect meals and one woman's data was not included?
In any clinical trial, mistakes are bound to happen. In this case, one male and one female participant received the wrong meals on the first day the reduced-carb and reduced-fat diets were administered. The researchers opted to keep these data in the final analysis, as removing them did not affect the statistical significance of any comparisons.

Two of the male participants also dropped out of the study after finishing the reduced-carb phase of the trial. Their data for the reduced-carb portion was kept in, but obviously they did not contribute any data to the reduced-fat phase.

Curiously, one female subject saw some unusual measurements on her DXA scans that prompted the research team to exclude her data from that particular analysis. This was because the DXA showed her fat mass had increased on both the reduced-carb and reduced-fat diets despite the fact that she had experienced weight loss and was in negative energy balance. Gaining fat mass while in substantial negative energy balance is something that is physiologically impossible, making it a clear outlier and hence leading to the decision to exclude those data points.

Why did they compare a low-fat to a moderate-carb diet instead of a low-fat to low-carb diet?

The baseline diet was set at 35% fat, 15% protein and 50% carbohydrate and about 20% of those total calories were from sugar. This is believed to represent a typical American diet composition. Because of this composition, it was impossible to make subtractions from carbs any lower in the low-carb group without having to add fat calories back in while keeping total calories constant between groups. The researchers did not want to do this, as the whole point of the trial design was to change just one macronutrient level while leaving the others untouched. This is why the macro composition was askew after the pre-set number of calories and been subtracted from each group.

There were too few participants in the study.
Usually, before a trial is conducted, a power calculation is used to determine how many people may be needed in the trial to reach adequate statistical power. That is to say, how many people will be needed to ensure that a statistically significant difference can be found in a study when there is one in reality. This method is how Dr. Hall reached the number 19 for participants needed in this study. It should be noted that due to the complexities and costs of running a metabolic ward study, 19 is actually a large sample size, comparatively.

Why did the reduced-fat group experience a greater drop in fasting blood glucose compared to the reduced-carb group (and other oddities in Table 4)?
You would expect the reduced-carb group to have the greater decrease in endpoints such as fasting blood glucose and fasting insulin levels. In this case that did not happen, as the reduced-fat group dropped their fasting glucose by 7.1 mg/dl and the reduced-carb group only experienced a 2.69 mg/dl drop. Decreases in fasting insulin were comparable between groups. So what's going on in Table 4? Try not to read too heavily into the blood data presented, as they were all exploratory secondary endpoints of the study. The p-values were also uncorrected for multiple comparisons.

Can we see the individual data?
Dr. Hall has said that he will be publishing future papers exploring the correlates of individual responses seen in this study. We look forward to seeing these data too!

What if this restarts the low-fat diet trends again? I loved the 80s!
Please, no more high vs low-fat diet shenanigans! Both dietary approaches are perfectly healthy. Pick what works best for you in the context of your food preferences, environment, and health status.

What should I know?

The most direct takeaway from this study is that carbohydrate restriction and insulin decreases are not required for fat loss. For a more real-world implication, we can extrapolate that you should pick whichever diet you can adhere to in the long run. This study is not showing that low-carb diets are ineffective, but rather demonstrates that both a low-carb and low-fat option may be equally efficacious for those seeking fat loss (at least as far as your body is concerned). Decreased insulin in otherwise healthy subjects will not provide an additional fat loss advantage, so do not fret that you must go low-carb or you will never lose weight ever again. ◆

Gluten-intolerant? There's a pill for that

Randomised clinical study: Aspergillus niger-derived enzyme digests gluten in the stomach of healthy volunteers

Introduction

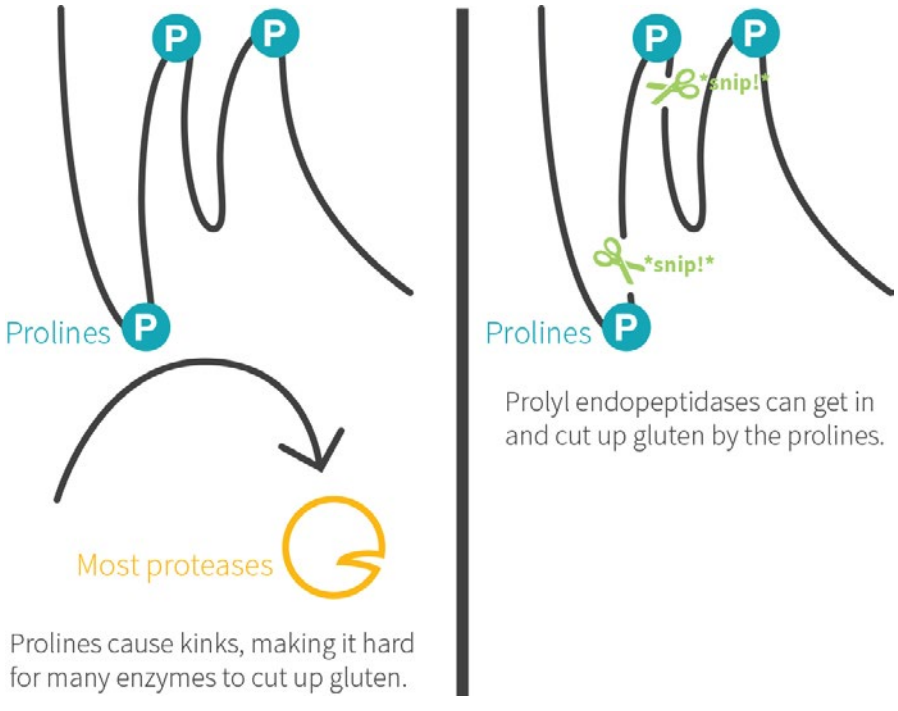
Gluten is a type of protein found in wheat and related grains such as rye and barley, making up about 80% of their total protein content. Normally, proteins are digested in the stomach and upper small intestine (duodenum). However, gluten's structure renders it highly resistant to most of our digestive enzymes, allowing fragments of the gluten protein to persist in the small intestine. More specifically, the gluten protein contains long stretches of proline and glutamine amino acids that require special enzymes to break apart, which humans do not possess. Interestingly, research has identified numerous microbes in both the mouth and colon that can degrade gluten.

It is estimated that at least 1% of the U.S. population suffers from celiac disease, an autoimmune condition characterized by the destruction of the small intestine in response to gluten. Immediate symptoms may include gastrointestinal (GI) distress, headaches, and muscle aches. And long-term gluten consumption can lead to malnutrition, weight loss, and possibly death. The only known treatment option is a lifelong gluten-free diet. However, many foods may contain hidden or unexpected sources of gluten, and food labels on products are not always present. Even items labelled "gluten-free" only need to be below a certain threshold, making them not truly gluten-free. And although non-celiac gluten sensitivity is a controversial diagnosis, research suggests that gluten may damage the guts of people who don't have celiac disease (as explored in ERD issues #7 and #8).

There has been a recent interest in prolyl endopeptidases (PEP, shown in Figure 1), which are a type of enzyme capable of breaking down the proline-glutamine chains within gluten. While early research suggests that PEPs derived from bacteria don't function well due to the stomach's acidity, are rapidly broken down by our own digestive enzymes, and are unable to efficiently prevent the passage of gluten through the intestinal tract, there has been increasing interest of PEPs derived from alternative sources.

In this respect, the *Aspergillus niger*-derived PEP (AN-PEP) has shown promising cell culture results. Additionally, it has proved itself in a digestive model that closely mimics the human GI tract. Most recently, AN-PEP appeared to be well-tolerated in celiac disease patients consuming gluten daily for two weeks, but its efficiency compared to placebo could not be evaluated. The authors of the study under review sought to evaluate how efficiently AN-PEP breaks down gluten in the stomachs of healthy volunteers.

Figure 1: Prolines in gluten make cutting it hard



Gluten is a digestion-resistant protein found in wheat and related cereal grains that can cause extreme distress for people with celiac disease. This study evaluated how efficiently a type of enzyme called AN-PEP breaks down gluten in the stomachs of healthy volunteers.

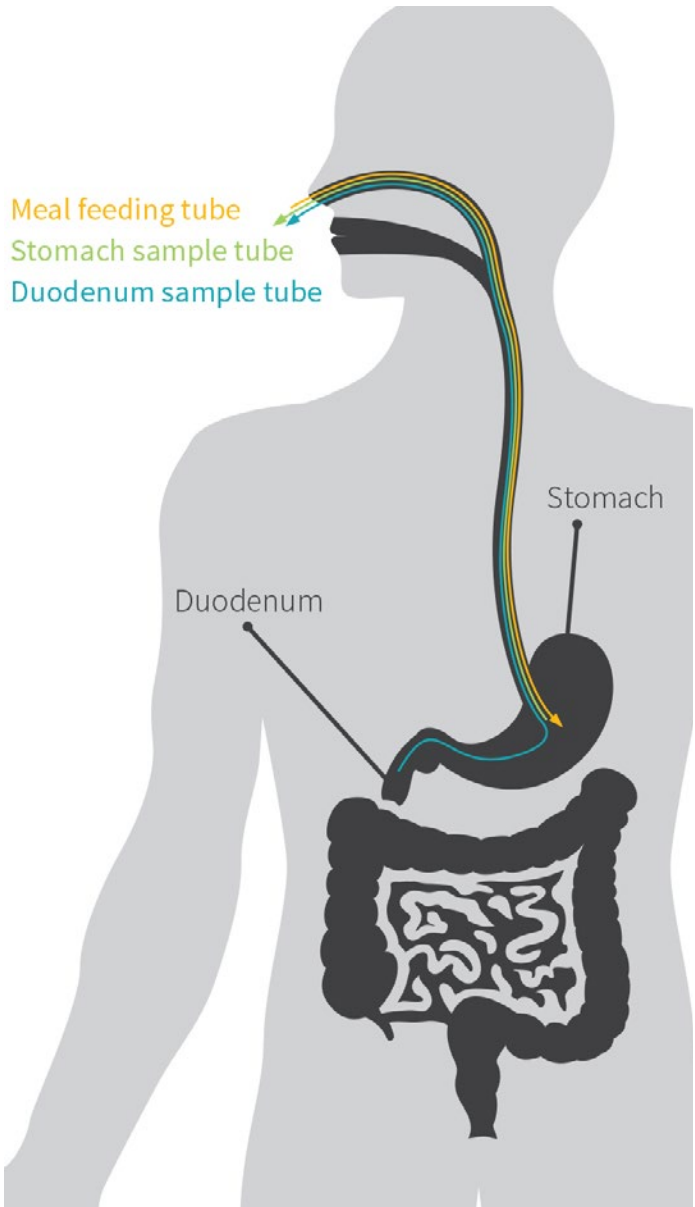
Who and what was studied?

In this double-blind, randomized, placebo-controlled,

crossover study, 12 healthy men and women with no history of gastrointestinal disorders and major diseases underwent four test days with a one-week washout period, where they consumed a high- or low-calorie test meal with AN-PEP or placebo. Each test meal was a powdered mixture of four grams of gluten protein (roughly equivalent to one slice of whole wheat bread), along with added sodium caseinate to balance protein content for the meals, maltodextrin to balance energy content, refined olive oil to add fat content, and acetaminophen to assess gastric emptying rate (through measuring its absorption into the bloodstream). Each group also had either AN-PEP or placebo dissolved in tap water.

The participants didn't actually eat this possibly disgusting concoction. Instead, the "meal" (for lack of a better word) was infused directly into their stomachs by way of a tube going through the nose and into the stomach (shown in Figure 2), along with AN-PEP or placebo. The participants also had other tubes in their stomach and duodenum, where test meal contents could be recovered for analysis.

Figure 2: Down the tube!



This method allowed for the direct measurement of actual gluten content in the GI tract, and allowed for standardized infusion of the test meal and AN-PEP or placebo so as to avoid differences in gluten degradation between interventions from variable meal consumption rates. However, this is obviously not representative of a real meal, where solid food and AN-PEP are ingested separately and undergo the normal physiological processes of mixing in the stomach.

In addition to measuring actual gluten content in the stomach and duodenum through two separate lab procedures, the rate of gastric emptying, and the presence of AN-PEP in samples, the researchers had participants complete a GI symptoms questionnaire.

In this double-blind, randomized, placebo-controlled, crossover study, 12 healthy men and women with no history of gastrointestinal disorders and major diseases consumed a high- or low-calorie test meal containing four grams of gluten (equivalent to the amount in about one slice of wheat bread) with AN-PEP or placebo.

What were the findings?

The results are summarized in Figure 3. Regardless of the caloric content of the meal, AN-PEP ingestion was associated with significantly reduced gluten concentrations in both the stomach and small intestine, compared to the placebo. In fact, gluten concentrations in the duodenum with AN-PEP were so low that they were below the detectable limit for the two lab procedures used (ELISA assay and Western blot).

With the placebo, gluten was detectable within the stomach for three hours after meal consumption,

regardless of caloric content, but significantly less gluten was detectable in the duodenum after the high-calorie meal versus the low-calorie meal. It's possible that the greater fat content of the high-calorie meal increased the secretion of pancreatic enzymes and facilitated gluten degradation. By contrast, gluten was broken down within the stomach in about 60 minutes in both the high- and low-calorie meals when consumed alongside AN-PEP, which consequently led to undetectable amounts of gluten in the duodenum.

AN-PEP itself was detectable only in the stomach, with none found in duodenal samples of any test meal. When food moves from the stomach to the duodenum, it is showered with bile, pancreatic buffers, and enzymes that act to reduce the acidity of the contents. It is therefore possible that under the more neutral conditions of the duodenum, AN-PEP becomes vulnerable to and is degraded by pancreatic enzymes.

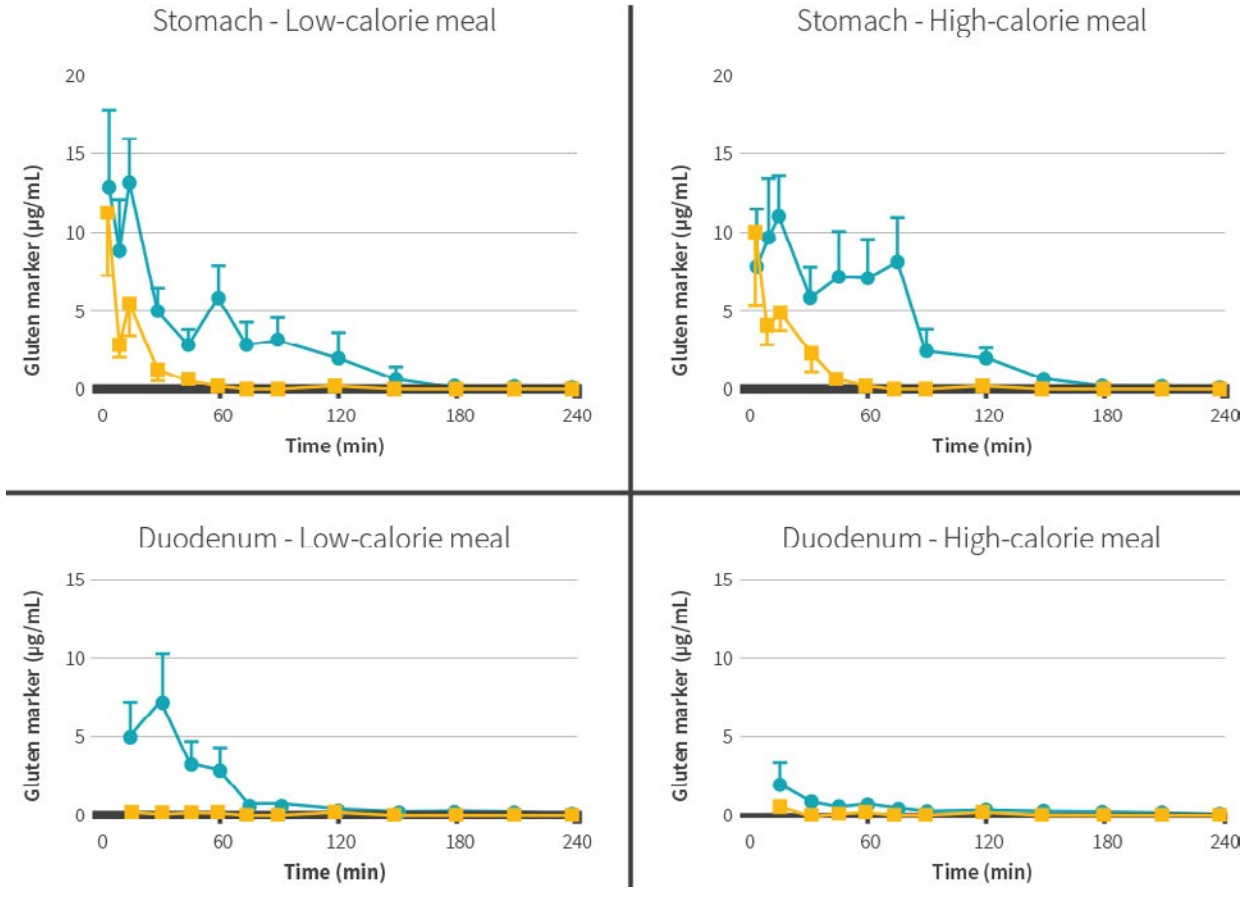
Finally, some mild GI symptoms were reported during the interventions, but there were no differences between AN-PEP and placebo or low- and high-calorie meals.

AN-PEP was able to degrade nearly all the gluten in the stomach within one hour, and then was likely destroyed by the body's own enzymes upon entering the small intestine. It was well tolerated by the participants regardless of the meal's caloric content.

What does the study really tell us?

This study tells us that AN-PEP effectively and safely degrades gluten in the stomach of healthy volunteers. The amount of gluten that entered the stomach was equivalent to about one slice of wheat bread, and the amount reaching the duodenum when ingested alongside AN-PEP was below detectable limits regardless of the caloric content of the meal. Despite these promising results, future research will need to evaluate the effectiveness of AN-PEP in individuals who are most sensitive to gluten – those with celiac disease. It is possible that even the low levels of gluten that entered the duodenum are enough to cause an autoimmune response in this vulnerable population.

Figure 3: Results



Future work will also need to evaluate how AN-PEP interacts with the amount of gluten consumed under more normal conditions, as the current study used a relatively small amount of gluten delivered directly into the stomach alongside AN-PEP, which is not representative of the normal digestive process. It appears unlikely that application of a gluten-degrading enzyme would be within gluten-containing foods themselves, and the most likely supplemental route would be a pill. Since people take pills at various times around a meal and the pill shell takes time to degrade in the stomach, more research is needed to determine how AN-PEP would perform when consumed like most supplements.

Finally, it must be noted that this study was entirely funded by DSM Food Specialties, who currently owns the patent for AN-PEP and have recently introduced it into the American marketplace. Additionally, two of the ten authors are associated with the DSM Biotechnology Center and were responsible for study design and critical revision of the manuscript. No authors declared a conflict of interest.

Despite these limitations, the results are encouraging and suggest that AN-PEP may be a useful adjunct to a gluten-free diet, in order to protect against unintentional and minor intakes of gluten.

Research using gluten-intolerant target populations with normal meal consumption and AN-PEP supplementation patterns will be needed before AN-PEP can be considered safe and effective, but initial results are encouraging and suggest AN-PEP may be a useful adjunct to a gluten-free diet.

The big picture

A gluten-free diet is a necessity for people with celiac disease. More recently, its application has expanded into the treatment of numerous other autoimmune and gastrointestinal diseases with promising results in alleviating symptoms. However, many individuals following a gluten-free diet do not have any of these conditions and yet claim to experience a very similar range of symptoms after eating gluten.

The sea of anecdotal reports eventually spurred clinicians to coin the term non-celiac gluten sensitivity (NCGS), with clinical trials results both supporting the condition and suggesting that it may be overblown. This may be because the mechanism of NCGS remains unknown, making diagnosis reliant upon a recurrence of symptoms when gluten is reintroduced into the diet after removal for a period of time. This is in contrast to celiac disease, for which we have a clear mechanism and mechanism-based diagnostic tools.

In otherwise healthy individuals, gluten consumption has been linked to markers of inflammation, and cell-culture studies have shown gluten to cause increased intestinal permeability, albeit to a lesser extent than in people with celiac disease. Interestingly, having a "leaky gut" is associated with several autoimmune diseases, which may explain why a gluten-free diet has been used successfully to help reduce symptoms of non-celiac autoimmune conditions such as rheumatoid arthritis. However, the overall link between gluten and inflammation in the general population is weak, despite some animal data suggesting a gluten-free diet reduces fat mass, inflammation, and insulin resistance.

Regardless of the true health effects of gluten, some individuals feel better following a gluten-free diet. Whether this is a placebo effect or if they truly suffer from NCGS does not undermine the choice to be gluten-free, since subjective improvement in wellbeing is reason enough to avoid gluten. With that in mind, a pill that may help reduce the likelihood of experiencing gluten's ill effects would be very beneficial.

AN-PEP is not the only gluten-degrading enzyme under

investigation. A barley-derived endoprotease (EP-B2) has been shown to be remarkably effective at digesting gluten in the rat stomach. ALV003, a mixture of EP-B2 and a second complementary protease, has also been shown to be effective in rats, as well as healthy humans. In fact, ALV003 has been shown to prevent biopsy-confirmed small intestinal mucosal injury in patients with celiac disease when consumed alongside two grams of gluten daily for six weeks. Researchers continue to look for bacterial enzymes with gluten-degrading activities.

Gluten is a well-researched compound when it comes to celiac disease, but its role in other conditions, like NCGS, is still being investigated. Regardless of pathology, some individuals may not feel well after eating gluten, which makes having a pill available that could reduce the fallout of accidental gluten consumption invaluable. Clearly the benefits escalate with the degree of harm gluten would cause.

Frequently asked questions

Can gluten be processed out of grains? What are some gluten-free grain sources?

Wheat protein contains about 80% gluten, and because it is bound to the starch within the endosperm of the kernel, processing does not remove it. All wheat varieties contain gluten, as well as relatives such as rye, barley, triticale, malt, brewer's yeast, and basic wheat starch. Additionally, many gluten-free foods, such as oats, are commonly cross-contaminated with gluten because of processing in a facility that also handles wheat-related products.

Ignoring cross-contamination, numerous grains are gluten-free. This includes rice, tapioca, corn, sorghum, quinoa, millet, buckwheat, arrowroot, amaranth, teff, and oats.

What should I know?

Gluten-free diets are contentious: some see them as fads with unsupported claims, and others see them as necessary for optimal gut health regardless of who you are. But there is a subset of the population for which gluten avoidance is absolutely necessary to maximize quality of life.

However, eating gluten-free is not always convenient or possible, putting many individuals at risk for adverse effects. The current study provides encouraging results to suggest that there may soon be an effective enzyme on the market that can successfully break down gluten before it reaches the intestines to cause problems. However, the AN-PEP enzyme isn't likely to be a complete replacement for a gluten-free diet. While it could help offset accidental gluten consumption, its effects as a real-life supplement given with a normal meal need to be further evaluated, in order to fully assess benefits and limitations. ◆

All up in your krill

Supplementation with a blend of krill and salmon oil is associated with increased metabolic risk in overweight men

Introduction

Fish oil. By now, most of our readers have heard of it. Fish oil has been tested as a potential cure for just about everything under the sun. From treating epilepsy (covered in ERD issue #1), to preventing cognitive decline in the elderly, to reducing fatigue. The Examine.com page on fish oil currently has more than 700 citations and over 90 categories in the Human Effect Matrix, which covers each of the known effects of a supplement.

However, not as many people know about krill oil: fish oil's lesser known cousin. Both contain the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are the primary catalysts for many of fish oil's benefits. However, krill oil has a distinct advantage in that it may be better absorbed than fish oil. One trial has suggested that the EPA in krill oil may be taken up better depending on the type of oil. A preliminary trial has even indicated that krill oil could potentially be superior at improving cholesterol profiles over fish oil. Krill oil also contains astaxanthin, a potent antioxidant that gives salmon and krill their reddish pigmentation. Figure 1 shows a selection of health effects that astaxanthin has been researched for (albeit not combined with krill oil, and at varying doses).

And yet, research into krill oil is not terribly extensive. The study under review helps expand krill oil's evidence base by examining its use as a means to improve insulin sensitivity. Insulin sensitivity refers to how much insulin the body needs to produce in order to manage blood sugar levels. Being insulin-sensitive is a sign of metabolic health, while insulin resistance can be a warning sign of metabolic dysfunction. People with insulin resistance would need their body to pump out a lot of insulin to bring blood glucose down to a normal level. Before the publication of this study, no human trials had investigated the outcomes of krill oil supplementation on insulin sensitivity. Previous research had found omega-3 fatty acids to exert little positive or negative change on insulin sensitivity, but these studies

Figure 1: Some human effects (or lack thereof) of astaxanthin

used a number of different omega-3 sources, varying doses, assorted control oils, and had confounding variables, like restricting participant calories in addition to administering an omega-3 supplement. Complexities like these make it hard to determine the true relationship between insulin sensitivity and omega-3s.

Although the sum of evidence to date shows little change in insulin sensitivity after omega-3 supplementation, there are many confounding variables that make it hard to say if omega-3s have an effect or not. Krill oil may possess some unique properties that could give it an advantage over fish oil, since it is easily absorbed and contains astaxanthin, an antioxidant.

Who and what was studied?

This study was a double-blind, randomized, controlled, crossover human trial investigating the effects of a krill and salmon oil blend (88%/12%, respectively) on insulin sensitivity in overweight middle-aged men. In a crossover trial design, all participants receive the treatment and the placebo in a randomly assigned order. For this 24-week study, three eight-week periods were used to test the krill/salmon oil blend. In the first eight weeks, half the participants received the krill/salmon oil and the other half received canola oil. After an eight-week washout period, during which no supplements were taken, participants switched to the opposite treatment for the last eight weeks.

The 47 participants were middle-aged men (35-55 years old) who were classified as being overweight (BMI 25-30) but were otherwise healthy (e.g. did not have diabetes, high blood pressure, high cholesterol, and did not use tobacco). The researchers noted that women were excluded from participating because menstrual cycles and contraceptives can affect insulin sensitivity, which could have confounded the study results. Men who took medications that could affect insulin sensitivity were also excluded.

The two study groups received one of the following: five grams of a krill/salmon oil blend or five grams of canola oil. Both oils were tested to ensure that they had not oxidized or gone rancid. Omega-3 fats in fish and krill oils are particularly susceptible to oxidation. Once these fats oxidize, their many potential health-promoting properties are diminished. These effects are explored further in the sidebar.

Insulin sensitivity was measured on four occasions, before and after the first and last eight-week periods, using an oral-glucose-tolerance test (OGTT). To take this test, participants are brought in after an overnight

> “...research into krill oil is not terribly extensive. The study under review helps expand krill oil’s evidence base by examining its use as a means to improve insulin sensitivity.”

Oxidized Fish Oil

Fish oil can go rancid and oxidize when exposed to oxygen, heat, or light. These oils are particularly susceptible to oxidation because of their very long chain polyunsaturated fatty acids. The oxidation level is measured using three values: peroxide value (PV), anisidine value (AV), and TOTOX value.

The PV is a measure of primary oxidation products (peroxides) and AV a measure of secondary oxidation (aldehydes and ketones). The TOTOX values is calculated using the formula AV + 2PV. The lower the TOTOX value, the better the oil quality will be. The Global Organization for EPA and DHA Omega-3 recommends no more than a TOTOX of 26.

Oxidation of fish oils is more common than you may suspect. One study found that almost 50% of commercial fish oils exceeded the maximum recommended TOTOX value. Evidence for the health effects of consuming oxidized fish oils is a bit mixed though. For healthy individuals, it would appear that there is a lack of obvious short-term health damage from consuming oxidized fish oil. One study showed no difference in circulating levels of oxidized LDL or inflammatory markers after seven weeks of oxidized fish oil supplementation. However, in people with high levels of cholesterol and triglycerides, consumption of highly oxidized fish oils can minimize its efficiency at improving metabolic markers like fasting glucose, total cholesterol, and triglycerides.

fast and drink a solution containing 75 grams of glucose. Five blood samples are then taken over the next two hours to measure glucose levels and insulin response. The responses are used to calculate insulin sensitivity via two methods, the Matsuda Index and HOMA-IR. These measures give us a short-term snapshot of how the body responds to ingested carbohydrate and provides an overall approximation of insulin resistance. Blood samples were also taken to assess metabolic disease risk (free fatty acids, C-reactive protein) and lipid profile (total cholesterol, HDL, LDL, triglycerides).

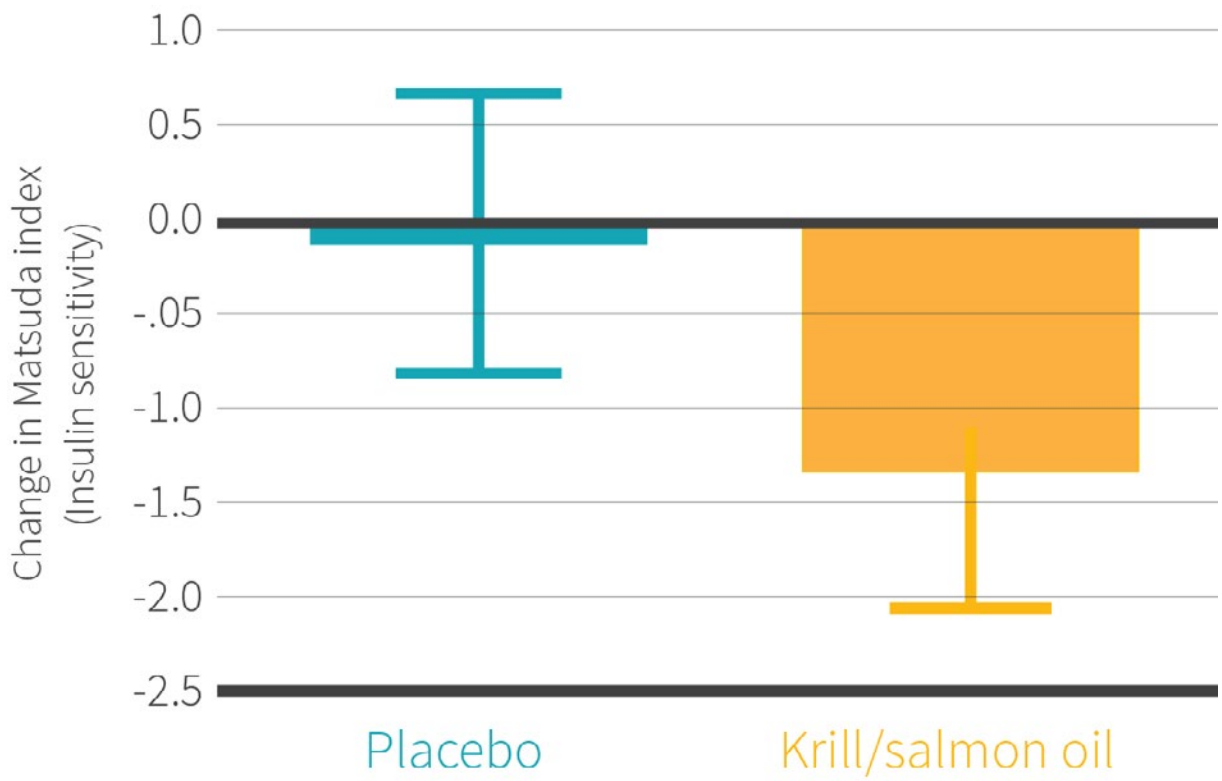
This randomized, controlled crossover trial was conducted in overweight but otherwise healthy middle-aged men. One group received a krill and salmon oil blend while the other received canola oil, which acted as the control. Participants were crossed over into the other group after an eight-week washout period. Insulin sensitivity was measured using an oral-glucose-tolerance test.

What were the findings?

The main finding is shown in Figure 2. Surprisingly, insulin sensitivity worsened, dropping 14% after krill oil supplementation, when compared to the control oil (Matsuda index - Control: 5.33, Krill Oil: 4.57). After receiving these findings, the researchers adjusted their analysis by controlling for the potential positive effects that DHA and EPA can have on insulin sensitivity. This analysis showed an even greater reduction of 27%, when compared to the control oil.

Researchers found no significant changes in the metabolic disease risk or lipid profile measurements. Results for total cholesterol, HDL, LDL, triglycerides, free fatty acids, and C-reactive protein did not significantly differ between groups. The most severe adverse event reported was a high frequency of eructation, which may sound scary, but is actually just the medical term for a belch. The authors described these belches as “fishy burps.”

Figure 2: Effect of Krill/salmon oil mixture on insulin sensitivity



The "fishy burps" experienced by participants lead to an interesting twist in the study. The majority of participants (51%) were able to guess which supplement they were taking at the end of the trial, causing the double-blinding of the treatment to be unsuccessful. One participant even admitted to cutting open his krill oil capsules to identify the contents. The researchers had been very diligent throughout the blinding process, going as far as to minimally coat the control canola oil capsules in fish oil to mimic the odor and taste of the krill oil pills. Unfortunately, their efforts were foiled by fish burps. However, there is no evidence to suggest this unintentional unblinding caused the participants to change any dietary or physical activity behaviors that could have influenced insulin sensitivity.

Krill oil supplementation caused a 27% reduction in insulin sensitivity when adjusted for the potential positive effects DHA and EPA can have on insulin sensitivity. No changes were detected in any other measures, including lipid profiles and markers of metabolic disease risk. Many participants experienced "fish burps," a common side effect of taking fish oil, which thwarted the researchers' attempts at blinding.

What does the study really tell us?

This study demonstrates that within certain subgroups, such as the overweight men examined here, krill oil may have a detrimental effect on insulin sensitivity. It is hard to determine if krill oil supplements would persistently lower insulin sensitivity when taken by overweight men, but there were certain trends and associations in participant biomarkers that could shed some light on this question. While participants supplemented with krill oil, their fasting insulin, carotid artery thickness, cholesterol, and apolipoprotein B all increased slightly.

All of these changes could potentially indicate future risk for developing metabolic syndrome. People who develop metabolic syndrome are more likely to have heart disease and to develop type 2 diabetes. These changes were only seen in a within-group analysis conducted post hoc and should be interpreted with a grain of salt. Future studies are needed to verify these findings.

The authors proposed that the unfavorable results seen in insulin sensitivity are not likely due to the omega-3

content of the krill oil, but some other chemical compound, since the reduction in insulin sensitivity got even worse when blood levels of omega-3s were taken into account. Narrowing down that compound proved to be an elusive endeavor. Since the trial used a blend of krill and salmon oil, it is hard to say which components of the two, or both, are to blame. In all likelihood, the krill oil may be the culprit, as previous fish oil studies have not shown adverse effects on insulin sensitivity. Furthermore, in one small study comparing krill and fish oil, the krill oil group saw an upward trend in fasting insulin levels, an indicator of insulin resistance. Though the researchers were not able to identify the compound responsible, they suggest it may be a protein that is not filtered out during the krill oil extraction process.

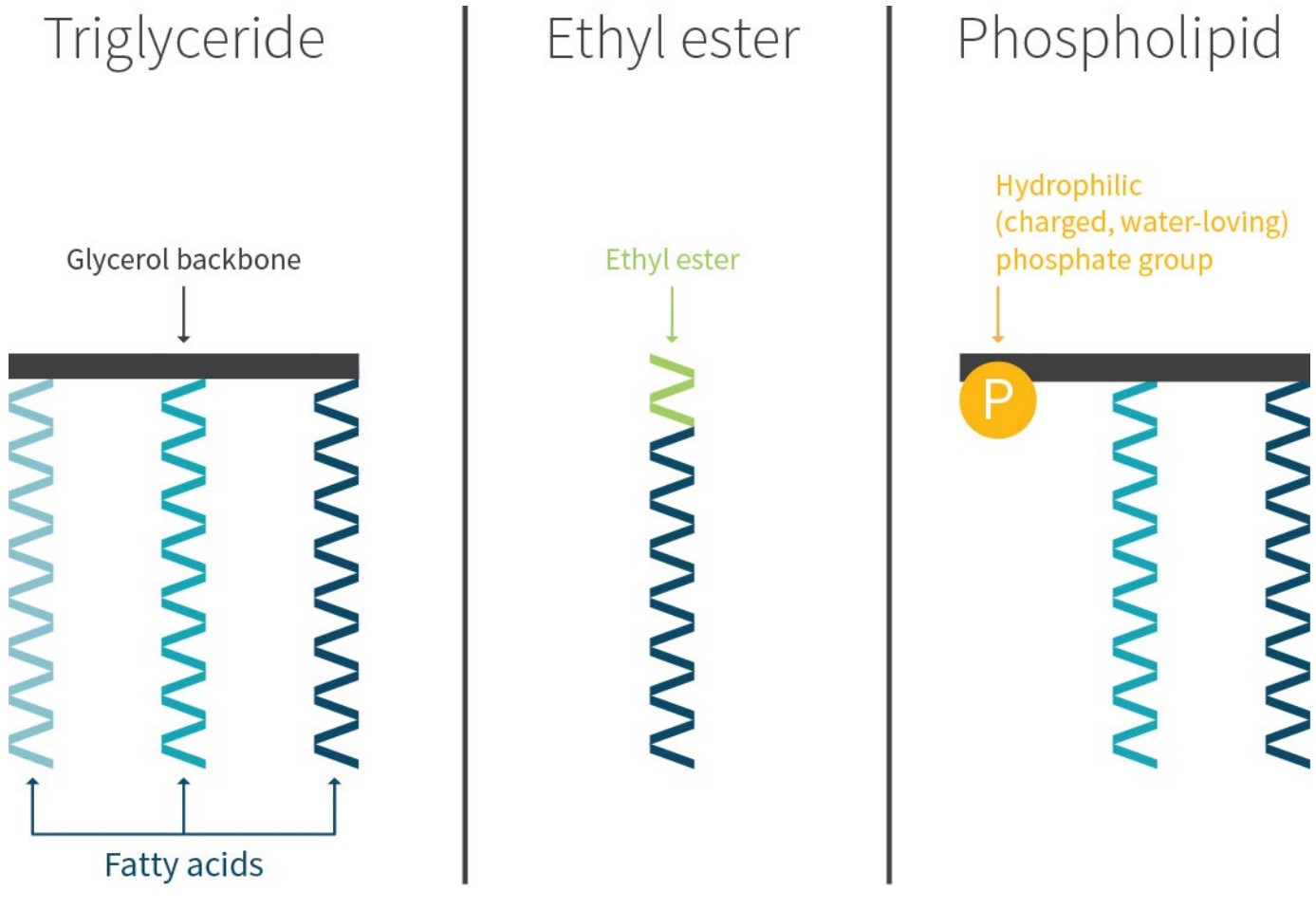
One finding of note was that the krill oil did not lower participant triglyceride levels (which are compared structurally to other fatty acids in Figure 3). Omega-3 fatty acids have been shown to be a potent treatment for decreasing serum triglycerides, but the participants in the study only experienced a tiny drop, from 105.4 to 104.5 mg/dL (1.19 to 1.18 mmol/L). This is most likely due to the fact that the participants' triglycerides were already in a healthy range at the beginning of the trial, and the dosage of omega-3 being administered was very low (230 milligrams of EPA and 154 milligrams of DHA). Trials that have shown large reductions were done on participants that already had high fasting triglycerides, who had been administered sizeable doses of EPA or DHA, usually around four grams of total fish oil. EPA tends to have a slight advantage in terms of lowering serum triglycerides.

While the krill oil did reduce insulin sensitivity, these effects may not have been brought about by its omega-3 fatty acid content. Rather, a yet to be identified protein could be the culprit. Omega-3 fats, particularly EPA, have the ability to reduce triglycerides, but only in people who have high levels and take substantial doses.

The big picture

The various studies that have looked at fish oil's effects

Figure 3: Three forms of fatty acids



on insulin sensitivity have been fairly equivocal to date. The study under review adds to the body of evidence that suggests omega-3 supplementation is unlikely to benefit insulin sensitivity. It is the first krill oil study to show significant harm to insulin sensitivity though. However, these findings have limited applicability, especially to women, as the studied participants were overweight but healthy middle-aged men of European ethnicity. Some studies have shown that some effects of omega-3s could differ between sexes.

It is not yet known if the decreases in insulin sensitivity caused by the krill oil would have long-term detrimental changes on health. Since the trial did not last long enough to give a concrete answer to this question, it would be advisable for people who fall into the "at risk" category for metabolic syndrome to seek out a fish or algal source of omega-3 oils (if they choose to supplement at all). Future research to identify which compound(s) in krill oil interfere with insulin sensitivity could lead to a better understanding of the different metabolic properties omega-3 oils can have, depending on their source. Identification of these compounds could also lead to manufacturing processes designed to remove them from the oil.

Outcomes like the krill oil-mediated insulin desensitization demonstrate why it is important to investigate krill oil and fish oil separately. As this study shows, they can have strikingly different results even when researchers are looking at the same endpoint. While fish oil is currently the more studied of the two, additional benefits of krill oil may be seen in future trials, as it contains the antioxidant astaxanthin and has may have greater EPA absorption compared to fish oil.

It is unlikely that omega-3 fatty acids can substantially improve insulin sensitivity. Due to krill oil's negative effects on insulin sensitivity, people at risk for metabolic syndrome should seek out different sources of omega-3 supplementation, such as fish or algal oil.

Frequently asked questions

I'm allergic to shellfish. Can I still take krill oil?

"[...] these findings have limited applicability, especially to women, as the studied participants were overweight but healthy middle-aged men of European ethnicity. Some studies have shown that some effects of omega-3s could differ between sexes."

The prevalence of shellfish allergy in the US has been reported at about 2% of the population.

There are trace amounts of protein in krill oil that could cause an allergic reaction. Two participants in the study under review had to withdraw due to reactions while on the krill oil supplement. Anyone with a shellfish allergy may want to seek out a non-krill oil source for their omega-3 supplementation, such as fish oil or algal oil.

Should I choose krill over fish oil since it is better absorbed?

As noted earlier, the EPA in krill oil may be absorbed better than fish oil. The current study showed that krill oil supplementation increased blood concentrations of EPA by 60% and DHA by 10%. The obvious choice may be to go with the krill oil, but at least in healthy people, there may be little difference between the effects of krill and fish oil. One study noted omega-3 blood concentrations between people taking either fish or krill were no different, nor were there any significant differences between markers of inflammation or oxidative stress. The doses used in this study may have confounded the results, as the krill group received 543 milligrams of omega-3 versus 864 milligrams of omega-3 in the fish oil group, but since blood concentrations of omega-3 were the same it indicates the krill oil may be more bioavailable. In the end, rather than fretting over which source may be minutely "better" for you, try to focus on finding a quality source for either type of oil and ensure your dosage is adequate.

What are the potential implications of unsuccessful blinding?

Blinding in clinical trials is a crucial aspect in eliminating sources of potential bias. This trial used a double-blinded protocol so neither the participants nor the trial investigators knew who was receiving which supplement. There are many benefits to this method for all blinded individuals. It makes participants more likely to comply with the full trial regimen and less likely to bias their psychical or psychological responses to the given supplement. For the researchers, blinding allows them to ensure similar and non-preferential treatment when interacting with the trial participants. Additionally, blinding helps prevent bias from creeping into the final assessments.

The blinding in this trial was unsuccessful, as the "fishy burps" experienced by many of the participants was a dead giveaway as to which supplement they were receiving. Although the participants stated they did not alter any diet or exercise habits that could affect insulin sensitivity, there remains the distinct possibility that they could have subconsciously made small, but significant changes. Participants may have slightly increased vegetable intake, daily activity, or reduced junk food, all of which can alter insulin sensitivity. Without successful blinding, the accuracy of the results of the trial can become skewed. Further replication trials would be needed to corroborate the findings.

What should I know?

Krill oil is a perfectly viable option for otherwise healthy individuals looking to supplement their diet with a quality omega-3 source. Until there is better data on the metabolic effects of krill oil, people with increased risk of type 2 diabetes or cardiovascular disease should stick with either a fish or algal source of omega-3. People with crustacean allergies should also seek out alternative sources. Additionally, omega-3 supplementation will likely not significantly improve insulin sensitivity or bring down your triglyceride levels unless they were elevated to begin with. ◆

Beet out your competition with dietary nitrate!

Dietary nitrate improves sprint performance and cognitive function during prolonged intermittent exercise

Introduction

If you thought the benefits of consuming beetroot were limited to the lowered blood pressure effect you read about in the ERD last March, guess again! Beetroot juice is also one of the hottest (relatively) new supplements that may provide an ergogenic boost during exercise. Nitrates in beets can be converted to nitrites, which then serve as a precursor for the production of nitric oxide, a signaling molecule that plays an important role in a number of physiological processes that can impact exercise performance, including muscle contractility, mitochondrial efficiency, and the regulation of blood flow to working muscles. This has led to athletes of all levels consuming beetroot juice in an attempt to improve performance.

Recent research has shown improved running, swimming, and rowing performance after beetroot juice consumption. Studies have frequently used short duration protocols, none of which mimic the longer durations typical of team sport play. To draw more relevant conclusions for athletes competing in sports like soccer, rugby, or field hockey, a study design that incorporates two 30-45 minute 'halves' is helpful.

Cognitive ability refers to a person's ability to react, make decisions, learn, and communicate. During intense exercise these skills, particularly reaction time, deteriorate. Along with the traditionally sought-after benefits of an ergogenic aid, like improved strength, power, and endurance, improved reaction time is something that could be of benefit to athletes in nearly every sport. Because nitrate supplementation has been shown to improve blood flow to the brain (particularly to areas associated with executive function) and enhance the blood flow response to visual stimuli, it is thought that nitrates could be beneficial during athletic competition.

As this had not been previously studied in a model of team sport performance, researchers attempted to study the effects of nitrate supplementation on cognitive performance during intermittent high-intensity exercise. Previous research has shown no improvements in cognitive function during moderate intensity cycling exercise after a single dose of beetroot juice supplementation. However, exercise tolerance in that study was increased by 16%.

The purpose of this study was to examine the effects of one week of dietary nitrate supplementation on exercise performance and cognitive function during a repeated sprint test protocol designed to reflect work and recovery patterns that typically occur during team sport play.

Beetroot juice is rich in nitrates, and may be useful as an ergogenic aid. However, most studies to date have tested only its shorter-term performance, which may not be relevant to longer-duration activities like rugby or soccer. Nor has there been much research on the effects of beetroot juice on mitigating the impact of physical activity on cognition and reaction time. The goal of this study was to test the effects of beetroot juice on performance and cognition during longer-duration physical activity.

Who and what was studied?

Sixteen male, recreational athletes who played field hockey, soccer, or rugby participated in this study. The participants were an average of 24 years old, 5'10" and 172 pounds, with a VO2 max of 50 mL/kg/min. To give some perspective, these were above average athletes but far from elite level. None of the participants had been taking any ergogenic supplements for six months prior to the start of the study.

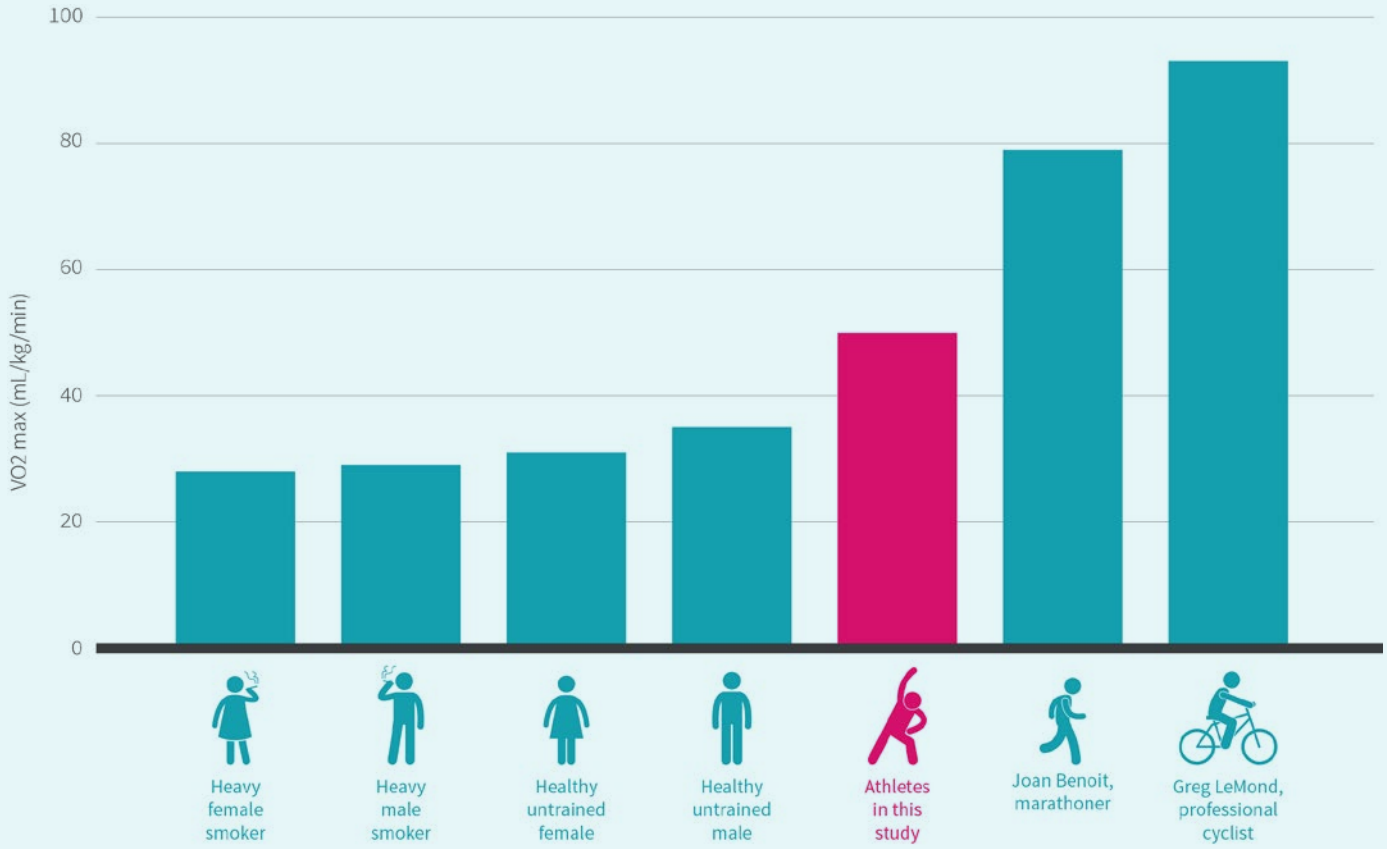
This was a double-blind, randomized study with a crossover design. A crossover design means participants were given either nitrate-rich (12.8 mmol of NO3−) or nitrate-depleted (placebo, 0.08 mmol NO3−) beetroot juice for seven days. After a wash-out period

VO2 max and fitness level

The majority of published studies indicate that nitrate supplementation has no significant effect on performance in highly trained athletes.

While there are varying ways to classify someone as elite, VO2 max, or the maximal volume of oxygen that can be consumed during exercise, is commonly used for comparison. Subjects in this study had an average VO2 max of 50 ml/kg/min, which is higher than most people, but far lower than the scores of 80-97 seen in the world's greatest athletes. Figure 1 shows VO2 scores of average people compared against subjects used in previous ERD sports studies.

Figure 1: VO2 max Measurements



of at least 10 days, they received the opposite drink (supplement or placebo) for seven days and repeated the tests. Participants consumed one serving each morning and evening for six days. On the seventh day, they consumed two servings 2.5 hours before completing an intermittent sprint test designed to simulate a team sport like rugby or football.

The test used two 40-minute "halves" of repeated two-minute blocks consisting of a six second maximal sprint, followed by 100 seconds of active recovery and 20 seconds of rest, on a cycle ergometer. A cycle ergometer is a stationary bike that can measure the amount of work done by the person pedaling. Twice during each half, participants completed five blocks of four second maximal sprints separated by 16 seconds of active recovery. During the recovery periods, as well as before, after, and during half-time, computerized tests were performed to determine the effects of fatigue (and the supplementation) on cognitive impairment during exercise. The tests alternated between the Stroop and Decision-Reaction tests.

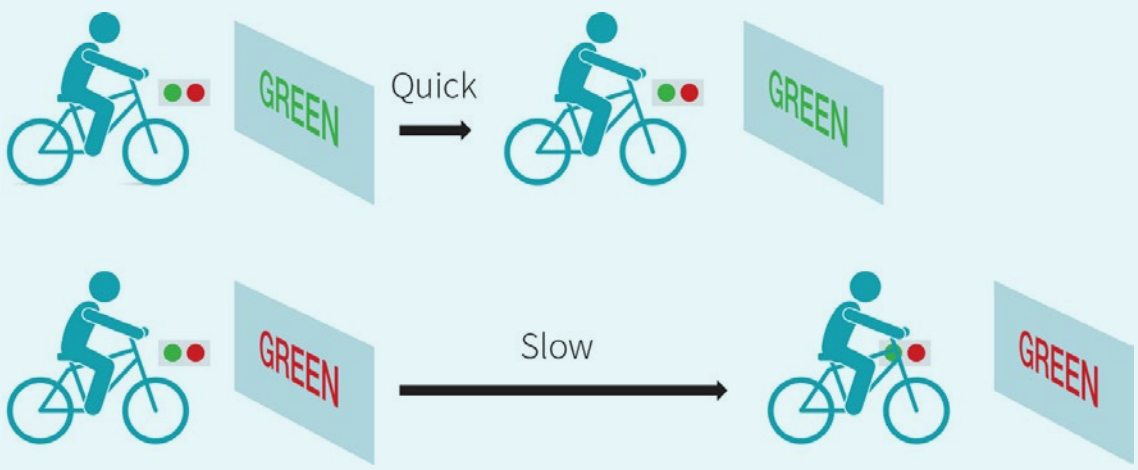
The Stroop and Decision-Reaction tests

Stroop test. This test (depicted in Figure 2) is used to measure information processing speed, executive abilities, and selective attention. The words 'RED,' 'YELLOW,' 'GREEN,' or 'BLUE,' or a non-color related word were presented on a screen, in a font color of red, yellow, green, or blue, which doesn't necessarily correspond to the word (e.g. "YELLOW" could be presented in a green font). Participants were instructed to press a button that corresponded to the color the text was written in, regardless of the color the word was describing. Words that correspond to their font colors are selected more quickly than non-corresponding words.

Decision-Reaction test. This test also studies information-processing speed. Arrows pointing either to the left or right are presented on a screen, either on the left or right side. At the top of the screen is a display that reads either 'LOCATION' or 'DIRECTION.' So, an arrow appearing on the right side of the screen with the header of 'LOCATION' required participants to press the right button on the control box irrespective of the direction of the arrow, while an arrow pointing to the right appearing with the header 'DIRECTION' required participants to press the right button irrespective of the location of the arrow.

Participants recorded their diet during the 24 hours before the first test and were asked to repeat that intake before the second. Participants were also told not to chew gum or use antibacterial mouthwash during the study, as this reduces the effects of dietary nitrate.

Figure 2: The Stroop Test



Sixteen male athletes participated in this randomized, crossover trial. Beetroot juice or nitrate-depleted beetroot juice as a placebo was consumed for seven days prior to performance testing, which consisted of two 40-minute "halves" consisting of sprints, active recovery, and rest on a cycle ergometer, on which power output was measured. Cognitive information-processing speed was also measured several times during the course of the study.

What were the findings?

The experimental results are summarized in Figure 3. Participants in the beetroot group completed about 3.5% more work overall during the repeated sprints than participants drinking the placebo (123 vs. 119 kJ). This difference was small, but statistically significant. This effect was due to greater work output in five of the 20 sprints during the first half, compared with placebo. In contrast, there were no differences between groups for total work done during the second half. Elevated plasma concentrations of nitrite (NO_2-)and nitrate (NO_3-) appear to be a requisite for any performance benefits seen with supplementation. Plasma concentrations of both were significantly greater after beetroot supplementation compared with placebo.

Although NO_2- decreased by the end of each half, it was still elevated compared with placebo.

Improvements in cognitive testing were seen with beetroot juice only during the second half. This protocol is fairly unusual in that researchers used two 40-minute halves to simulate game play. Reaction time on the Stroop test was the same in each group during the first half, but beetroot juice mitigated the slowing of reaction time by about 25 milliseconds in the second half, equal to a 3% improvement. While 3% may seem small, it can actually be very important during athletic competition. Further analysis showed that improvements in reaction time occurred during the final third of each half, when fatigue would be expected to have a greater effect. Accuracy of responses was not affected by supplementation, nor were there any differences in reaction time during the tests performed before, at halftime, or after the end of the sprint testing. No significant differences were seen between groups for the Decision-Reaction test, mean oxygen uptake (VO2), heart rate, or concentrations of blood lactate, glucose, sodium, or potassium.

It is unclear why differences were seen during the Stroop test but not the Decision-Reaction test.

Beetroot juice increased the total amount of work generated by about 3.5%, mostly during the first half of the experiment. It also improved reaction time by about 3%.

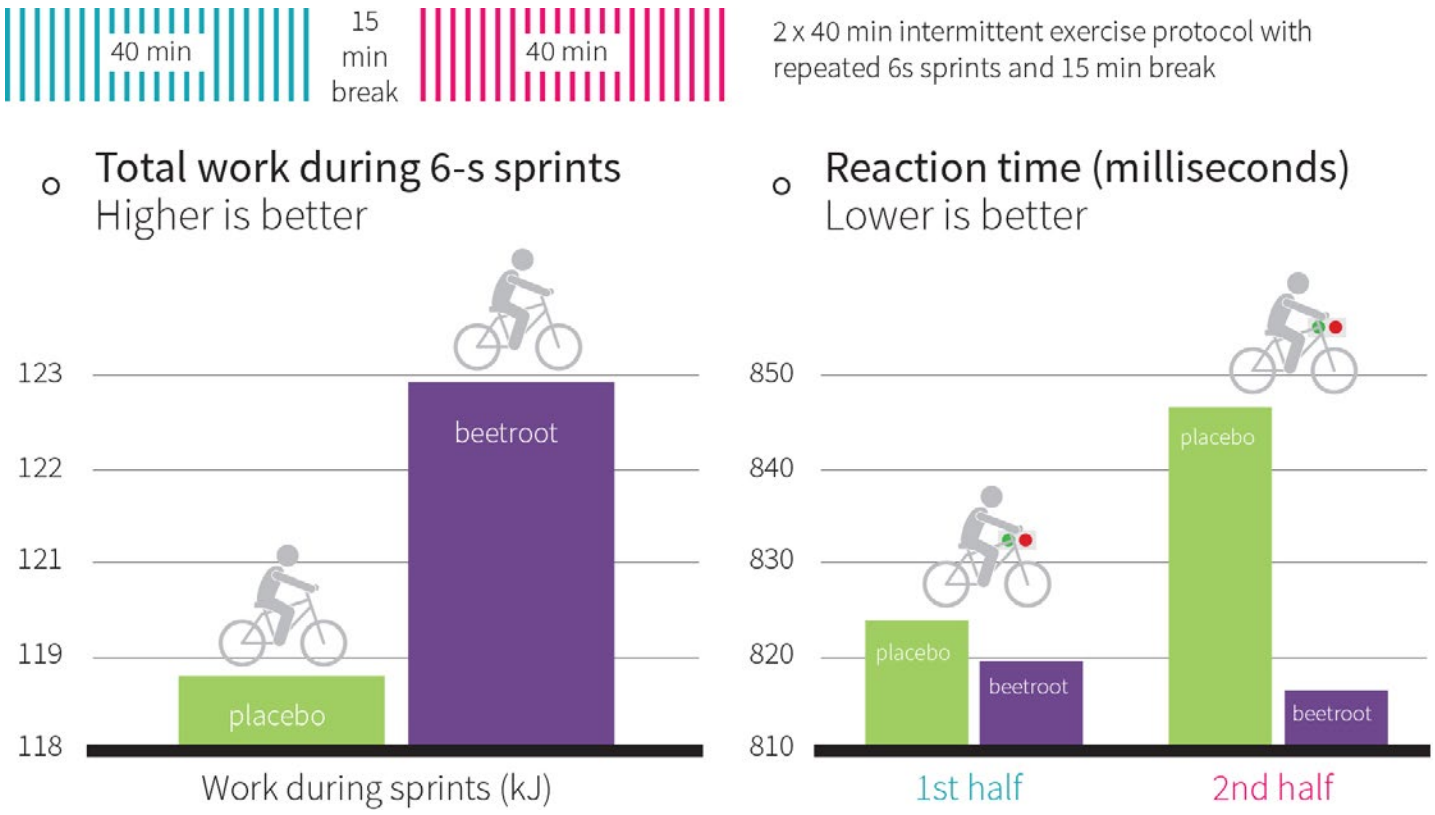
What does the study really tell us?

"These findings suggest that dietary nitrate enhances repeated sprint performance and may attenuate the decline in cognitive function (and specifically reaction time) that may occur during prolonged intermittent exercise."

Beetroot's ability to extend exercise tolerance at sub-maximal intensities, as well as during short-duration high-intensity intermittent exercise, has been well-documented. This is the first study, however, to mimic the repeated sprint-recover-rest intervals occurring over two 40-minute 'halves' that occur during many team sports.

This study shows that dietary nitrate supplementation

Figure 3: Summary of experiment and results



Adapted from www.mysportscience.com entry on 4/10/15

for seven days can increase the total amount of work performed during a repeated cycling sprint test. This study was done in younger athletes, so it is appropriate to consider that these effects might not extend to older non-athletes. Since this experiment was a lab test on a cycle ergometer, it can't accurately reflect the real-life dynamics of a sport like rugby or soccer, but it can attempt to provide a reasonably close approximation with regard to the work to rest ratios. This also allows the researchers to detect small differences that may not otherwise be seen if the participants were out on a field running around, but could still be important in edging out the competition.

The second aspect of this study was to determine if beetroot supplementation could improve reaction speed or accuracy. Successful athletic performance requires split-second decision making while performing at very high exercise intensities. While supplementation didn't improve reaction time at rest or early in the exercise period, it did prevent the decline in reaction time that occurred later in the exercise test for the placebo group, an effect which could be beneficial for competitive athletes.

> "Generally speaking, more impressive results are seen in untrained and recreationally trained athletes, compared with elite athletes."

While this lab study can't exactly reflect sports performance on the field, its design allowed for measuring small differences in work output and reaction time, which may not have been possible to measure on the field. The study design was also fairly effective at creating a sport-like environment involving multiple bouts of sprinting and resting.

The big picture

The area of research dedicated to the effects of beetroot juice and supplements related to nitric oxide on sports performance is rapidly growing. Overall, it has become quite clear that dietary nitrate can have beneficial effects. The most typical effects seen with supplementation are a reduction in the oxygen cost of exercise (less oxygen required to sustain a given work rate) and increased time until exhaustion during a submaximal effort. Reasons for performance enhancement can include improved mitochondrial efficiency, improved blood flow (thus, more oxygen) to the muscles, improved contractile function in type II fibers (fast twitch fibers, used for sprinting and explosive movements), or some combination of these.

It is also possible that the beneficial effects on performance may become more apparent during high-intensity exercise, when a greater percentage of fast-twitch muscle fibers are activated. These effects, however, are contingent upon appropriate dosage, duration and type of exercise performed, environment (temperature and altitude), and training status of the individual. For example, even among trained athletes, both positive and no effects from beetroot juice sup-

plementation have been reported. Generally speaking, more impressive results are seen in untrained and recreationally trained athletes, compared with elite athletes. For more in-depth reading, a number of good reviews are available on the subject.

In this study, the elevations of plasma nitrite and nitrate were higher than previous studies, likely due the higher dose of nitrate given. This supports the previously reported dose-dependent relationship between nitrate supplementation and plasma nitrate/nitrite levels, which occur up until a certain point and will then level off. The decrease in plasma nitrite during both the first and second halves of the test are consistent with the idea that plasma nitrite serves as a reservoir for nitric oxide production during times of low oxygen availability, like high intensity exercise.

The effects of supplementation seem to be diminished in highly-trained endurance athletes with a VO2 max of more than 70 ml/kg/min. Trained athletes demonstrate greater muscle blood flow and better metabolic efficiency during exercise (less oxygen is needed at a given workload) compared to untrained people. As dietary nitrate supplementation improves that efficiency, there is potentially less room for additional improvements in trained populations. Additionally, the effects of nitrate supplementation on muscle contractile efficiency and blood flow occur mainly in type II muscle fibers. As endurance athletes mainly use type I fibers (slow twitch), improvements in blood flow may not be observed. Furthermore, endurance athletes have higher levels of basal nitrite, which could mean higher doses of nitrate are required to observe any performance benefit.

Dietary nitrate may work as an ergogenic aid through multiple possible mechanisms. It also tends to have a dose-dependent effect, although it eventually levels off. Elite endurance athletes also see a more limited benefit.

Frequently asked questions

Do I need to follow a loading protocol or is a single dose effective?

While the benefits of supplementation are likely to depend on the type of activity performed, it appears that longer supplementation (and at larger doses) is more likely to be beneficial than acute (and smaller dose) supplementation. More research is needed to determine optimal dosing protocols for a given sport however, as both single dosage and loading have shown benefits.

A 2010 study that compared an acute dose (after 2.5 hours) with both five and 15 days of supplementation found that longer periods (15 days) of supplementation may confer greater benefits compared with one to five days. That same study also reported that improvements in exercise efficiency occurred as early as 2.5 hours after ingestion and were maintained throughout the 15 day study. A 2014 review article suggested that although chronic supplementation is not contraindicated, it may be best to avoid very high doses (10 mmol per day) for long periods of time (more than eight weeks). This is based on animal research suggesting a reduction in vascular function with long-term supplementation.

Also, while this hasn't been studied specifically studied, beets do contain low levels of fructans which can be problematic for people with IBS or other FODMAP intolerances. So if GI symptoms worsen while nitrate loading, then you may want to reconsider supplementation. Or consider other vegetables rich in nitrates, as seen in Figure 4.

What type of sports would benefit the most from supplementation?

People participating in most sports could find some benefit from dietary nitrate supplementation. Activities that are limited by oxygen supply to the muscles (i.e. high-intensity, short-duration) would be most likely to benefit from nitrate supplementation. In contrast, endurance athletes (particularly highly trained peo-

ple) may be less likely to benefit. However, recreational (non-elite) endurance athletes may find improvements due to the reduced oxygen cost of submaximal exercise with supplementation. It has also been observed that nitrate supplementation appears to be more effective in exercise activities in which the arms are utilized, such as rowing and kayaking, compared with cycling and running. This comparison is complicated by differences in the various study designs, however.

Do men and women respond differently?

As usual, studies on this topic have more often been done on males, but it is certainly worth trying out beetroot juice if you're a woman, as there don't appear to be downsides to short-term supplementation. A 2014 study reported an improvement in oxygen cost of exercise during cycling in females, while a 2015 study on female athletes using a combination of sodium phosphate and beetroot juice on repeated sprint ability found that it was the sodium phosphate, and not the beetroot juice, that led to improvements in performance (which brings to mind the sodium phosphate article in ERD #4 from last February).

What should I know?

Seven days of supplementation with beetroot juice led to an improvement in total work completed during a repeated cycle sprint test that was designed to reflect the start-stop nature of team sport play. Additionally, supplementation improved reaction time during cognitive tasks without changing the accuracy of the responses. This study adds to the growing body of evidence suggesting that beetroot juice can be an effective ergogenic aid in sports requiring repeated sprint–recovery efforts, and may also reduce the decline in reaction time that typically happens during prolonged intermittent exercise. ◆

Figure 4: Nitrate content of some common vegetables

Source:
Hord et al. Clin Nutr. 2009 Jul.

Got Milk(fat globule membrane)?

Potential role of milk fat globule membrane in modulating plasma lipoproteins, gene expression, and cholesterol metabolism in humans: a randomized study

Introduction

Cardiovascular disease (CVD) is a general term for any pathological condition that involves the heart or blood vessels. Many of these diseases, and certainly those most commonly associated with Western societies, are a result of atherosclerosis – the thickening of artery walls through the buildup of plaques of fatty material. Although CVD was once thought to be primarily due to elevated cholesterol levels, it is now recognized that inflammation of the arteries is a necessary prerequisite for plaque formation.

That said, LDL infiltration of the artery walls is a major cause of inflammation, and there is little debate among the medical community that high levels of LDL-cholesterol (LDL-c) is a risk factor for CVD. Two recent meta-analyses support this view. The first looked at over 38,000 patients taking statins and found a significant reduction in risk as LDL-c levels moved from above 175 mg/dL to below 50 mg/dL. Achieving an LDL-c below 100 mg/dL through statin therapy was associated with a 44% reduced risk of having a major CVD event, while levels below 50 mg/dL were associated with a 56% reduced risk. The second analysis looked at data from over 10,000 patients enrolled in 24 randomized, placebo-controlled trials of PCSK9 inhibitors and found that treatment reduced LDL-c by nearly half while simultaneously reducing the number of heart attacks by 51% and the odds of death from any cause by 55%.

What these studies serve to illustrate is that we now have two completely different drug therapies, statins and PCSK9 inhibitors, which reduce LDL-c by different methods and reduce the risk of CVD-related events. It stands to reason that other methods that reduce LDL may also reduce the risk of CVD. A first line of defense for the management of blood cholesterol levels is dietary intervention.

Dairy fat is typically around 70% saturated fat and makes up about a fifth of total saturated fat intake in the U.S. diet, making it a prime target for nutritional interventions. However, results from observational and experimental trials that evaluate the impact of dairy products on blood cholesterol levels are not conclusive. Notably, there is a lot of evidence from randomized controlled trials that diets high in saturated fat derived largely from butter fat increases LDL-c, whereas cheese intake results in lower LDL-c compared with butter of equal fat content, and may not increase LDL compared with a diet lower in saturated fat. Similarly, the results are fairly consistent in showing that whole milk

“ Dairy fat is typically around 70% saturated fat and makes up about a fifth of total saturated fat intake in the U.S. diet, making it a prime target for nutritional interventions. ”

increases LDL-c more than low-fat or skim milk, while whole-fat yogurt may reduce LDL-c.

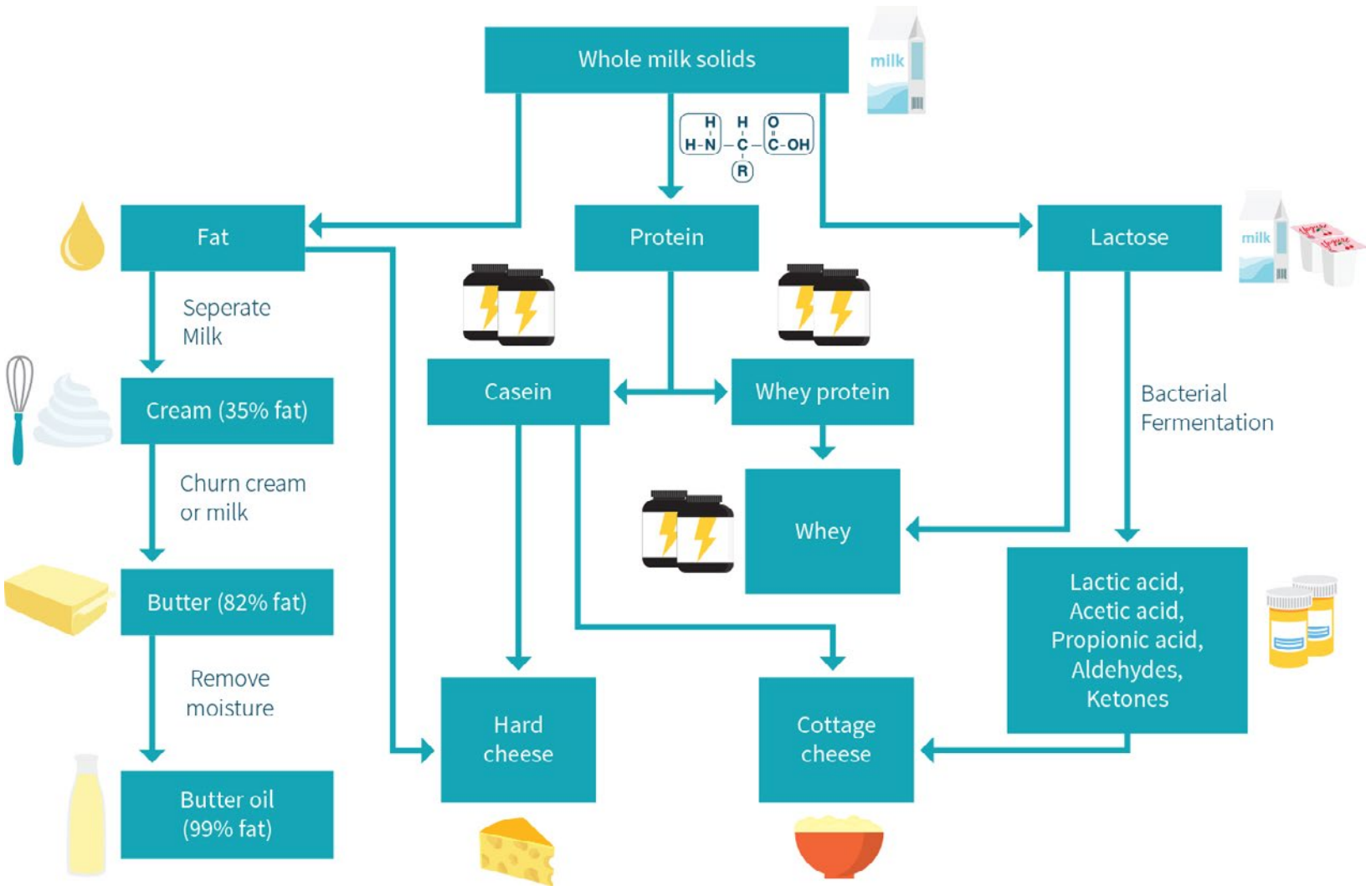
The inconsistent findings with regard to dairy fat's effects on blood cholesterol have been hypothesized to be owed, at least in part, to the milk-fat globule membrane (MFGM) – a three-layered membrane composed of proteins, lipids, and numerous minor bioactive sterols that encloses the milk fat globules. Figure 1 shows the basic processes used to make different dairy products. The MFGM is a very fragile compound that is preserved in cream and cheese, but destroyed during mechanical processing, such as the churning required to make butter or the homogenization of milk. It has been suggested to have numerous health benefits, including cholesterol-lowering effects. The current study was an attempt to test the hypothesis that the effect of dairy fat on serum cholesterol levels is mediated by the presence (or absence) of the MFGM.

Observational and experimental evidence investigating the effect of dairy fat on serum cholesterol levels is conflicting and depends on the dairy product consumed. An intact milk-fat globule membrane (MFGM) present in some dairy foods, such as non-homogenized cream and cheeses, may explain the inconsistencies. The study under review tested this hypothesis.

Who and what was studied?

Local advertising at Uppsala University Hospital, Sweden was used to recruit overweight but otherwise healthy men and women to undergo an eight-week, single-blinded randomized trial. The participants were 50-65 years old, had an average BMI of 28, did not regularly engage in heavy exercise (more than 3 times per week), and had no abnormal blood chemistry. After being stratified by baseline sex, age, LDL-c, and habit-

Figure 1: Dairy products in a nutshell



ual dairy intake (high vs. low), the participants were randomized to a MFGM or control group. Stratification means that the participants were put into categories based on the aforementioned variables, helping ensure an even distribution between groups. A total of 46 people completed the study (26% men and 74% women).

The dietary interventions were essentially the same except for the source of dairy fat. Both groups consumed 100 grams (just under ½ cup) of fat-free milk and one scone prepared by the research staff. The MFGM group also consumed 100 grams (about 6.5 tablespoons) of whipping cream (40% fat) per day, which was confirmed to have intact MFGMs, while the control group consumed 40 grams (about three tablespoons) of butter oil that had no MFGMs present, and a few grams of whey protein isolate to match up the protein and calcium content of the groups. The butter, oil, and whey were baked into the scones of the control participants. All participants were allowed to eat their food however and whenever they wanted through the day, provided the whipping cream was not heated, mixed, or whipped.

The participants were all free-living adults who visited the research clinic weekly for weight measurement, food distribution, and general support. They were instructed not to change their usual dietary habits, but to avoid consuming any dairy or margarine products not provided by the researchers.

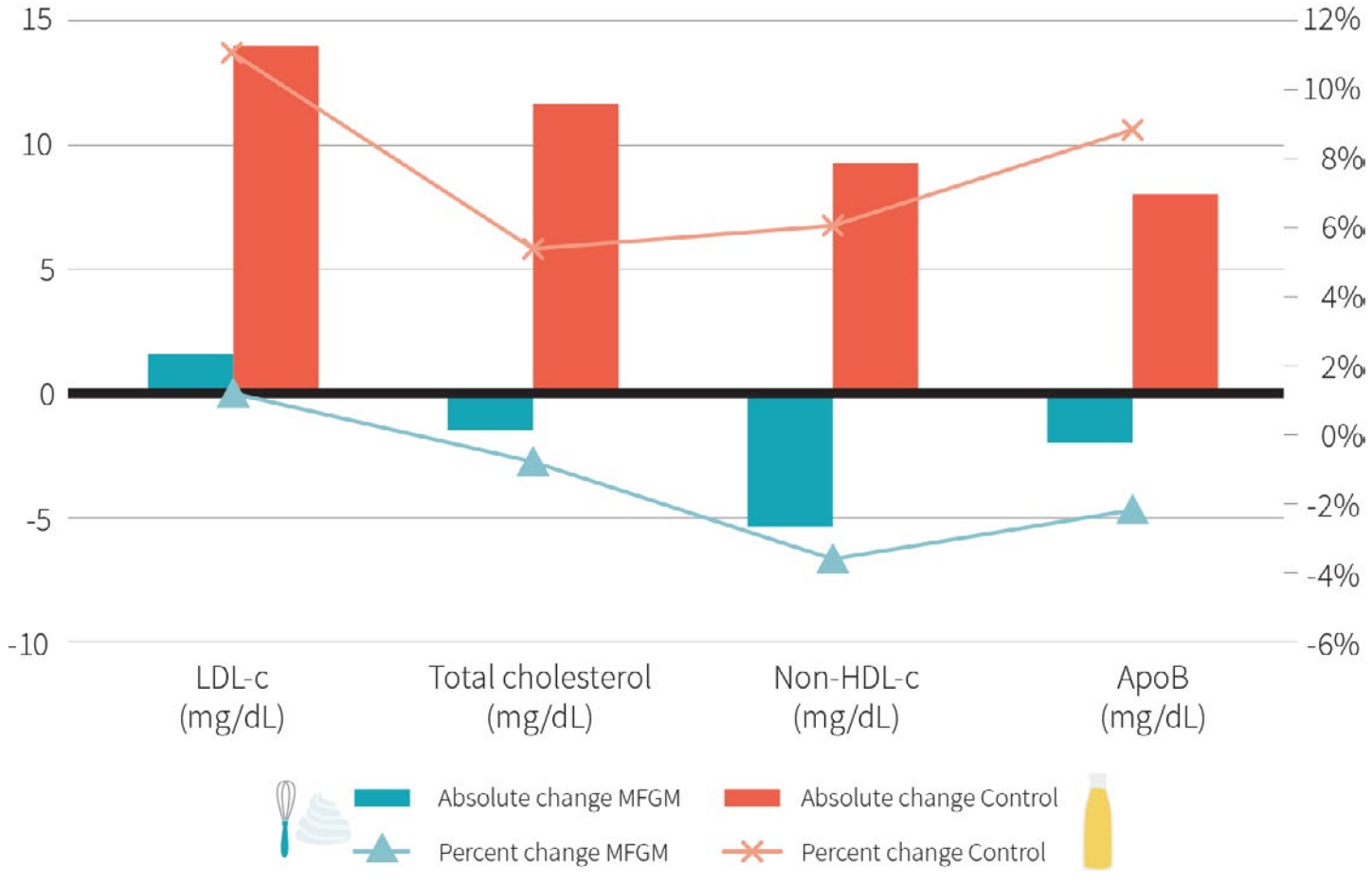
Table 1: Nutrient content of the intervention food items

	MFGM diet	Control diet
Energy, kcal	805	794
Carbohydrate, g	88.9	86
Fat, g	41.8	41.4
Protein, g	16.7	16.7
Phospholipids, mg	19.8	1.3
Cholesterol, mg	100	120
Calcium, mg	687	677

What were the findings?

The researchers evaluated numerous metabolic biomarkers, but the treatment affected only a few, which are shown in Figure 2. While the control group showed

Figure 2: Differences between study groups



a rise in total-, LDL-, and non-HDL-cholesterol and apolipoprotein (Apo) B, the MFGM group did not, and there was a significant difference between the groups after the eight week intervention.

The researchers also analyzed peripheral blood mononuclear cell (PBMC) gene expression in the women of each group and found the expression of 19 genes to be significantly reduced in the MGFM group and increased in the control group. Changes in most of these genes correlated with changes in one or more of the changes in blood lipids. Though the scientific understanding of most these genes is poor, some of them have been implicated in the regulation of the cell life cycle, including apoptosis (programmed cell death), and in the regulation of protein breakdown within cells.

What does this really tell us?

This is a limited but informative study. It shows that replacing the habitual dairy fat intake of older overweight-obese Swedish men and women with 40 grams of dairy fat from pasteurized but not homogenized cream has no effect on blood cholesterol levels and down-regulates the expression of numerous PBMC genes. However, replacing dairy fat with butter oil significantly increases blood cholesterol levels and PBMC gene expression. The small sample size, ethnical and geographical homogeneity, and inability to know how, when, and with what exactly the test products were consumed are significant limitations to the study. Although the presence of the MFGM in the cream is a plausible explanation for the outcomes, it is also possible that the physical state of the fats (fat globules vs. isolated fat) influenced the results.

Nonetheless, a strength of this study is that the results are directly translatable to common foods, although the effects may have been different if butter, rather than butter oil, was used in the control group. Still, the LDL-c-raising effects of butter oil are in line with those observed with butter. Of the most commonly consumed sources of dairy fat, butter and butter oil have the lowest MFGM content (see FAQ).

> “The small sample size, ethnical and geographical homogeneity, and inability to know how, when, and with what exactly the test products were consumed are significant limitations to the study.”

Another strength of the study was that plasma phospholipid and cholesterol fatty acid composition were unchanged in both diets throughout the intervention without any differences between the diet groups, suggesting that the milk fat dose used (40 grams a day) was

similar to the habitual dairy fat intake of the participants. This helps minimize the possibility that changes in dietary fat intake influenced the results.

In sedentary, overweight-obese Swedish people, consuming pasteurized but non-homogenized cream instead of butter oil prevented increases in blood cholesterol concentrations, possibly due to the intact MFGM within the cream. However, the small sample size and lack of geographic and ethnic diversity makes it difficult to generalize the results of this study.

Big picture

The potential mechanisms through which MFGM counteracts the cholesterol-raising effects of dairy fat are not well established, but animal models suggest that it involves reduced cholesterol absorption or phospholipid-induced alterations in liver gene expression. For instance, rats fed a high-fat diet supplemented with MFGM phospholipids display a 15% to 30% increase in fecal cholesterol excretion and a 20% to 60% decrease in liver cholesterol. In a separate rodent study, where the consumption of phospholipids was set to the estimated intake of a typical human, overall and per meal cholesterol absorption were reduced by half. Although the current study did measure surrogate markers of cholesterol absorption and synthesis and found no changes among the participants, the possibility of reduced cholesterol absorption and increased cholesterol excretion cannot be ruled out until more direct measurements in humans are made.

Rats that consume a high-fat diet supplemented with MFGM-rich milk extract display a reduction in liver fat accumulation and blood lipid levels attributed to a significant reduction in the expression of hepatic genes that regulate cholesterol synthesis (HMG-CoA reductase), bile acid synthesis (cholesterol 7α-hydroxylase), and fatty acid synthesis. This is in accord with the current study, which found all 15 tested PBMC genes to be down-regulated in the MFGM group. It has been suggested that PBMC gene expression after dietary interventions reflect changes within the liver and can be used for studying the response of certain genes related to fatty acid and cholesterol metabolism.

“ The potential mechanisms through which MFGM counteracts the cholesterol-raising effects of dairy fat are not well established, but animal models suggest that it involves reduced cholesterol absorption or phospholipid-induced alterations in liver gene expression. ”

The current study is novel in its attempts to test the MFGM hypothesis, but falls short of providing concrete evidence that the group differences are owed to it. Unfortunately, there are no other human studies on the MFGM. The main confounding variable was the different dairy sources used, which could be overcome in future trials by using products only differing in their level of processing (e.g., homogenized vs. non-homogenized whole milk). Of course, adding more strict dietary control would also aid in isolating the MFGM. Allowing the consumption of MFGMs through their natural food sources provides a degree of generalizability to the foods themselves. Future trials should also evaluate if supplementation with a MFGM extract would have similar effects on blood cholesterol and interact with other fat sources in the diet.

> “The world enjoys gravitating to the extremes of issues, and blood cholesterol levels are no exception.”

Frequently Asked Questions

If inflammation is a necessary prerequisite for atherosclerosis, then why worry about LDL-c?

The world enjoys gravitating to the extremes of issues, and blood cholesterol levels are no exception. Although many “anti-mainstream” nutrition enthusiasts claim that hypercholesterolemia doesn’t play a role in heart disease, understanding how arterial plaque forms may aid in illustrating why we should care about LDL-c and especially LDL particle count (LDL-p).

After an artery is damaged through any of the many risk factors for CVD (hypertension, free radicals, etc.), it begins to express certain proteins that allow for the accumulation of white blood cells. As the white blood cells arrive, they begin releasing chemicals (cytokines; this is the inflammation part) that signal more of their brethren to stop by. Under normal circumstances, the damage would resolve and the blood cells would leave: mission accomplished. However, under conditions of continuous damage through, for example, chronic inflammation, the artery will never fully repair and the white blood cells will continue to accumulate. This is where LDL-c and LDL-p come into play.

LDL-cholesterol enters the damaged area, where it is more prone to becoming oxidized. The oxidation signals to the white blood cells that they need to eat it, so as to protect the body. But this LDL-c-engulfing process turns white blood cells into “foam cells,” which can be thought of as obese white blood cells. That is, they are giant lipid-filled cells that can’t function properly and are ultimately part of what forms the plaque seen in atherosclerosis. If there there are fewer LDL particles with overall lower LDL cholesterol in the blood, then there is a reduced likelihood of it entering the damaged area and being oxidized and consumed. Similarly, if someone has a boatload of LDL-c in their blood along with high LDL-p, there is a far greater likelihood that some of it will become oxidized and consumed by white blood cells, even if the area is only temporarily (i.e. no systemic inflammation present) damaged or not damaged at all. So, the best bet against heart disease is keeping both inflammation and LDL-c / LDL-p low.

What dairy products contain an intact MFGM?

The MFGM in dairy fat prevents lipid droplets from

grouping together, therefore they remain dispersed in the milk. Only after destruction of the structure of the MFGM through mechanical force like churning do lipid droplets aggregate and subsequently form large fat clumps (i.e., butter). This is why MFGMs are only present in dairy products that contain dairy fat and have not been mechanically altered through churning or homogenization. For example, whole milk, cream, and cheese all contain MFGMs, whereas butter, butter oil, butter milk, whey protein, and any fat-free dairy products do not.

That being said, MFGM is only one of the many differences between dairy products that may influence their differential impacts on heart disease. For example, cheese and milk are more nutrient-dense than butter. Additional differences are shown in Figure 3.

What is the difference between butter and butter oil?
Butter oil is the fat concentrate obtained primarily from butter or cream by the removal of practically all the water and proteins. The terms anhydrous milk fat, dry butterfat, and dehydrated butter fat are used synonymously with butter oil, but the raw material used for their preparation is mainly cream. Ghee is also a form of butter oil.

What should I know?

Non-homogenized milk fat (cream) does not increase cholesterol levels, which, ironically, is the main reason we are told to avoid high-fat dairy foods. The MFGM may explain the inconsistencies of observational and experimental studies evaluating the impact of dairy fat on blood lipid levels, and this study provides preliminary evidence to support this hypothesis. However, no concrete conclusions can be made because of a handful of study limitations, such as the physical state of the dairy products used. Animal research suggests any potential effects may be owed to the ability of the MFGM to reduce cholesterol absorption and synthesis and increase excretion. ◆

Figure 3: Differences between butter and cheese impact health effects

	CHEESE	BUTTER
CHOLESTEROL EFFECT	LOW/ MODERATE	MODERATE/ HIGH
MFGM	HIGH	LOW
PROTEIN	MODERATE	ABSENT
VITAMINS/ MINERALS	MODERATE	LARGELY ABSENT
FERMENTATION	PRESENT	ABSENT

Vitamin (K)ardiovascular health?

Menaquinone-7 supplementation improves arterial stiffness in healthy postmenopausal women. A double-blind randomised clinical trial.

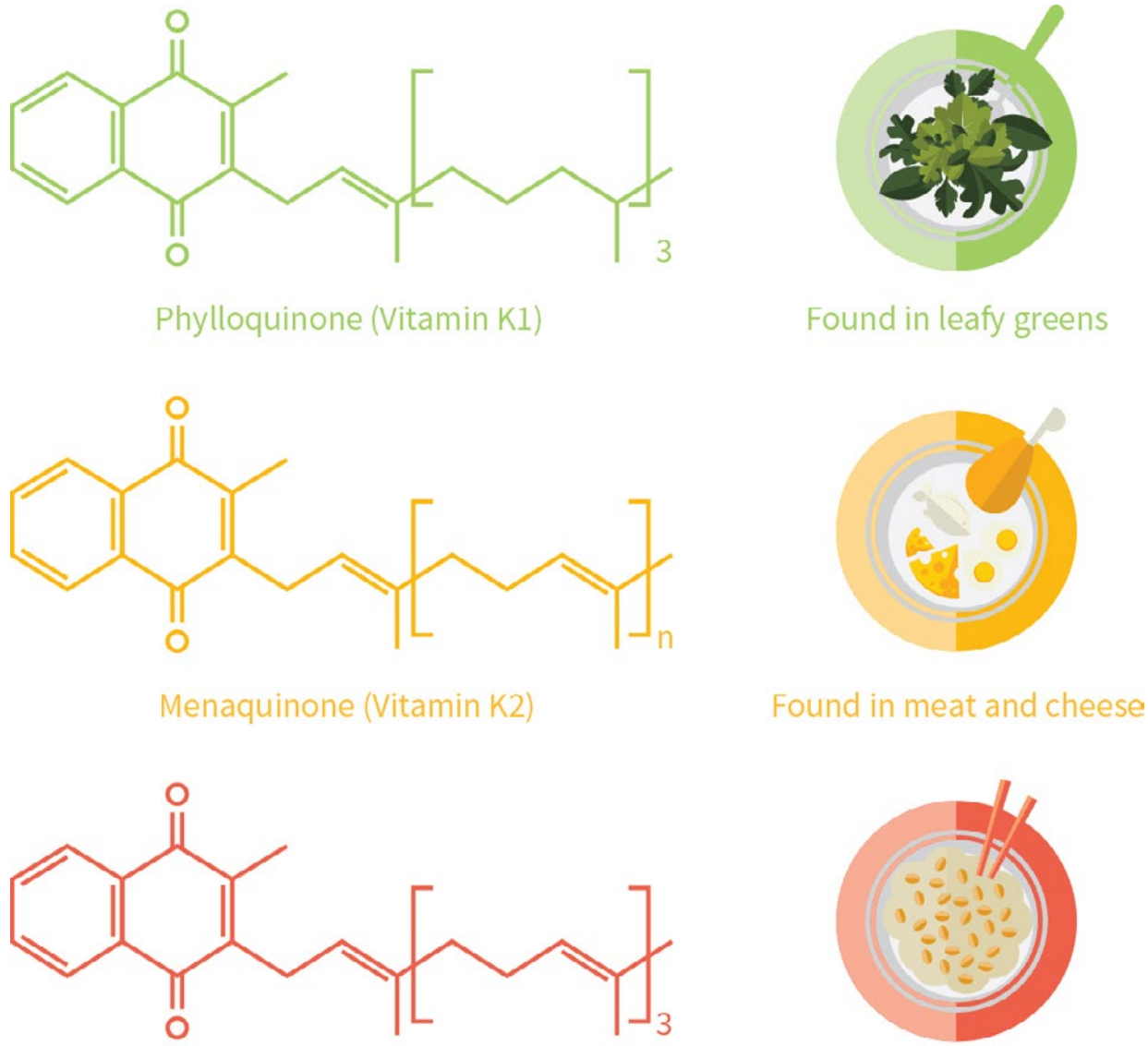
Introduction

As you age, there's a lot to look forward to: career development, watching your children and grandchildren grow, and retirement, are some things that come to mind. But along with the good, there's also the bad. For post-menopausal women, an increased risk of heart attack or stroke is among the bad. The drop in estrogen after menopause is an independent risk factor for cardiovascular disease in women. Hormone replacement therapy comes with its own set of risks and its efficacy at reducing cardiovascular disease is controversial, though. So, is there another way to lower cardiovascular disease risk in women?

Some studies are starting to suggest that vitamin K may be one possible route. There are different forms of vitamin K (shown in Figure 1) known as *vitamers*. Vitamin K1, also known as phylloquinone, is found in leafy green vegetables. There is also a group of vitamin K2 vitamers, known as the menaquinones (MKs). This is a family of molecules that differ through how many isoprene units make up their side chain. The name of a specific type of vitamin K2 is based on the length of this side chain. So, vitamin K2 with seven units in its side chain is called MK-7. The forms of vitamin K2 have different physiological effects than vitamin K1, which largely benefits clotting and was named for the German term "koagulation."

Recent observational evidence suggests that dietary intake of MKs may be associated with a lower risk of cardiovascular disease. Interestingly, these studies found no such association with the intake of vitamin K1. Another observational study found that the higher-chain MKs, specifically MK-7, MK-8, and MK-9, were particularly associated with decreased cardio-

Figure 1: The K Vitamins



vascular disease risk. Many of the MKs, especially the longer-chain ones, are found in meat and cheese, with MK-7 mostly found in a type of fermented soybean from Japan called natto (a food that is unappetizing to those unaccustomed to the slimy texture and funky taste), although it can also be found increasingly as a dietary supplement.

In terms of interventional trials for the prevention of cardiovascular risk factors, only vitamin K1 has been tested to date, with mixed results and at five to ten times the recommended daily allowance. One trial found some effect on blood vessel elasticity when combined with vitamin D, and another found no effect in its primary endpoint measuring artery hardening, but found some promise in a subgroup analysis of those who were highly adherent to the supplementation regimen.

For vitamin K2, things look different, though. The observational studies mentioned above suggest that vitamin K2 intake that is associated with decreased cardiovascular disease risk, not K1. Furthermore, one of the MKs, MK-7, has been found to have properties that suggest it would make a better supplement than vitamin K1. Specifically, it lasts longer in the blood stream, is absorbed well, and may be more potent by some measures such as inducing bone-building. But up until now, no interventional studies tested the effect of MK supplementation on cardiovascular outcomes. The purpose of the trial we're reviewing here was to fill this hole in the research.

There are two different kinds of vitamin K: vitamin K1 and vitamin K2. Vitamin K2 also has different subtypes depending on the length of its chemical side chain. These are known as the menaquinone Ks, or MKs. Observational studies have suggested that a high dietary intake of MKs are associated with a reduced risk of cardiovascular disease, but this hypothesis has not been tested in a clinical trial up until this one.

Who and what was studied?

The study involved 244 healthy post-menopausal women between 55 and 65 years of age. However, the trial was actually not designed around measuring vascular outcomes. Instead, the main hypothesis being tested was to see whether MK-7 affected the rate of bone loss in this population. During the bone study, measures of vascular health, to be discussed below, were also taken to explore MK-7's effects on the vascular system. In other words, the vascular health hypothesis was an add on to this study - two studies were being conducted simultaneously, but the study was primarily designed around MK-7's effects on bone health.

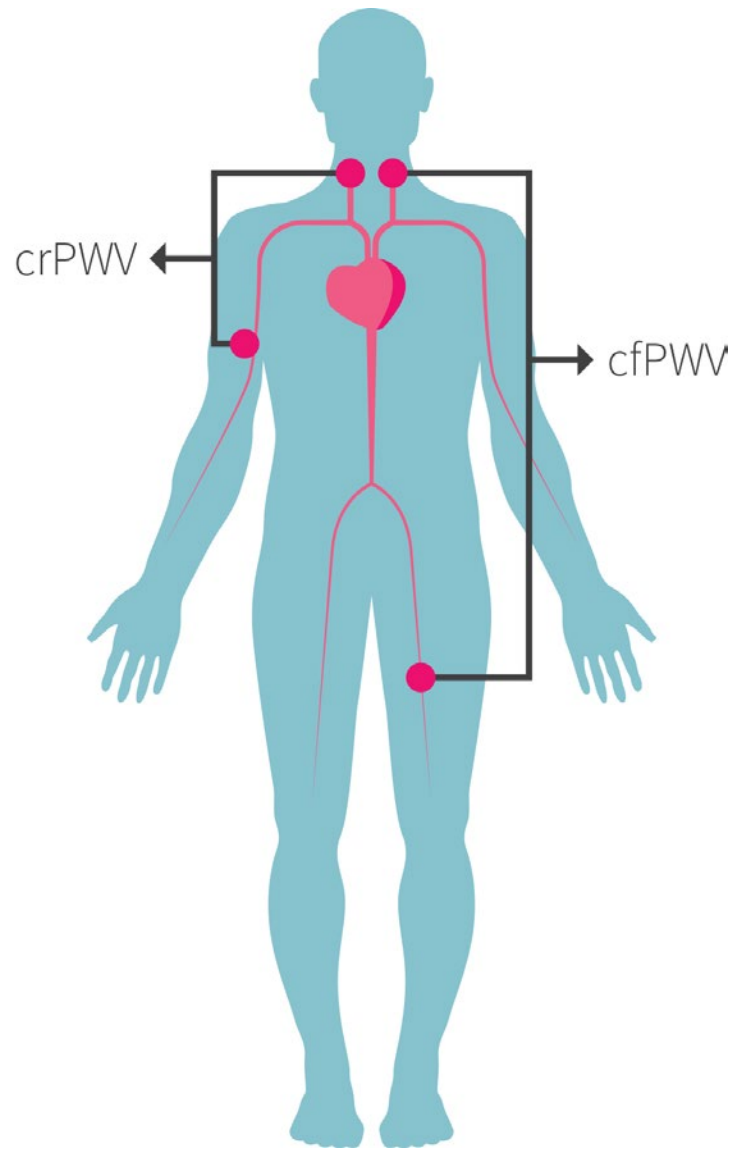
The women were randomly assigned to either take placebo or 180 micrograms of MK-7 once daily at either breakfast or dinner for three years. While this study wasn't completely designed around measuring vascular outcomes, the authors of the study had good reason to suspect that the dose of MK-7 used may have cardiovascular effects. They knew from a previous study that MK-7 is about three times more effective in facilitating blood clotting than vitamin K1. And they also knew from another study that 500 micrograms per day of vitamin K1 slowed coronary artery calcification (hardening of the arteries) in older adults. Putting these two facts together gave the authors some reason to think that the dose of MK-7 they were using would be effective.

No other restrictions or instructions concerning diet were given to the women. They came to the clinic once a year to have measurements taken. The measurements fell into three groups: measurements of regional arterial stiffness, measurements of the local stiffness and elasticity of the common carotid artery (the main artery supplying the head and neck with its blood supply), and blood work.

"Regional arterial stiffness" is a measure of how stiff an artery is across an entire region. Arteries can run in pretty long stretches, and so this measure takes

a long stretch of artery and gets its average stiffness. This was measured using pulse wave velocity (PWV) through two regions shown in Figure 2: from the femoral artery to the carotid artery (cfPWV) and from the radial artery in the arm to the carotid artery (crPWV). The concept of PWV was explained in ERD#6 in the Introduction of "Blueberries every day keeps high blood pressure at bay." Briefly, it measures how long it takes for a wave a pressure to travel through the walls of the artery. The more stiff the artery is, the faster the wave will travel. The cfPWV is considered the "gold standard" measurement of arterial stiffness overall. Also, since the pulse wave travelling through this section of the arterial system passes through the aorta, its travel speed is a function of aortic stiffness as well. This is a big deal, since the aorta is the major artery of the body through which the heart pumps blood to the entire body. For this reason, the cfPWV can be considered the primary outcome of this study.

Figure 2: How cfPWV and crPWV are Measured



Local carotid artery stiffness was also measured a few different ways. Its local PWV was measured. Also, ultrasound was used to measure and calculate several of its properties, such as its diameter, how its diameter changed when the heart beat, and how elastic the artery was. While we won't discuss every measurement of the carotid they made in detail here, one warrants further mention: the stiffness index β. This number is calculated by seeing how much the carotid artery changes in diameter under systole (the pressure in the blood vessel when the heart ventricles are contracting) relative to diastole (the pressure when the heart is relaxed). Overall, it's a measure of how much the carotid artery changes in diameter under pressure. A lower number means the artery is healthier since it's less stiff.

Finally, blood draws were done to test for three different things: inflammation, endothelial dysfunction, and vitamin K status. Inflammation as a whole was examined by measuring several different markers for inflammation in the blood and averaging the results together. This was done in order to increase the power of the study to find a difference between the MK-7 and placebo groups. Markers for endothelial dysfunction (damage to the inner lining of arteries) were measured and averaged together in a similar way. Vitamin K status was studied by measuring plasma levels of a protein called desphospho-uncarboxylated matrix Gla-protein (dp-ucMGP). This is an inactive form of the matrix Gla-protein (MGP). It is activated by a chemical reaction called carboxylation, which is mediated by vitamin K. Thus, the more dp-ucMGP there is in the blood, the worse vitamin K is doing its job, which is why it's a good marker for vitamin K status. Activated MGP is responsible for preventing hardening of the arteries by inhibiting calcification, so the more of the inactive form there is, the less elastic the arteries are. This relationship between dp-ucMGP, arterial calcification, and cardiovascular disease has been observed in several studies.

Healthy, post-menopausal women supplemented either placebo or a form of vitamin K2 known as MK-7 for three years at a dose of 180 micrograms per day. Regional and local stiffness of their arteries were measured as well as blood markers for inflammation, arterial damage, and vitamin K status.

What were the findings?

The authors found some interesting correlations between the measurements at baseline, before supplementation began. In particular, they found an association between markers of inflammation, which is thought to contribute to the development of arterial stiffness, and cfPWV, the "gold standard" measurement of regional arterial stiffness. They also found that that higher levels of dp-ucMGP (indicating poorer vitamin K status) were correlated with inflammation and cfPWV. These baseline measurements are just correlations, though, they don't tell us if changing vitamin K status would lower cfPWV or inflammation. That's what the rest of the trial was designed to test.

After three years of supplementation, the authors found that the stiffness index β decreased significantly compared to placebo. Also, the levels of dp-ucMGP in the MK-7 group dropped by 50% compared to the placebo group. This means that treatment indeed improved vitamin K status. Aside from these changes, though, every other measurement in the MK-7 group was not statistically different from placebo, including all other measurements of local carotid artery stiffness, the blood markers of endothelial damage or inflammation, and the PWV measurements. The difference in the major measure of regional aortic stiffness, cfPWV, was of "borderline significance" ($p = 0.087$). The MK-7 group's cfPWV dropped compared to placebo, but this drop did not meet the researchers' pre-determined significance cutoff.

Since the main outcomes of the study weren't very strong, the researchers decided to delve into the data a little more after the study was completed to see if any subgroups may have responded to MK-7 more than others. The results from this post hoc (Latin for "after the fact," which means that the researchers didn't plan for this in advance) analysis turned up something a little more promising.

Why post hoc analyses should be taken with a grain of salt

The old saying "seek and ye shall find" is meant to be encouraging. However, in science, it's not. Humans (and scientists are indeed human!) are great at finding patterns where there may be none, and this pattern-seeking can easily be fueled by wanting to find something. Even if it isn't there.

One way to help curb this tendency in science is to lay out the rules of a study ahead of time. Researchers will state exactly what they'll take to be a positive result before they do an experiment so they can't change the rules of the game halfway through to get the results they want. However, that's essentially what post hoc analyses are. They offer an opportunity for unconscious biases to kick in, thus removing one of the failsafes for strong science. In addition, it offers an opportunity to see patterns in randomness that aren't there. And the more ways you slice and dice the data in a post hoc analysis, the more opportunity there is for this. It's kind of like playing the lottery: the chances of winning the lottery are small, and the drawings are random. But, if millions of people play, somebody somewhere is probably going to win.

This does not mean that the results of post hoc analyses are wrong, but it does mean that any positive results found through them should be followed up more closely before coming to any firm conclusions.

> “ [...] MK-7 supplementation didn’t result in obvious improvements beyond one measure of carotid stiffness. Does this mean that MK-7 isn’t much good for preventing cardiovascular problems? Not necessarily. ”

They divided the participants into two groups: participants who started the study with low and high stiffness index β. This subgroup analysis revealed that those who started out with a high β responded quite well to MK-7 treatment compared to placebo. Most of the local measures of carotid elasticity were significantly better at the end of three years, even after adjusting for confounders (which was a good thing to do, since those who started out with a high β were different from the low-β folks in many other ways, too). In addition, the stiffness index β dropped quite strongly in the group that started out with a high β, whereas it didn’t change in those who started out with a low β. The carotid PWV was also lower in the MK-7 group. Yet cfPWV still hovered around “borderline significance” ($p = 0.062$), never quite becoming clearly distinguishable from statistical noise.

The stiffness index β of the carotid was better in the MK-7 group after three years of treatment compared to placebo. However, no other measurements were meaningfully different between the two groups. When the women were divided up into those who started the study with low versus high β, MK-7 had a stronger effect on decreasing carotid stiffness and improving elasticity, although regional arterial stiffness was still unaffected.

What does the study really tell us?

Overall, this study tells us that MK-7 supplementation at a dose of 180 micrograms for three years can reduce one out of several measures of carotid artery stiffness in healthy post-menopausal women. But the study came up negative in terms of regional arterial stiffness, other local measures of carotid artery health, as well as markers of blood vessel damage and inflammation. Subgroup analysis also hinted that MK-7 supplementation may be more helpful in women who already had a higher baseline arterial stiffness, but we cannot ignore that supplementation did not do much for the average study participant.

In short, a lot of measurements were taken, but MK-7 supplementation didn’t result in obvious improvements beyond one measure of carotid stiffness. Does this mean that MK-7 isn’t much good for preventing cardiovascular problems? Not necessarily. There are several aspects that should be investigated before we give up on MK-7 as a preventer of cardiovascular disease.

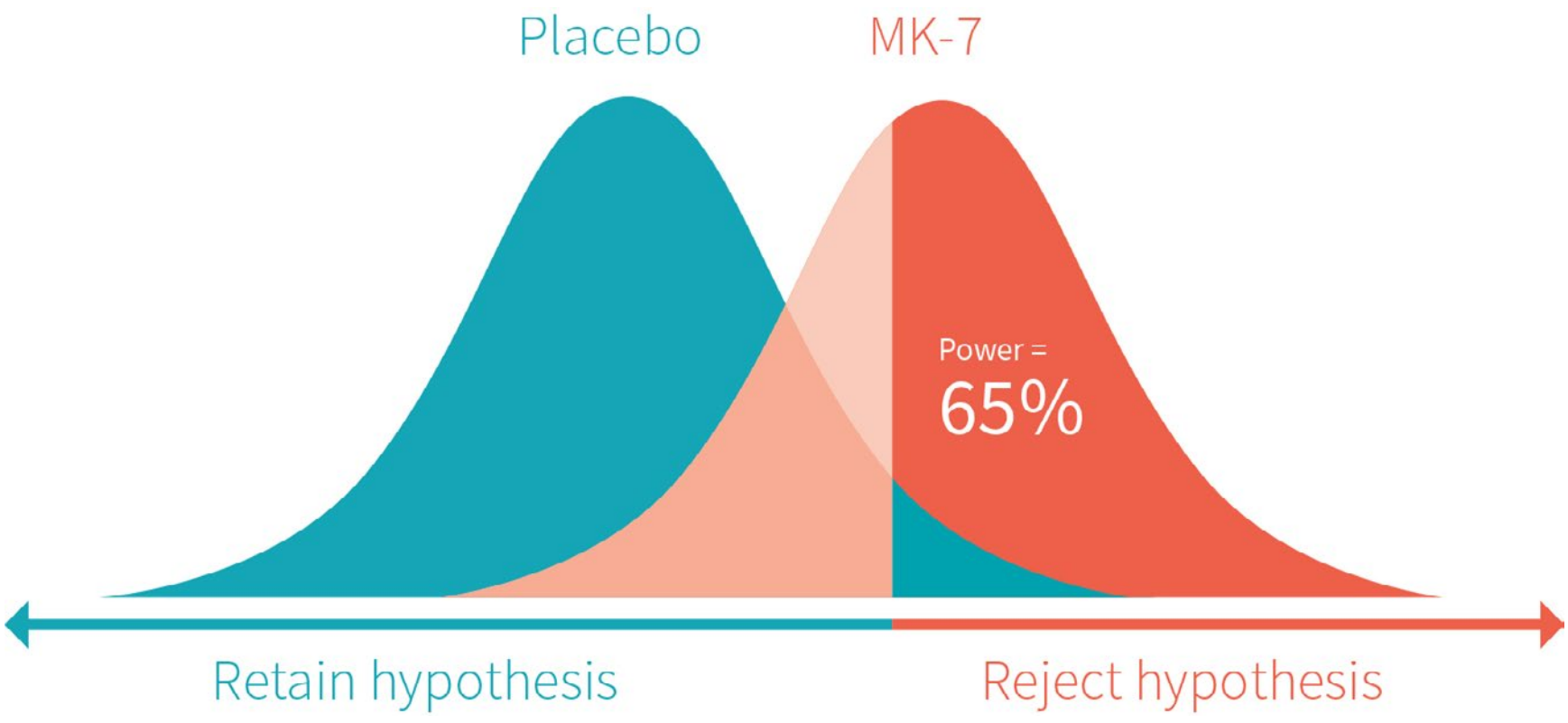
One such aspect is the dose. In this study, the 180 micrograms of MK-7 was used. This is in the range of a “nutritional dose” - the amount needed for good health

that could be obtained from diet. Supplemented doses could go much higher, since MK-7 has a very strong safety profile at higher intake levels. So, a higher dose of MK-7 could have shown an effect where the lower "nutritional" dose in this study didn't. Also, the "nutritional" dose is calculated based on the intake of vitamin K1 and assuming equivalency. It would be hard to get this much pure MK-7 in the diet without eating natto. Non-natto sources of MK-7 include pork liver at levels of less than 20 *nanograms* (1000x less than the microgram doses used here) per gram of liver, and turmeric at 6 micrograms per 100g.

Also, the population recruited for this study was overall a healthy one. It's possible that MK-7 would be more beneficial in a population with a higher risk of cardiovascular disease. There are a few good reasons to suspect that this may indeed be the case. For one, observational evidence indicates a strong association between dp-ucMGP levels and the either calcification, heart disease severity, and mortality in people who either have cardiovascular disease or are at risk for it (e.g. diabetics). In studies of healthy populations, these correlations did not appear, though. Thus, vitamin K status (and thus dp-ucMGP levels) may be a more relevant factor in at-risk or diseased populations. This would explain why even though MK-7-treated individuals in this study saw strong decreases in dp-ucMGP, there weren't many changes of statistical significance seen in arterial stiffness measures. It would also explain why those who entered this study with a higher stiffness measure β responded a little more to MK-7 supplementation according to the post hoc analysis.

Finally, keep in mind that this study was mainly designed to test the effects of MK-7 on bone loss, not arterial stiffness. The arterial measurements were done concurrently as a kind of "add-on" to the bone study so that the authors could get two studies for the price of one concerning MK-7, but the main design focused on effects on bone. That means that enough patients were recruited to see differences in bone measurements, not arterial measurements. The authors note that while the study's "power" (power is illustrated in Figure 3) was 90% for bone strength, the "power" for the primary outcome of arterial stiffness, cfPWV, was only 65%. This means that even if there was a clinically meaningful difference between the MK-7 and placebo groups, this study only had a 65% chance of detecting it. A larger sample size would have increased the power.

Figure 3: Statistical Power



Fortunately, the authors are in the process of conducting a new study that addresses these issues. They mention that a new study specifically designed to test cfPWV as the main outcome is being conducted in men and women at higher risk for arterial stiffening, using a higher dose of MK-7. This new study will provide much stronger evidence as to whether MK-7 is effective in preventing or reducing arterial stiffening.

That being said, no one trial or even collection of trials necessarily indicates the usefulness of a nutrient, since nutrients typically work in concert. Specifically, fat soluble vitamins (such as vitamin D) can interact with vitamin K2, and hence changing only one nutrient isn't that likely to improve either biomarkers or endpoints in a condition such as cardiovascular disease. In other words, foods and dietary patterns can be much stronger determinants of health than one lone vitamin, but that doesn't mean that vitamin isn't important.

It should be mentioned that funding for this study came from Nattopharma, a Norwegian company that manufactures MK-7 and provided the specific brand of MK-7 used in this study. No conflicts of interest were declared from the study's authors.

Plausible reasons why many of the outcomes measured in this study came up negative include the study's low statistical power, its use of a relatively healthy population, and the relatively low dose of MK-7. The authors of this study are currently conducting a follow-up study that addresses these issues.

The big picture

Few, if any, people wake up in the morning with the aim of decreasing their arterial stiffness ("Boy, my carotid's sure feeling stiff today! What can I take for that?"). They probably care about preventing heart attacks and strokes. So why did the authors of this study measure arterial stiffness and not how many people developed any of these diseases?

The authors themselves didn't say, but there are a few good and plausible reasons. For one, it would probably take longer and therefore be more expensive to run a clinical trial for these outcomes. Heart attack, stroke, and the like are somewhat common, but not too common, especially among a healthy population. A trial that looked at these outcomes would probably have to

> "A trial [...] would probably have to be a lot longer than three years and/or a lot larger than a couple of hundred of participants in order to see a statistically meaningful difference between the MK-7 and placebo group."

be a lot longer than three years and/or a lot larger than a couple of hundred of participants in order to see a statistically meaningful difference between the MK-7 and placebo group. Also, such a trial may have ethical issues. If the authors really expected MK-7 to work, then giving some people placebo and then watching them die would be morally questionable.

In addition, many of the measures taken in this study are plausibly related to cardiovascular disease outcomes both mechanistically and experimentally. Mechanistically, the carotid artery thickening and building up plaque, which can lead to stroke, makes sense, and the extra strain that regional and aortic stiffness puts on the heart is pretty well understood. Experimentally, PWV measurements of the aorta, including cfPWV, are quite strong predictors of cardiovascular disease and mortality, which is why it is called the "gold standard" measurement of arterial stiffness. Carotid stiffness is a pretty good predictor of stroke as well.

While this trial did not measure cardiovascular disease and death directly, the measurements they took are directly relevant to cardiovascular disease. There's a decent chance that any intervention that affects these measures will affect cardiovascular disease as well.

Local measures of carotid stiffness and regional measures of arterial stiffness, especially those that involve the aorta, are good surrogates to look at when considering cardiovascular disease risk. Given the difficulty in conducting long term trials that can pick up on hard cardiovascular outcomes, trials like these can still provide useful information.

Frequently asked questions

This study only involved women. Would MK-7 be effective in men?

This is the first interventional study involving MK-7 and cardiovascular outcomes, so there's no direct evidence of this. However, the authors claim that since cfPWV doesn't differ between men and women, there's not much of a gender difference here, and so one could expect to see the changes here extend to changes in men as well. This isn't a perfect argument, since other factors besides a lack of difference in cfPWV measurements may come into play, but the authors of the study seem to think it holds water, and it's something to consider.

This study was primarily designed to test MK-7's effect on bone loss in post-menopausal women. How'd that turn out?

Over three years, density was preserved in the lumbar spine and neck of the femur, but not in the hip. This conflicts with another study, which found that double the dose had no effect on bone loss.

Were there any side-effects to MK-7 use?

The authors said that there were no side-effects at the doses given over three years.

What should I know?

This study found that 180 micrograms of MK-7 over three years decreased the local arterial stiffness of the carotid artery in healthy post-menopausal women. Post hoc analysis found that the effect on carotid elasticity was stronger in those women who started the study with stiffer arteries, with a marginal effect on regional arterial stiffness. The authors are currently working to confirm these findings using higher doses of MK-7 in a higher-risk population of men and women. ◆

I get by with a little help from my friends: probiotics and depression

A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood

Introduction

For thousands of years, clinicians have observed a connection between the gut, brain, and overall health. Hippocrates is famously quoted as saying "all disease begins in the gut." This should not come as a surprise to anyone who has ever experienced gastrointestinal (GI) symptoms like diarrhea, indigestion, or abdominal discomfort in response to changes in their emotional state. Studies in both healthy patients and in people with functional bowel disorders have confirmed connections between emotional state and GI function.

The gut and brain communicate through neural, endocrine and immune pathways. It has become increasingly clear that interactions with intestinal microbiota are also an important part of this communication. A number of animal and human studies have examined the relationship between gut bacteria and mood symptoms such as anxiety and depression, leading to the idea that probiotic supplementation may be a potential strategy for reducing or preventing depression.

According to the cognitive theory of depression, an individual's negative and distorted thinking is the basic psychological problem at the root of depressive syndrome. Cognitive reactivity refers to the activation of dysfunctional patterns of thinking triggered by subtle mood changes. This is a key feature in the development and occurrence of depression, and as such would be a relevant target for interventions. Dysfunctional patterns of thinking can include thoughts of hopelessness, thoughts of hurting oneself or others, ruminating on the causes and consequences of anguish, and a general loss of motivation for life. These responses are thought to come from underlying negative thought patterns that get brought to the surface during times of low mood. Cognitive reactivity appears to be a cause of, rather than simply an association with, depression, since higher cognitive reactivity scores precede and predict the onset of depression, even in people with no prior incidence of depression.

Considering the vast potential to treat and prevent mood disorders by improving gut health, the objective of this Dutch study was to determine the effects of a probiotic supplement on cognitive reactivity to sad mood, as well as symptoms of depression and anxiety in non-depressed, healthy adults.

"Cognitive reactivity appears to be a cause of, rather than simply an association with, depression, since higher cognitive reactivity scores precede and predict the onset of depression, even in people with no prior incidence of depression."

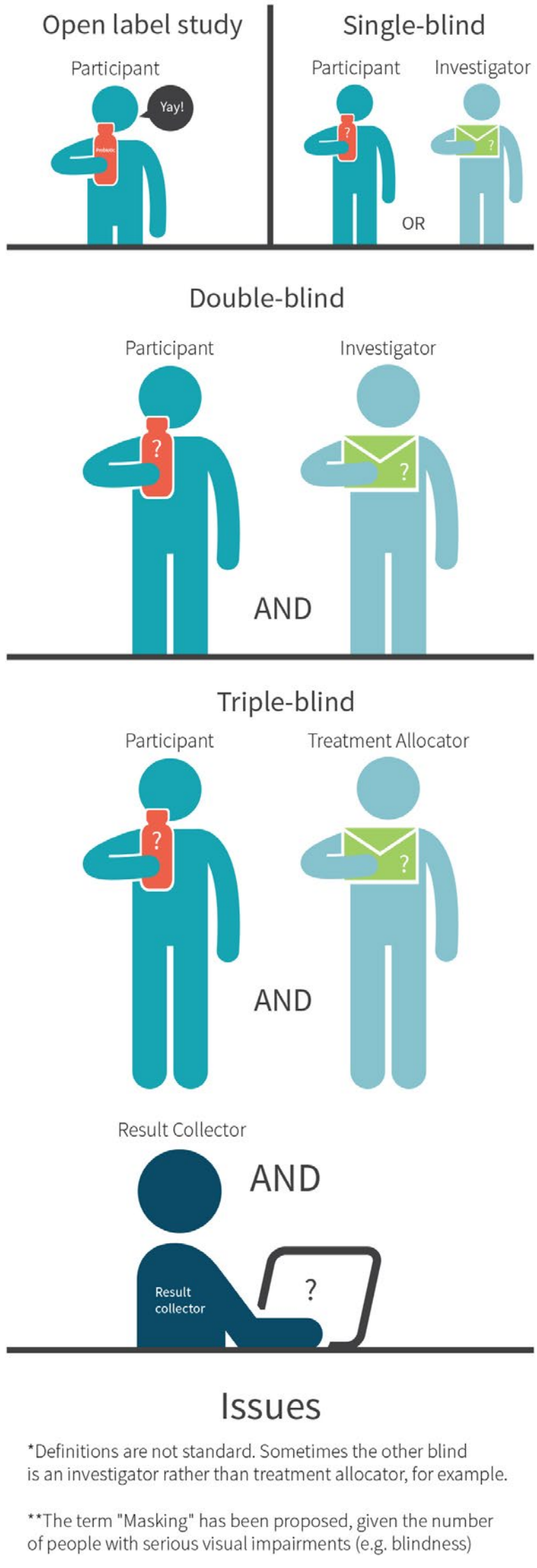
Cognitive reactivity, which involves negative thought patterns triggered by mood change, has been shown to predict the onset of depression. Since the brain and gut communicate through several pathways and the gut microbiome has recently been found to influence this communication, it is possible that probiotic supplementation could affect cognitive reactivity. This is what this study set out to test.

Who and what was studied?

Forty healthy, normal weight college-age adults participated in this triple blind study. A triple blind study means neither the participants, researchers, nor the people who organize and analyzed the data knew which group a participant was in. Different types of study blinding are depicted in Figure 1. The participants did not smoke, had no reported medical conditions, food allergies, medications, or drug use, and consumed no more than three to five drinks per week. Participants also did not have any psychiatric or neurological disorders, or any personal or family history of depression or migraines. They were randomly assigned to receive either a probiotic supplement (n=20, five males) or placebo (n=20, three males) for four weeks. The menstrual cycle was not controlled for in the female participants.

Interestingly, all participants were told they were receiving the probiotic supplement. This is different from many other trials, as participants would normally be told they have an equal chance of receiving the supplement being studied or placebo. The probiotic used was a mixture that is commercially available in the Netherlands, containing *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *L. casei* W56, *Lactobacillus salivarius* W24, and *Lactococcus lactis* (W19 and W58). The participants consumed either supplement or placebo for four weeks.

Figure 1: Triple blinding vs other blinding



Three different questionnaires were used before and after the intervention period to quantify the outcomes. The revised Leiden Index of Depression Sensitivity (LEIDS-r) measured the perceived cognitive reactivity to transient changes in sad mood, which indicates vulnerability to depression. The LEIDS-r is made up of 34 questions that assess the extent to which dysfunctional thoughts are activated when someone is experiencing a mild state of dissatisfaction with their life. Example questions include "*when in a low mood, I take fewer risks*," or "*when in a sad mood, I more often think about how my life could have been different*." Responses are given on a 5-point scale, with 0 being "does not apply to me" and a score of 5 meaning "very strongly applied to me." The scale measures vulnerability to depression overall, and consists of six different subscales: regard to aggression, hopelessness/suicidality, acceptance/coping, control/perfectionism, risk aversion, and rumination.

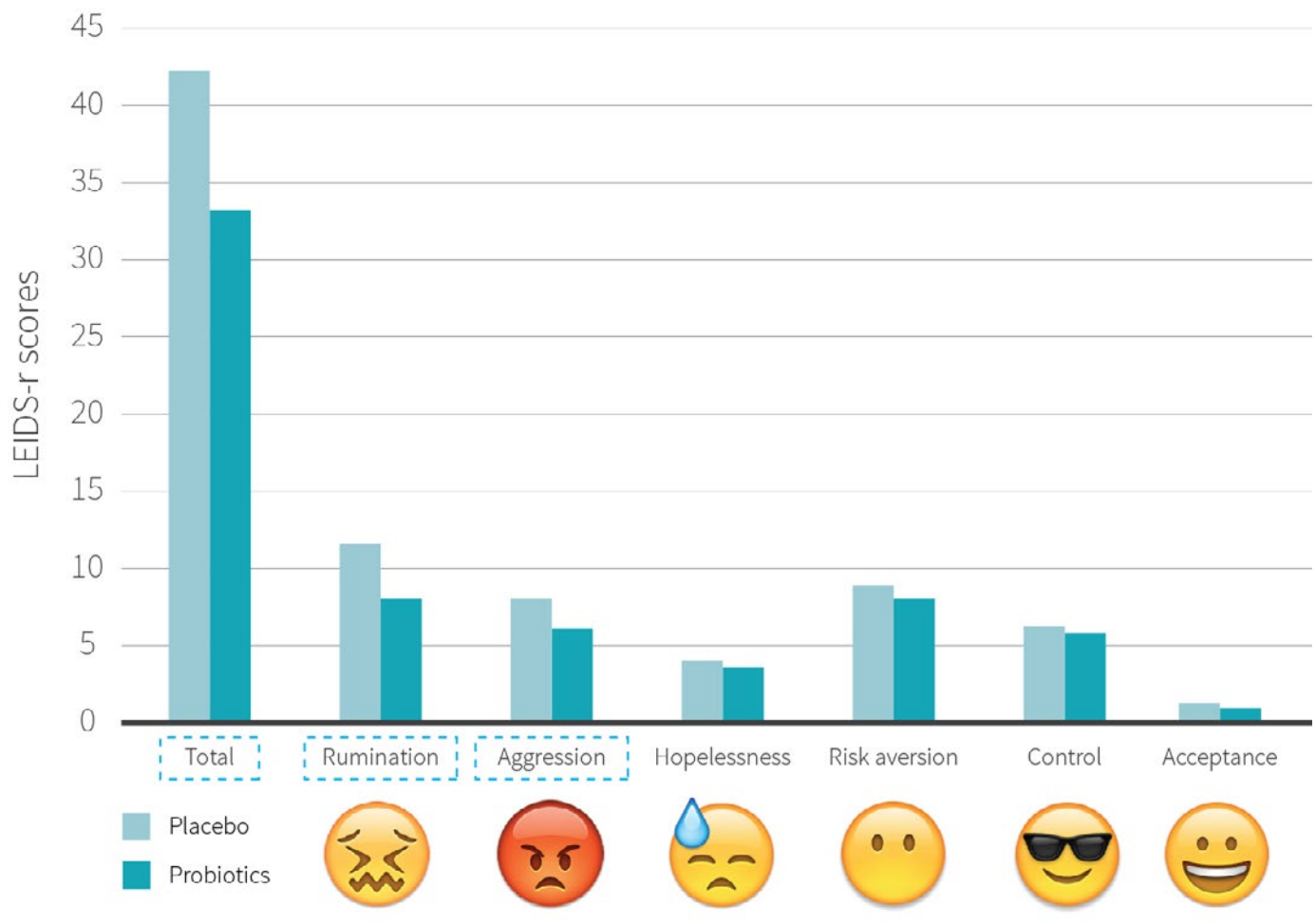
The Beck Depression Inventory II (BDI-II) is a 21-item questionnaire that assesses the existence and severity of depressive symptoms occurring during the previous two weeks. Similar to the LEIDS-r, questions are rated on a 4-point scale ranging from 0 to 3 in terms of severity, and the total score is added up to classify the level of depression (minimal, mild, moderate, or severe). The Beck Anxiety Inventory (BAI) is also a 21-item questionnaire, used to assess the existence and severity of anxiety symptoms occurring during the previous week. Questions are also answered on a 4-point scale ranging from 0 to 3 in terms of severity.

Forty healthy college-age adults with no personal or family history of depression took either placebo or a probiotic mixture. Cognitive reactivity was measured with the LEIDS-r questionnaire before supplementation began and after four weeks of supplementation. Anxiety and depression were also measured using questionnaires.

What were the findings?

The study findings are summarized in Figure 2. None of the participants (in either group) showed any signs

Figure 2: Study results



of depression (using the BDI-II) or anxiety (using BAI) at baseline or follow-up. Differences were seen on the LEIDS-r, however, which measures vulnerability to future depression. Participants who received the four-week probiotic supplement showed a significantly lower score for overall cognitive reactivity to sad mood, mainly accounted for by reduced rumination and aggressive thoughts. No differences were found between groups for hopelessness, control, risk aversion, or acceptance.

What does the study really tell us?

"*The present results indicate, for the first time, that probiotics intervention can influence cognitive mechanisms that are known to determine vulnerability to mood disorders.*"

This study set out to determine the effects of a multi-species probiotic supplement on cognitive reactivity, an important marker in predicting future depression, in healthy young men and women without any personal or family history of mood disorders. Though none of the participants showed any signs of current anxiety or depression, a four-week probiotic intervention showed significantly reduced cognitive reactivity scores, suggesting a reduced vulnerability to future depression. Reductions in total cognitive reactivity score were largely due to decreases in the aggression and rumination subcategories. This is relevant because the people who ruminate about the causes of being in a sad mood may have a harder time recovering from depression.

These participants were healthy and had no diagnosable anxiety or depression at baseline, so improvements in these scores would not necessarily be expected. The lack of any existing mood disorders in the participants is important because it allows the researchers to test for any influence on *future* depression, which the LEIDS-r questionnaire has been shown to do. Of course, further long-term studies using the probiotic interven-

> "Participants who received the four-week probiotic supplement showed a significantly lower score for overall cognitive reactivity to sad mood, mainly accounted for by reduced rumination and aggressive thoughts."

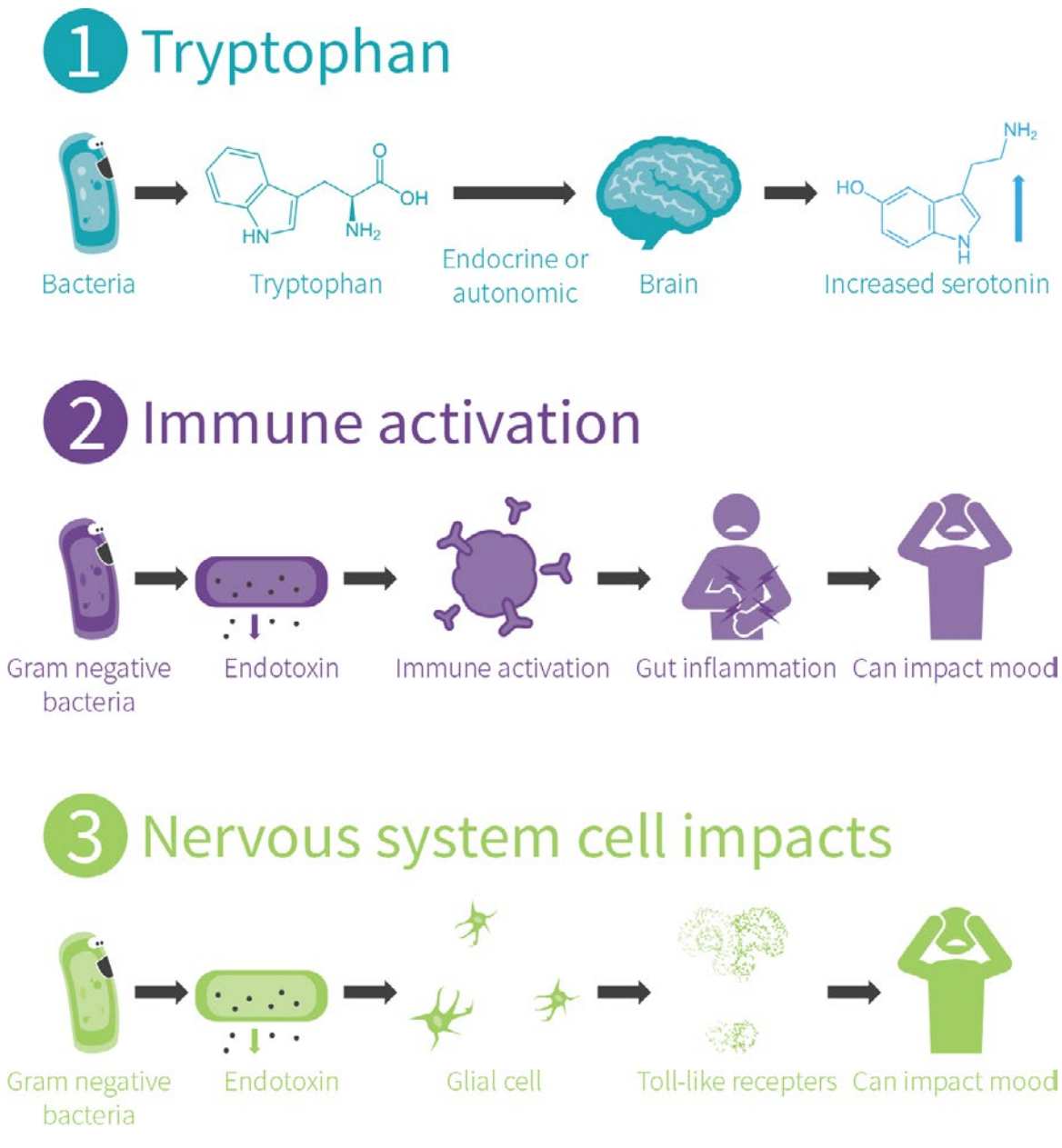
tion would be needed to confirm if these predictions become clinically relevant.

However, we can calculate a guesstimate of the odds reduction of developing depression based on the LEIDS-r score. The roughly nine-point reduction in the LEIDS-r score seen in this study due to probiotic supplementation translates to an odds ratio of 0.76, in terms of developing depression over a two-year period, based on previous research.

While no mechanisms of action were studied, a number of hypotheses can be considered, which are shown in Figure 3. Cognitive reactivity scores can predict the depressive response to serotonin depletion, and gut bacteria may increase serotonin in the brain by increasing plasma tryptophan levels. Decreased intestinal permeability from the probiotic supplementation could also play a role, as increased gut permeability can lead to symptoms of depression. A review of the effects of probiotic supplements on intestinal permeability found a positive effect in 48% of the controlled studies.

While compliance was not confirmed by stool analysis and dietary control did not include consideration for other probiotic-rich foods (i.e. yogurt), the biggest limitation in being able to draw wider conclusions from this study is the disproportionate female to male balance. Participants in this study were 80% female, which is opposite to the male gender bias often found in the scientific literature. This is relevant because men and women have different gut microbiomes due to differences in sex hormones, and we are also somewhat different both emotionally and cognitively. This is also a fairly small study in a young population, which makes generalization difficult. Although ethnicity wasn't

Figure 3: Three of the ways that microbiota can impact the brain



explicitly mentioned in this study, this study was conducted in the Netherlands, which may warrant caution when generalizing to broader populations As we've seen in ERD #6 in "The gut microbiome's role in type I diabetes," nationality and ethnicity can correlate with microbiome differences.

This study suggests that a multispecies probiotic supplement reduces cognitive reactivity, which is associated with a lower risk of future depression. This is plausible, since there are several mechanisms by which the gut microbiome may affect vulnerability to depression, although these mechanisms were not examined in the study under review. The small sample size and limited diversity in age, gender, and ethnicity makes the results difficult to generalize.

The big picture

A number of human and animal studies show reduced signs of depression and anxiety with probiotic supplementation, though improvements are often seen only with pre-existing anxiety or depression.

Taking a probiotic supplement made up of multiple strains of bacteria can have increased effectiveness through an additive or synergistic effect of the individual strains, compared with mono-species supplements. However, some probiotics may work in an antagonistic manner, so combinations of strains should be studied both individually and in combination before creating a multispecies product. A previous study by one of the co-authors of this study reported improvements in intestinal barrier function by each probiotic strain used in this study separately, as well as in the combined product. Another study that included that same co-author found a decrease in migraines during the second and third month of taking the same supplement. However, no control group was used and no placebo was given.

As mentioned in last month's ERD article on HMB supplementation, the fact that a company sponsors the research does not automatically taint the results. However, it is worth noting that the aforementioned study about intestinal barrier function was done by the company's own internal research and development scientists. Both the study about migraines, as well as the study under review, featured the same employee from Winclove Probiotics as a co-author. Despite this, the authors of this paper state that "no competing interests exist."

Conflicts of interest

Conflicts of interest occur when the people who design, conduct, or analyze research have a motive to find results that suit their needs. The most obvious source of a conflict of interest is monetary. Sometimes, such as in this paper and the vitamin K2 study reviewed in this issue of the ERD, possible conflicts may exist even though the authors claim there are none. But these authors aren't alone. One study suggests that nondisclosure of possible conflicts of interest is somewhat common.

However, having competing interests doesn't automatically negate the results of studies. For instance, one review of major cardiovascular trials found that conflicts of interest had no impact on the results.

Conflicts of interest must be evaluated carefully. Don't automatically assume that they don't exist just because they're not disclosed, but also don't assume that they necessarily influence the results if they do exist.

Many studies to date have suggested that probiotics may have an effect on anxiety and depression, especially in animals, although human trials were mostly conducted on populations with pre-existing anxiety or depression. Multistrain probiotics could be more beneficial than individual strains in some, but not all, cases. Ideally, research should compare single versus multi-strain supplementation.

Frequently asked questions

Would probiotic supplementation have the same effect in men and women?

This study recruited both men and women, though it was predominantly (80%) made up of women and results were not listed separately by gender. Other trials using probiotic supplements have found both similar and differing effects among men and women. It is difficult to say how this product would compare between genders.

Could the benefits of this supplement extend beyond depression and anxiety?

Previous research using the same supplement has shown improvements in gut barrier function and a reduction in migraines. Additionally, a number of the species used in this product (but different strains) have shown cholesterol lowering effects, as well as contributing to improved immune function.

What I should know?

This study showed that healthy individuals consuming a multispecies probiotic for four weeks experienced a reduction in cognitive reactivity scores, which are a marker of vulnerability to future depression. In particular, these reductions were characterized by reduced aggressive and ruminative thoughts in response to sad mood.

This trial could provide a basis for larger trials in more diverse populations, possibly also testing the efficacy of multi-strain versus single species probiotics. ◆

> “Previous research using the same supplement has shown improvements in gut barrier function and a reduction in migraines.”

Do BCAAs and arginine prevent central fatigue during exercise?

Branched-chain amino acids and arginine improve performance in two consecutive days of simulated handball games in male and female athletes: a randomized trial

Introduction

Exercise fatigue is complex, stemming from both central (brain) and peripheral (muscular) origins. For example, skeletal muscle under repeated stress may deplete local energy stores and/or accumulate metabolites that disrupt enzymatic function and therefore may not be able to provide the necessary ATP in order facilitate contraction.

The skeletal muscle may also send inhibitory feedback to the brain under high effort loads via the golgi tendon apparatus in order to prevent injury, which may lead to muscular failure during resistance training. Alternatively, fatigue may involve the accumulation of serotonin via tryptophan uptake into the brain. Tryptophan is the biological precursor to serotonin, and increasing levels of serotonin are linked with drowsiness, lethargy, and changes in motivation. Bioaccumulation of serotonin is the basis of the exercise-induced central fatigue hypothesis, which is shown in Figure 1.

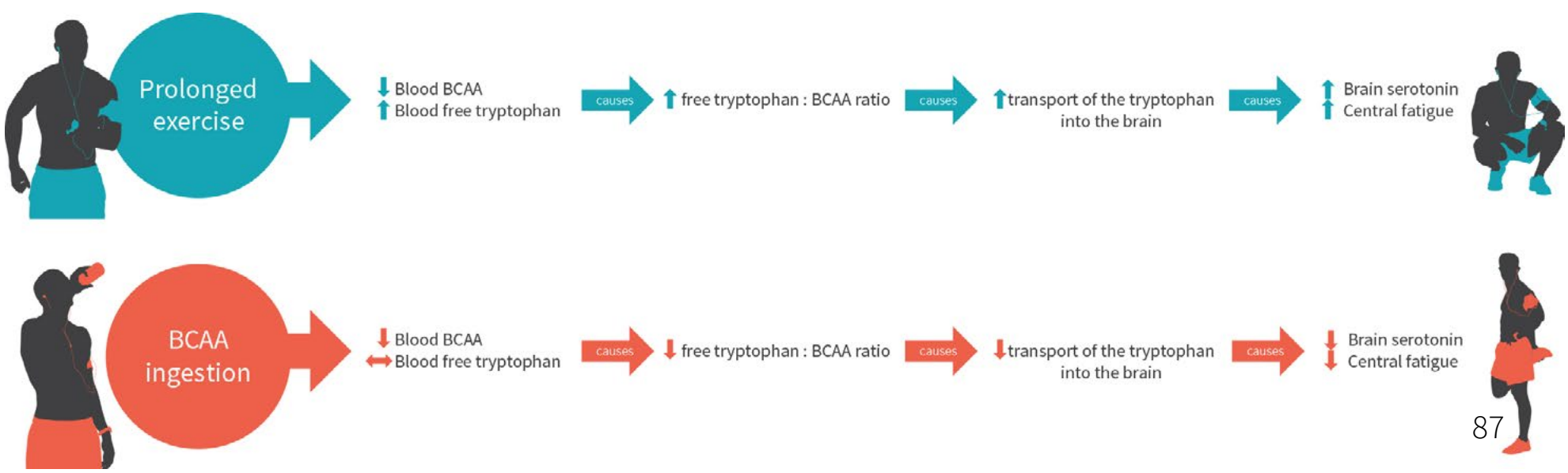
The rate-limiting step in central serotonin synthesis is tryptophan transport into the brain. Tryptophan enters through the large neutral amino acid transporter (LNAAT). Therefore, it has been proposed that the competition between tryptophan and other compounds for the LNAAT may decrease intra-exercise serotonin production and decrease central fatigue.

Interestingly, branched chain amino acids (BCAAs) also use the LNAAT to pass through the blood-brain barrier (BBB), making them a viable option for competitive inhibition of tryptophan uptake. If this were the case, then BCAA supplementation would be an effective method for decreasing exercise-induced central fatigue via bioaccumulation of serotonin. However, previous studies on humans had found no ergogenic benefit.

In theory, BCAAs have a potential in inhibiting central fatigue. However, BCAA degradation leads to ammonia production. It has been postulated that the increase in circulating ammonia may be a limiting factor in applicability of BCAA supplementation to prevent central fatigue. Research groups may have come up empty handed due to the ammonia accumulation that occurs during periods of BCAA supplemented exercise. It has been hypothesized that BCAA degradation into ammonia would effectively lead to exercise decrement by affecting the brain's central fatigue mechanisms. In this case, the introduction of arginine may mitigate the proposed effects of accumulated ammonia by stimulating the urea/ornithine cycle, converting the ammonia to urea and ornithine.

In addition, there has not been any data accumulated on the effects of BCAA supplementation and central fatigue with multiple days of controlled activity. A Taiwanese group proposed that BCAA and arginine would prevent the onset of central fatigue in consecutive days of exercise. This would imply that the serotonin synthesized during exercise would induce a refractory period that would affect performance on sequential day(s).

Figure 1: How BCAAs might combat central fatigue during exercise



The short of central fatigue

When we exercise, fatty acids are mobilized in order to provide energy to produce ATP. A percentage of these mobilized fatty acids bind to circulating albumin. Albumin also binds the amino acid tryptophan. If a larger percentage of circulating albumin is binding fatty acids then there is a larger concentration of free circulating tryptophan. Tryptophan can pass through the blood brain barrier and be converted to serotonin, which may increase the rate of perceived exertion during exercise.

Exercise-induced fatigue is thought to be partially caused by tryptophan entering the brain. BCAAs should compete with tryptophan transport into the brain, decreasing central fatigue. However, human studies to date have not found such an effect. This may be due to accompanying increased ammonia production, which could theoretically be offset by adding arginine. This study was designed to test this hypothesis.

Who and what was studied?

Twenty-two elite-level handball players were recruited for a double-blind, placebo controlled, randomized cross-over experiment.

After initial testing the players were split into two groups, with supplements taken one hour before the game:
Amino acids (AA)
- 0.17 g/kg BCAA (2:1:1 leucine:isoleucine:valine)
- 0.04 g/kg arginine

Placebo (PB)
- An equal weight of starch as the AA group has BCAA and arginine

A controlled diet was provided the day before the trial and the two days during the trial. Researchers controlled fluid intake on the second day by mandating consumption similar to the amount consumed on the first day.

Each group played one simulated handball game two days in a row. The simulation was essentially two thirty-minute blocks of calisthenics (bodyweight/dynamic movements) with ten minutes of rest after the first block. The end of each thirty-minute block had a timed 20 meter sprint, which was used as the performance metric. Heart rate was measured throughout each game.

Blood samples were taken for analysis before breakfast and after the simulated handball game on both days. The free tryptophan was measured via fluorescence. Tryptophan is an aromatic amino acid with a special series of conjugated double bonds in its ring. Aromatic amino acids have specific emission spectra if certain wavelengths of light are shone on them. If one color of line is shone, another color shines back! This allows for calculation of their concentration in complex mixtures, like blood. Ammonia, glycerol, free fatty acids, and lactate levels were all determined by commercial kits.

After the washout period (one to two weeks) the groups were switched and another set of simulated handball games was performed.

This was a placebo-controlled, crossover trial where elite handball players took either placebo or BCAAs plus arginine one hour before playing a simulated handball game for two days in a row. Twenty meter all-out sprint times were measured halfway through the game and after the game, and were the main study outcome. Blood chemistry and heart rate were also measured. After a one to two week washout period, players who took the placebo repeated the experiment with BCAAs plus arginine, and vice versa.

What were the findings?

The study's findings are summarized in Figure 2. The average 20 meter sprint time was not significantly different between groups, but there were significant differences in improvement (about a tenth of a second) for the AA group between days. There was an improvement of about 1.3% and 1.7% between the first and second halves, while there was no improvement in the PB group.

Also, the rate of perceived exertion (RPE) was lower in the AA group, which averaged 14/20 over the two days compared to 15/20 in the PB group. Unsurprisingly, the average heart rate was not significantly different between groups.

Post-exercise BCAA levels were elevated in the AA group, but this is also unsurprising considering they supplemented BCAA before the simulation. However, plasma tryptophan remained unchanged between groups in both trials. The unchanging tryptophan levels could indicate that there was no prevention of uptake and therefore no decreases in serotonin production. The tryptophan to BCAA ratio was lower in the AA group post exercise, but the AA group had supplemented with BCAA prior to the game, which may account for this difference.

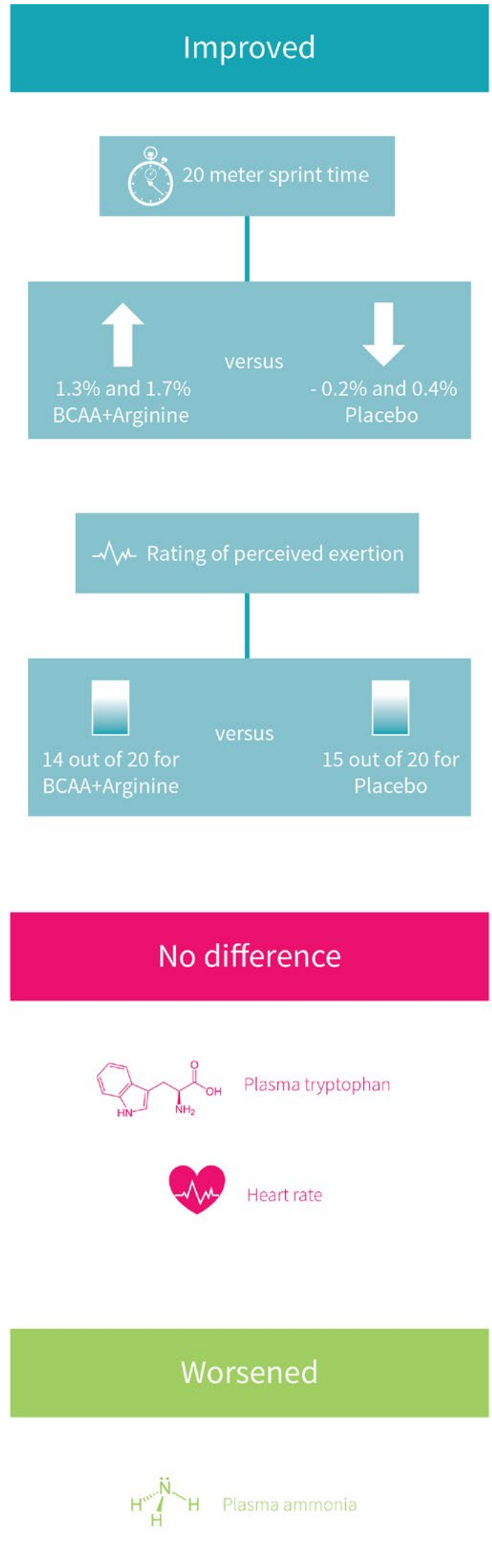
Plasma concentrations of ammonia post exercise were significantly higher in the AA group when compared to the PB group. Elevated ammonia in the AA group is expected, as there is more nitrogenous substrate for oxidation during exercise. However, not enough circulating ammonia is produced to induce exercise-mediated central fatigue. It is not possible to determine whether or not the arginine helped attenuate ammonia accumulation because there was no control for arginine in the form of a BCAA-only group.

The BCAA+arginine group showed a small but significant improvement in sprint time and rate of perceived exertion. However, there was no change in heart rate or serum tryptophan levels between the AA and PB groups. Circulating ammonia was greater in the AA group after exercise when compared to PB, but it did not appear to negatively affect sprint performance, which was better in the AA group during the second day's sprinting.

What does this study really tell us?

This study provides a great example of research that shows serious promise in rodents but does not quite pan out when the concept is applied to humans.

Figure 2: Trial results



Pharmacological intervention has shown that stimulating the 5-HT1A receptor may decrease performance, but there does not appear to be any evidence that preventing its synthesis, in the form of tryptophan depletion via dietary supplements, increases performance in humans.

This study also tells us that the accumulation of circulating ammonia, as a result of BCAA oxidation during exercise, may not have a strong impact on performance. Researchers included arginine in their pre-workout formulation in order to mitigate ammonia accumulation, but there was no control group given only BCAAs to establish whether or not it had a measurable effect.

In the study's introduction, the researchers mention that cognition and reactive abilities were metrics for central fatigue in exercise. It is curious that the researchers had decided to use an all-out 20 meter sprint combined with circulating tryptophan as the metric for central fatigue. Other studies have used similar metrics with different exercise modalities, but it may speak to the lack of standardization as to how central fatigue is tested. For example, giving an athlete an attention capacity test while they are physically exhausted may be testing other confounding variables that have little application to an athlete's capability to perform a sport-specific task while centrally fatigued. Furthermore, the group concluded that performance was maintained/improved in the AA group due to delayed central fatigue, but the non-supplemented group did not have meaningful performance decrements. Because of this, it is possible that the AA group had "improved" over the second day due to some other factors unrelated to central fatigue.

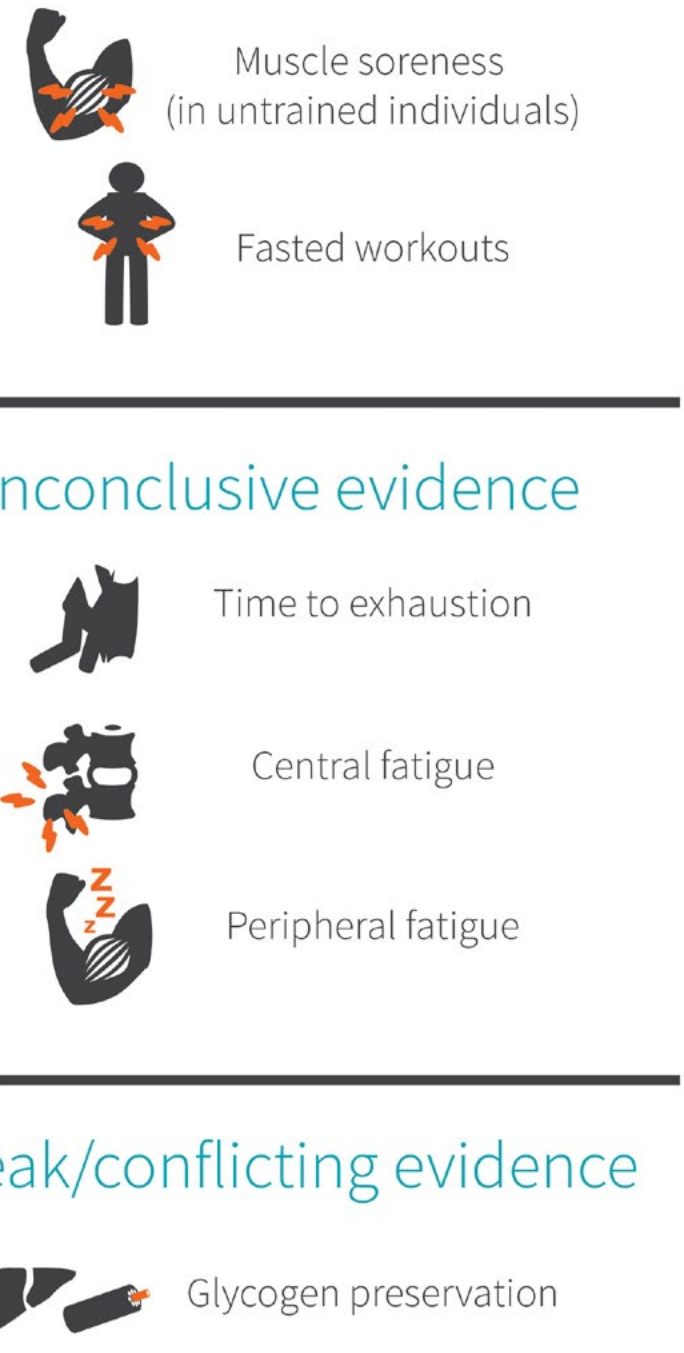
Supplementing BCAAs and arginine did not improve performance in humans to the extent previous animal research suggested it would. The study design also had some issues, making it difficult to say for certain whether the added arginine necessarily lead to decreased central fatigue.

The big picture

Central fatigue in exercise is a real phenomenon. However, how the brain regulates our rate of perceived exertion is likely much more complicated than an increase in cerebral serotonin during extended activity. Interaction and regulation via other neurotransmitters, such as dopamine and acetylcholine, is almost certainly part of the larger issue.

While it's possible that BCAA supplementation may be a viable option for improving various aspects of exercise, as is shown in Figure 3, the evidence is mixed. Alternatively, they may prevent muscle protein breakdown due to exercise.

Figure 3: Evidence for BCAAs improving exercise



However, it would appear that acute ergogenic benefits have yet to be unequivocally established.

Well-trained athletes may simply have the mental grit necessary to overcome the feelings of fatigue associated with these changes in brain chemistry. This may be the mental barrier athletes push through on a daily basis. If there is no fuel for a muscle, or if the enzyme function is interrupted due to the metabolic environment, then a muscle simply cannot function. However, everyone has witnessed athletes pushing through extreme mental barriers in order to perform. It is unlikely that a muscle had magically repleted itself of glycogen of phosphocreatine. It is entirely possible that this is part of the desensitization process of central fatigue in well-trained athletes. Finding out what is going on in the brains and bodies of athletes during this stage of performance may hold part of the answer scientists seek.

Frequently asked questions

Will supplementing BCAAs and arginine increase exercise performance?

Supplementing these amino acids does not appear to have meaningful ergogenic benefit for trained athletes. However, supplementation may provide long-term benefit with respect to muscle growth and preservation, which may lead to accumulated performance benefits in the long term.

Is it possible to be centrally fatigued and not peripherally fatigued?

In theory, yes! If the muscles were fully repaired and energy stores (fat and glycogen) were repleted within the muscle then a confounding factor in performance decrement may be related to central fatigue.

Does the serotonin hypothesis of central fatigue apply to strength athletes?

There isn't any controlled research that looks at this phenomenon in well-trained strength athletes. However, due to the glycogen-intensive nature of such exercise, it is much more likely that peripheral fatigue is the limiting factor in work capacity.

Does arginine supplementation prevent performance decreases related to hyperammonemia?

Clinically relevant hyperammonemia is unlikely to occur in healthy individuals with a fully functioning urea cycle. Ammonia levels high enough to cause cognitive dysfunction (without artificial administration) are commonly seen in people with hepatic encephalopathy due to liver failure.

What should I know?

Central fatigue is a complicated issue that will require a multi-faceted approach to prevent.

Central fatigue is currently postulated to be a build up of the neurotransmitter serotonin during physical activity. This may cause an increase in one's rate of perceived exertion, causing a decline in performance.

Tryptophan is the precursor to serotonin, so scientists have tried to prevent onset of central fatigue by supplementing with BCAA which may compete with tryptophan for brain uptake, but haven't had much success.

In this study, there was no meaningful change in circulating tryptophan in athletes that were supplementing with BCAA vs placebo control. Yet there was a small improvement in performance in the BCAA group. The lack of an arginine control group limits the conclusions that can be made.

BCAA supplementation may have benefits, but there is little evidence to show supplementation causes an ergogenic effect, or a substantial decrease in central fatigue. ◆

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