ERD

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From the Editor

Does an industry-funded study mean that the results aren't true? Simply put, no.

If you're a primary investigator researching ways to help sick people get better, getting industry funding bumps your chances of getting a grant up considerably—from ludicrously tiny to just plain small. If, on the other hand, you're researching ways to help relatively healthy bros and she-bros gain muscle and lose fat, then things get a lot harder. Is there a proper non-male-centered term for women with bro mentalities? If you are an expert on this topic, please write to me at erdeditor@examine.com.

The NIH is interested in helping sick people more than gym rats, and that probably won't change anytime soon. You know who cares if you have striations in your quads and visible ab veins? You. Maybe your partner. That's probably about it.

Another entity with skin in this game is supplement manufacturers. They are not categorically evil just because they sell products. And similarly, they are not evil because they fund studies.

Typically, the research team puts strict limits on the funder's involvement. Sure, give us money. But help writing the manuscript? Designing the study? Analyzing the results? Absolutely not, this is strictly a situation where an interested party provides the means to do research.

… or is it? While industry-funded research isn't by any means of lesser quality, there are pitfalls to watch for. Pharmaceutical research has been heavily interrogated in recent years, after research oversights in reporting and methodology resulted in massive controversies dealing with recalled drugs.

There are more ways to influence research than just writing the paper. The very research question itself can be influenced by the chances of getting funding, whether from government or private sources. The government isn't going to fund many studies discrediting its dietary guidelines, and similarly, industry participants aren't likely to fund research comparing a supplement against its food-based alternative.

Perhaps more importantly, not all studies get published. Some trials stop early due to low enrollment or obvious results that warrant cessation in order to help people in the control group. But others simply don't get published, often due to a lack of significant findings. How are you to know how many studies were started on a food/diet/supplement/drug that weren't published? Nobody has the free time to scour clinicaltrials.gov for such trials, and hence you can't know whether that enticing study showing awesome results is the only such study that's been done. And that's without the methodological issues present in many studies, that can mitigate their impacts.

On a final note, let me mention our April Fools joke email. Some people got very angry that we were seemingly going against all we stand for and selling supplements (including crispy chicken and spray cheese flavors … I guess they didn't read all the way through). But on the positive side, at least there's a contingent of people who are hypersensitive to industry influence, even if they don't correlate emails very well with joke-related holiday dates. For those people, I have one final tip: a study doesn't have to be funded by industry to overstate its results. We run across them every day. Many, many valid studies are funded by industry and conducted by brilliant investigators. Research quality is really a crapshoot, and that's part of the reason we're around, to help separate the wheat from the chaff.

Kamal Patel, *Editor-in-Chief*

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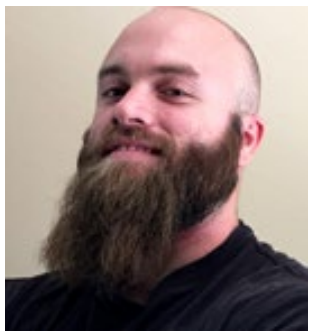

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Blueberries every day keeps high blood pressure at bay

Daily blueberry consumption improves blood pressure and arterial stiffness in post-menopausal women with pre- and stage 1-hypertension: A randomized, double-blind, placebo-controlled clinical trial

> “In the U.S., it’s estimated that about 29% of adults have high blood pressure and roughly another third of adults have pre-hypertension and are at risk of developing high blood pressure.”

Introduction

Your heart beats around 100,000 times a day. As blood moves through your body, it exerts pressure on the veins and arteries. While blood pressure tends to increase with age and can vary transiently based on physical activity and consumption of substances like caffeine, a “healthy” resting blood pressure for adults is 120/80 ‘millimeters of mercury’ or mmHg. The top number, or systolic blood pressure, is the pressure in your blood vessels during heart contraction, whereas the bottom number, or diastolic blood pressure, is the pressure sustained while the heart is in the relaxed state.

Chronic high blood pressure is a risk factor for a number of disorders throughout the body, such as stroke, glaucoma and retinopathy in the eye, heart attacks, heart failure, and kidney failure. In the U.S., it’s estimated that about 29% of adults have high blood pressure (greater than 140/90 mmHg) and roughly another third of adults have pre-hypertension (greater than 120/80 mmHg) and are at risk of developing high blood pressure. Two of the five most commonly prescribed drugs in the U.S. treat high blood pressure, with nearly 46 million Americans taking at least one drug for the condition.

Studies on blood pressure in older women are extremely important. While high blood pressure is more prevalent in younger men than in younger women, by age 65 more women than men are affected. The goal of this study was to examine blueberry consumption in postmenopausal women at risk of developing hypertension to determine if blood pressure and arterial stiffness could be decreased.

As we get older, our arteries tend to get stiffer and less elastic. Since the arterial vascular tone plays a key role in maintaining adequate blood circulation, when elasticity is affected, an increased stress is placed on the heart. High blood pressure and arteriosclerosis, or hardened arteries, often go hand in hand. The most common type of arteriosclerosis is known as atherosclerosis, where fatty plaque deposits build up on the inner surfaces of hardened arteries. Arteriosclerosis is also linked to increased risk of stroke, heart attacks, and kidney failure.

Blood pressure is measured with a sphygmomanometer, the cuff that health professionals put around your arm at each doctor’s visit. Arterial stiffness is a little trickier. One way to measure this non-invasively is called Pulse Wave Velocity (PWV). Imagine your arteries as a plastic tube, and the ‘wave’ of pressure caused by your heartbeat as a ball bouncing back and forth between the sides of the tube. If the plastic is very hard, the ball will bounce down the tube faster than if the plastic is soft - just like if your arteries are very stiff, the wave of blood pressure will move through the artery faster.

This can be quantified by placing pressure sensors at two different points in the body, and then measuring the time it takes for a blood pressure wave to travel between the

two sensors. Since the distance between the two sensors is known, the travel time of the wave can be used to calculate the stiffness of the artery through which it traveled. The two most common PWV measurements are between the carotid artery in the neck and the femoral artery in the leg (cfPWV), and between the brachial artery in the upper arm and the anterior tibial artery in the ankle (baPWV). The cfPWF measurement is more relevant to the central arteries, while the baPWV is more relevant to the peripheral arteries. Increased arterial stiffness has been shown to be a risk factor for cardiovascular disease and strokes.

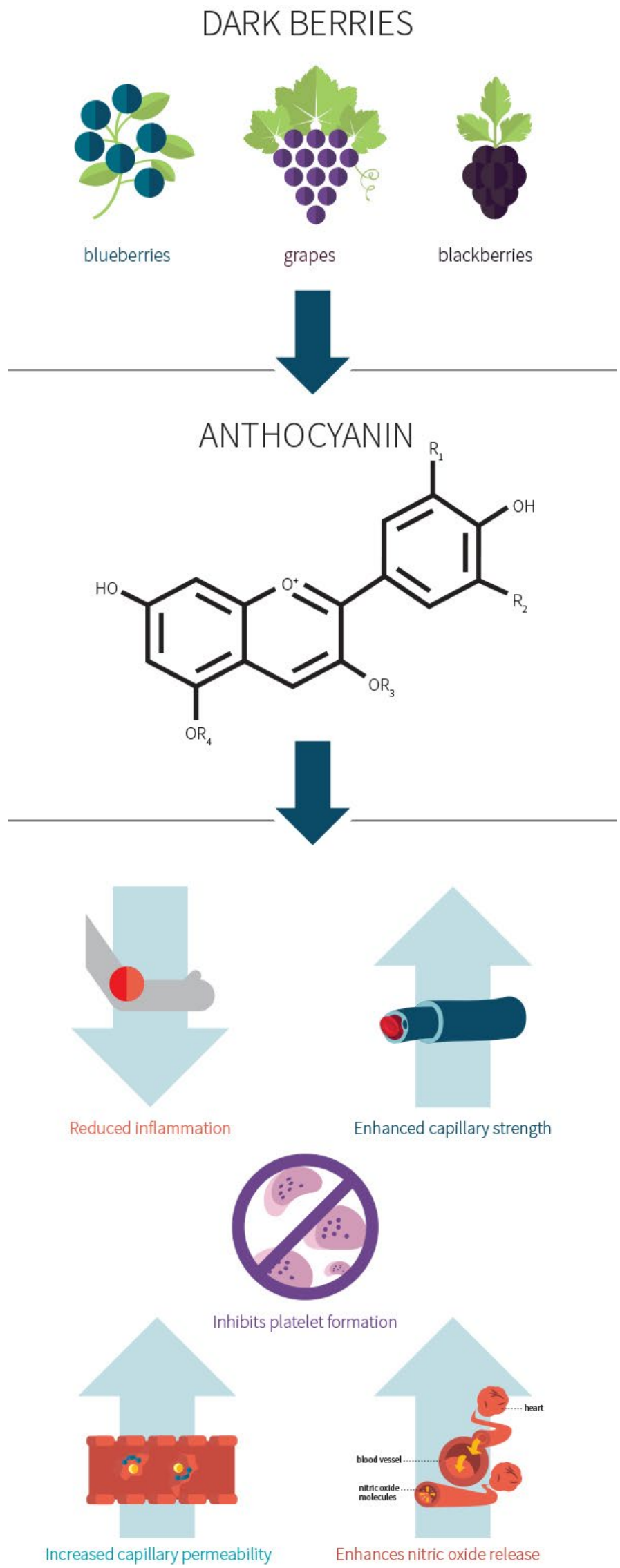
High blood pressure and arterial stiffness have been linked to a number of disorders, including heart disease and stroke.

Why blueberries? Several studies have shown that blueberry consumption is linked to a modest decrease in blood pressure in various groups of people with hypertension. Blueberries contain a number of compounds, including anthocyanins (which put the "blue" in blueberry) and other flavonoids that act as antioxidants. Figure 1 shows some potential mechanisms by which anthocyanins may help with heart disease prevention. An especially important one may be that these compounds increase the production of nitric oxide, which acts as a vasodilator and lowers blood pressure. Since current recommendations for pre-hypertension are to make dietary and lifestyle changes (e.g. decreasing sodium intake and increasing exercise) before turning to medication, evaluating blueberries in additional populations is an important area of research.

Who and what was studied?

This study evaluated 40 postmenopausal women (aged 45-65). All of the women had either pre-hypertension or stage 1 hypertension, meaning that their blood pressure was over 120/80 mmHg but under 160/90 mmHg. The women were, on average, overweight to slightly obese, but otherwise healthy and not taking medications for hypertension. All

Figure 1: Mechanisms linking anthocyanins to heart disease prevention



study participants were told to maintain their usual diet and exercise habits. However, adherence to these recommendations was not evaluated.

The women were randomized to receive either packets of powder made from freeze-dried blueberries, or a placebo powder matched for calorie, carbohydrate, and protein content with artificial sweeteners and colors added so that neither the researchers or the participants knew which packet they had received. The content of the blueberry packet was equivalent to one cup of fresh blueberries, while the placebo powder lacked calcium, potassium, and fiber in addition to the missing anthocyanins and other flavonoids. The women consumed half of the day's powder in the morning and half in the evening, for a total of eight weeks. At study enrollment, four weeks and eight weeks, the participants had their weight and waist circumferences measured, cardiovascular measurements taken, and provided a blood sample to test for the presence of blood markers.

Postmenopausal women consumed blueberry powder or a nutritionally-equivalent substitute for eight weeks to determine the effects of blueberries on blood pressure and other cardiovascular measurements.

The study was funded by 'Big Blueberry' (aka the U.S. Highbush Blueberry Council) and the U.S. Department of Agriculture, but the study authors are independent of both organizations.

What were the findings?

The mean weight and waist circumference measurements of either group of participants did not change significantly over the course of the study. Also, there were no statistically significant changes seen in any of the study measurements at the initial four-week follow-up.

At eight weeks, the blueberry group saw a seven-point decrease in systolic blood pressure and a five-point decrease in diastolic blood pressure compared to their pre-study measurements. The control group saw no changes in either systolic or diastolic blood pressure measurements. The blueberry group also saw a decrease in their baPWV, of approximately 6.5%, but no changes in their cfPWV, arterial pressure, or heart rate. None of these measurements changed in the control group.

The researchers also looked at three blood markers of cardiovascular health: c-reactive protein (CRP), superoxide dismutase (SOD), and nitric oxide (NO).

The CRP levels did not change in either group over the course of the study. Oddly, the SOD levels rose significantly in both the blueberry and the control groups, nearly doubling by the eight-week follow-up. The researchers had no explanation for this phenomenon other than "a time effect." The NO levels were significantly increased by 68% in the blueberry group, whereas in the control group, no significant changes were observed.

Studied cardiovascular health markers

C-reactive protein is a protein generated by the liver in response to inflammation. Elevated CRP levels are associated with increased risk of cardiovascular diseases.

Superoxide dismutase is an enzyme that converts superoxide free radicals into either oxygen or peroxide, protecting cells from damage.

Nitric oxide is an important signalling molecule in the body that plays a variety of roles and among other things, drives blood vessel dilation, thus, playing a crucial role in the process of blood pressure regulation.

Another reason for control groups

The SOD measurement shows the importance of having a control group in clinical trials. If researchers had just looked at a group of people consuming blueberries, they could have mistakenly associated the increase in SOD levels with consumption of blueberries.

Because levels rose in both groups, the increase in SOD is likely not related to blueberry consumption, and instead is due to something that is similar in both groups (anything from the particular time of year to the fact that they were participating in a clinical trial).

Consuming the equivalent of one cup of blueberries per day was associated with decreased blood pressure and peripheral arterial stiffness, possibly as a result of increased NO levels.

What does the study really tell us?

Overall, this study showed that consuming blueberries was associated with a drop in blood pressure and arterial stiffness, and an increase in NO levels.

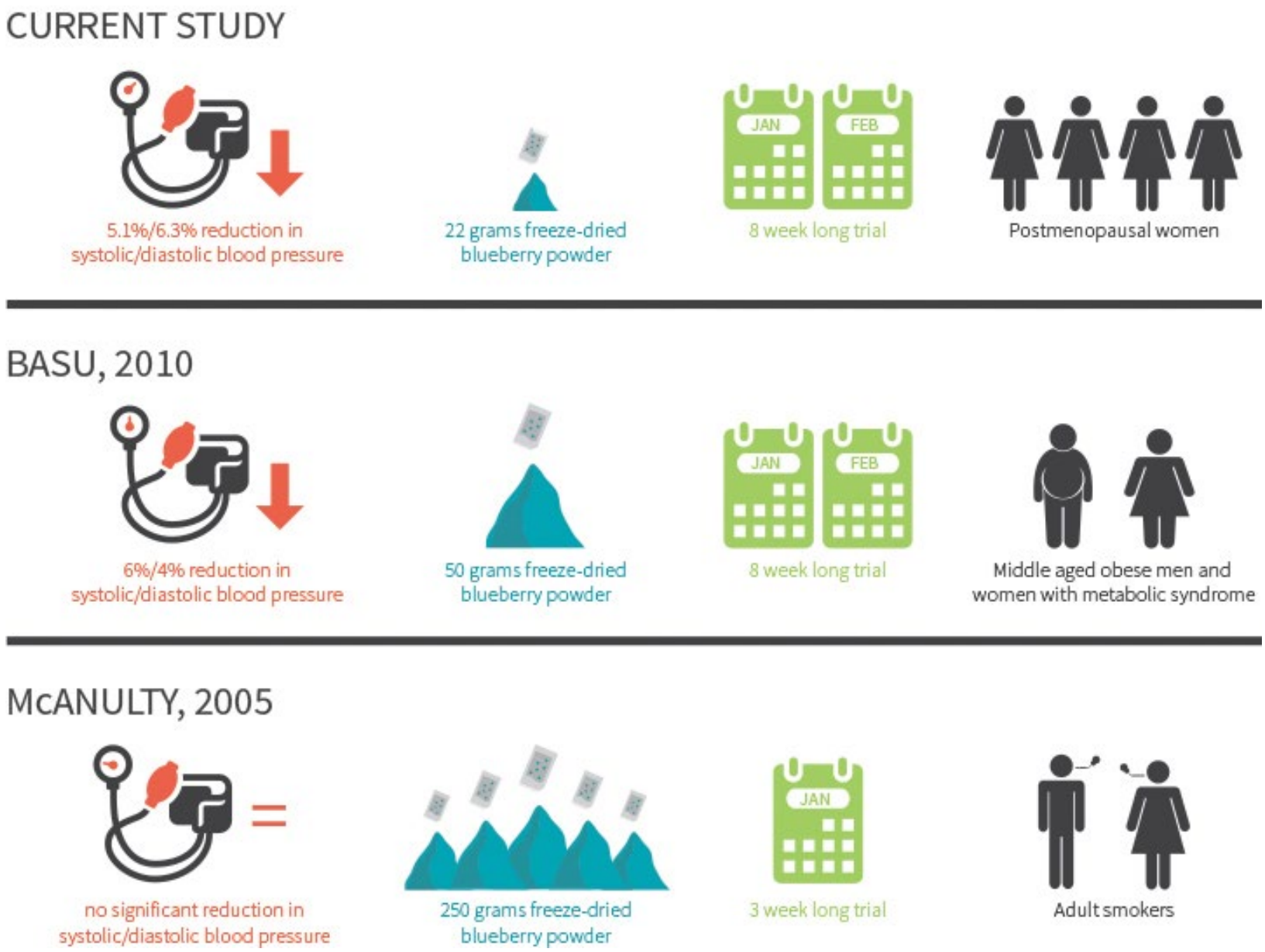
While heavy smokers (more than a pack a day) were excluded from the study, the data were not normalized based on current or past smoking habits. This is an important factor to consider, as it has been previously shown that smokers may have an impaired response to antioxidants when compared to non-smokers, even with blueberries specifically (shown in Figure 2). One study specifically evaluated blood pressure in smokers, and found that eating fruit high in antioxidants had no effect on blood pressure and biomarkers of oxidative stress when compared to a group that did not consume the fruit.

Another factor that was not controlled was the dietary and exercise habits of the participants. As noted before, the participants were generally overweight to slightly obese with an average BMI of 30 in the blueberry group and 33 in the control group. The participants' diets were recorded at the beginning of the study but not tracked over the course of the eight-week study period, and exercise was not evaluated at all.

While the study authors hypothesize that the antioxidants are responsible for the increase in NO and subsequent drop in blood pressure, additional studies with comparable amounts of isolated anthocyanins and flavonols would need to be conducted to further validate that this is the mechanism driving the observed changes. It's possible that other components of the blueberry powder contributed to the lowered blood pressure. The DASH diet (Dietary Approaches to Stop Hypertension) recommends increased potassium, magnesium, and calcium, protein, and fiber in addition to reduced sodium levels, and the freeze-dried blueberry powder used in this study had higher levels of potassium, calcium, and fiber compared to the placebo. It is possible that any of these could have influenced the cardiovascular measurements as well.

These results may not extend to heavy smokers, who were excluded from this study and may have altered responses to antioxidants. While the authors hypothesize that the antioxidants from the blueberries caused the changes seen, it is possible that other components of the powder could partially account for some of the changes.

Figure 2: Trials on blueberries and blood pressure



Sources: Basu et al., J Nutr. 2010 McAnulty et al., Free Radic Res. 2005

The big picture

The research results from this study are in line with other trials and observational studies that have looked at blueberry consumption as a direct intervention, or have linked dietary patterns that had a high consumption of blueberries and other berries to decreases in blood pressure. In this study, a modest reduction of about 5-6% was seen in blood pressure after blueberry consumption. It's not yet known if earlier dietary intervention would be sustained through the aging process, where increases in blood pressure are very common.

Arterial stiffness has been studied less often in dietary interventions, but since it's strongly correlated with cardiovascular events, it's likely a better marker than even blood pressure for predicting an individual's risk. Since the participants had changes in their baPWV but not their cfPWV, this might suggest that dietary changes (particularly short terms ones like this) have a bigger effect on peripheral arteries than central arteries.

Even with the study's potential limitations, it still appears to be a promising intervention. The reductions in blood pressure, NO levels, and arterial stiffness that were seen in this study are all consistent with the known effects of antioxidants, although other nutritional differences between the two powders could have also contributed to the effects, particularly the fiber. Luckily, blueberries are available in a variety of forms (fresh, frozen, freeze-dried, juice, supplements), and "eat more blueberries" is easy advice to give and to follow.

Frequently asked questions

Could I get the same effects from blueberry pie?

Unfortunately, probably not. Processing blueberries causes

the levels of anthocyanins and other compounds to decrease. The most nutritional loss comes from the application of heat. While freeze-drying causes the least amount of nutrient loss, the researchers point out that even their test product could have been less efficient than the fresh fruit. It would be more challenging to design a double-blinded, placebo-controlled study to evaluate the impact of fresh blueberries, but additional observational studies could confirm these results. Additionally, the caloric effect of all the sugar and crust might outweigh any effects of the blueberries themselves.

Are there other foods that provide anthocyanins?
Yes - other blue-purple foods also contain the anthocyanins that make blueberries blue. These foods include eggplant, other dark berries like acai, blackberries and black raspberries, and certain varieties of grapes and potatoes. It's possible that these foods could also provide a similar benefit if eaten by people with slightly elevated blood pressure.

A variety of fruits are high in antioxidants (as seen in Figure 3). While the antioxidant level may not be the direct cause of health benefits, high-ranking fruits are typically rich in bioactive phytonutrients, and "eating the rainbow" does ensure that you get regular consumption of dark, bright, and light-colored plant foods.

Does this study apply to the general population?
The researchers caution that this study was designed to evaluate the effects of blueberry consumption in a specific population in terms of age, sex, and medical history. The study was also of a very short duration, and it's not known if either higher or lower "doses" of blueberries would have the same or more pronounced effects.

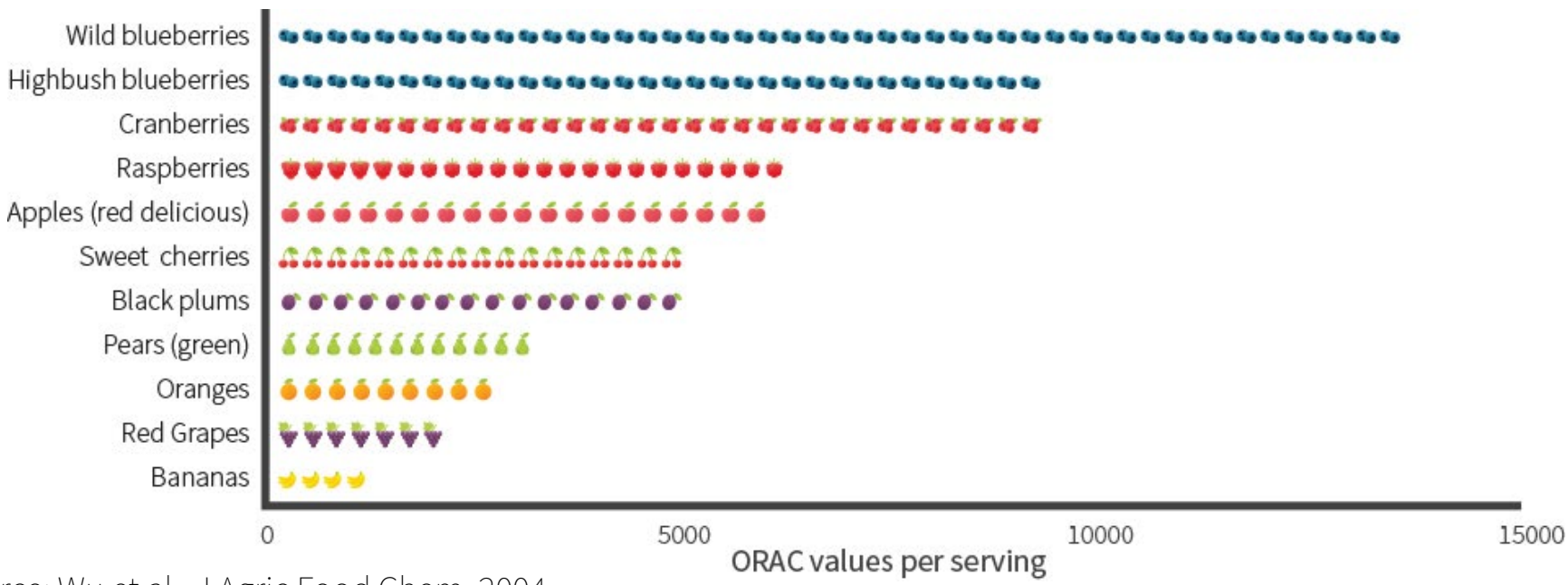
There is epidemiological evidence for effects in other populations though, as well as a number of research studies on polyphenols from other food sources, such as red wine and green tea. In general, polyphenols seem to have positive health benefits, and unlike some supplements, blueberries are a widely-available (and delicious!) healthy addition to any diet, so there are very few downsides to eating up.

What should I know?

Postmenopausal women with slightly elevated blood pressure experienced a decrease in both systolic and diastolic blood pressure measurements after consuming freeze-dried blueberry powder daily for eight weeks. The blueberry group had increased nitric oxide levels, which offers a plausible mechanism for the blood pressure effects, possibly due to slight vasodilation. The amount of blueberries consumed was equivalent to a reasonable one cup per day. ◆

Read more about anthocyanins on the Examine.com website here and here, and visit the ERD Forum on Facebook to discuss this study.

Figure 3: Antioxidant capacity of common fruits



Source: Wu et al., J Agric Food Chem. 2004

Driving a car blindfolded: the neurobiology of appetite

By Margaret Leitch, Ph.D.

Dr. Margaret Leitch completed her Ph.D. in Experimental Psychology in Eating Behaviour and Obesity related research. Margaret has devoted over a decade of research to investigating the psychobiological basis for overeating behavior. With both clinical, private and publicly funded research grants, she has conducted studies that range from projects directly linked with the food industry, for example with Wrigley's International, to BBSRC and Robert Wood Johnson funded projects.

When it comes to protecting public health, 'Eat Less Move More' is a pretty flimsy strategy. This quirky adage entirely neglects a mention of the environment, which is a critical point. Although we are getting better at public health messages for obesity, we've still not reached the gold standard, like what was achieved with seat belts. We may want to focus more on seat belts, than to draw similarities between eating and smoking, compelling as it may be to infuse the obesity conversation with aspects of addictive behavior.

Today, in Canada, you'd be hard pressed to find someone who felt that wearing a seatbelt was a sign of weakness or seat belt laws were infringing on civil rights. Nor do we view those who wear seatbelts as a rare group of actual science nerds, clinging to their calculators projecting grim mortality contingencies, on behalf of the big bad insurance companies.

'Eat Less Move More' doesn't really capture the consequences of our unsustainable food environment. Not only does it minimize the difficulty of weight loss, but it fails to recognize the faults in our food production model. It's an enormous feat of human engineering to have created a system for mass produced, energy-dense, nutrient-poor food. Somewhere along the way, we need to learn how to buckle up, and protect ourselves against the onslaught of hyper-rewarding palatable food.

I became interested in the neurobiology of feeding behavior a few years ago, in order to better address the 'Eat Less' part of the obesity equation. In my career as a health researcher, I have learned the way the brain responds to pleasure, and how neural circuitry can be exploited (and perhaps exhausted) by palatable food. I have also learned about food politics and the immense pressure experienced by people who work in the food industry. Everyone is trying to collectively deal with the consequences of obesity. Unfortunately, we don't have solutions such as cheap, calorie-free chocolate. Not yet, anyway. We also don't have a road system where we can go seatbelt free. I personally feel nervous being in a taxi without buckling up, and it's something I learned to do from an early age.

"Reward circuitry in the base of the brain in obese individuals looks eerily similar to those with methamphetamine addiction, and, as a few researchers have discovered more recently, porn addiction."

In my work, I learned a few important points that I'll sum up quickly:
Cheetos can disrupt satiety signaling, and too many Cheetos too often can lead to habitual overconsumption. Reward circuitry in the base of the brain in obese individuals looks eerily similar to those with methamphetamine addiction, and, as a few researchers have discovered more recently, porn addiction. So, when people try to 'just' stop eating Cheetos, the process might be similar to if a person 'just' stopped taking stimulants. From a psychosocial perspective, it's important to note the association between obesity and poverty in high income countries is both robust and bidirectional.

Certain components of food such as sweetness, mouth feel, and the broad term 'palatability' will enhance the experience of eating. With chronic exposure, we become tolerant to the taste of 'hyper-palatable' foods. The use of the term 'tolerance' is telling. It's a description typically reserved for drugs and alcohol, referring to the diminished sense of delight or momentary escape we get when we use either substance. The fact that 'tolerance' is used in step with eating habits demonstrates the biological liability presented by our food environment today.

But why does food taste good? And why does unhealthy food taste great? In the most basic sense, food tastes great, because it delivers a lot of energy. Tastiness has very little to do with micronutrient content.

> "[...] this explains why both food and drugs 'feel' better when we're hungry: an energy depleted state enhances the feeling of pleasure, because the magnitude of the contrast is larger."

Our brain's energy requirements are astronomical. The brain demands 20% of total energy consumed at rest (or, 20% of Resting Metabolic Rate [RMR]). For children, this is even higher, varying between 43%-85% of RMR. Therefore, kids are particularly sensitive to the rewarding aspects of food. Their brain is continuing to grow quickly and thus energy demands are huge. This is why very sweet foods taste great to kids.

Chronic exposure to hyper-rewarding stimuli can lead to changes in brain plasticity. The brain's plasticity can be a blessing and a curse. On the one hand, plasticity means a person can recuperate full function and even increase function into adult life. On the other hand, if exposed to the 'wrong' inputs (e.g. hyper-rewarding stimuli that exploit hedonic/pleasure driven motivation) the person will find themselves attracted to the reward, at the expense of other adaptive behavior (or other healthier food choices).

We now appreciate the fact that the brain can be reprogrammed to continue a cycle of chronic overconsumption. The hypothalamus regulates a host of physiological responses and behaviours, and is regarded as the primary nutrient 'sensor' in the brain. Together, with reward circuitry, the hypothalamus acts in concert with areas in the brain that sense pleasure to control our feeding behavior. For this reason, Pavlov was correct when he claimed that 'hunger is the best spice.' In addition, this explains why both food and drugs 'feel' better when we're hungry: an energy depleted state enhances the feeling of pleasure, because the magnitude of the contrast is larger.

The hypothalamus is the main point of convergence for regulating a host of behaviours. Like the entire brain, it is plastic. So, if you have learned to prefer high sweet foods, hypothalamic and reward signaling will be sluggish when you consume foods that aren't quite so 'nice.'

As humans, we are simply not very good at self-discipline when it comes to eating behavior. This is being capitalized

upon. Some might say exploited. Food companies can afford to make these kinds of foods because the ingredients and technology to are bought at scale. Human appetite has become an economy of scale.

I'd like to borrow a quote from Nora Volkow. Dr. Volkow is the current Director of the National Institute on Drug Abuse, and has used this analogy to describe the difficulty in behavior modification and overeating: 'When peripheral signals—such as leptin or insulin—are not released, or your brain becomes tolerant to them, you don't have a mechanism to counter the drive to eat. It's like driving a car without brakes.'

Without some appreciation of the effects of the food environment, we're driving our cars blindfolded. While we could argue that we have a conscious choice regarding the way we respond to our environment, we could also argue the food choices available perpetuate overeating, which leads to weight gain and obesity.

Obesity is the result of a series of endocrine, metabolic, neurocognitive, and physiological adaptations, fostered by an environment of abundance. Our default environment contributes to passive overconsumption, and my personal bias is that there is no 'body wisdom.' Rather, the body responds to the inputs it receives. For the same reasons I won't trust a five-year-old to drive a car, or myself to get through traffic blindfolded, I don't trust that as a group of over six billion, we're entirely capable of making decisions to just 'eat less' on our own accord.

But back to snappy strategies for public health: 'Total Car Crash' might be more apt for certain choices in our environment. Calling food out in this way might do a lot more for the public than 'Eat Less' ever has. ◆

> "Human appetite has become an economy of scale."

Margaret's expertise is unique insofar as she has cultivated both academic and industry related knowledge. She has extensive understanding of the psychological aspects of food choice, and cognitive mechanisms responsible for reward. In addition, she is well versed in food politics and commercial aspects of the food industry. Together, these afford a unique perspective on the 'decision' to overeat, and the steps needed to reform our food environment. In her co authored book, Fat Planet, she has had the opportunity to bring scientific insight, policy research, and public health experience to the forefront.

Can the paleo diet make metabolic syndrome ancient history?

Favourable effects of consuming a Palaeolithic-type diet on characteristics of the metabolic syndrome: a randomized controlled pilot-study

Introduction

Approximately 25% of the adult U.S. population has metabolic syndrome, a condition that greatly increases the risk of diabetes and cardiovascular disease (CVD). Though the exact definitions may vary, metabolic syndrome is characterized by insulin resistance and is classified as a cluster of at least three of the following risk factors:

- Abdominal obesity (waist circumference greater than or equal to 102 cm in men or 88 cm in women)
- Fasting blood glucose of 100mg/dL or higher
- Serum HDL cholesterol below 40mg/dL for men or 50mg/dL for women
- Serum triglycerides of 150mg/dL or higher
- Blood pressure elevated above 130mmHg systolic or 85mmHg diastolic
- Use of medication to achieve healthy ranges of blood glucose, cholesterol, triglycerides, or blood pressure.

When we consider the daily activity levels of people prior to the 20th century, it becomes clear that we are in the midst of a genetic mismatch between our genes and the environment. The modern lifestyle includes 24/7 availability of salty, sugar-laden, high-fat foods along with unnatural exposure to artificial light and the ability to go days, weeks, months, or even years without walking for longer than five minutes in a row. Acknowledgment of this genetic mismatch coupled with declining health statistics has led researchers to consider the foods that were being eaten prior to not only the industrial revolution, but also before the agricultural revolution (the past 10,000 years, when the cultivation of grains, legumes, and dairy began).

“ The modern lifestyle includes 24/7 availability of salty, sugar-laden, high-fat foods along with unnatural exposure to artificial light and the ability to go days, weeks, months, or even years without walking for longer than five minutes in a row. ”

An oft-cited criticism of the “paleo” diet is that there was not one specific ancestral diet. However, a number of research papers have used similar diets focusing on lean meats and fish, fruit, vegetables, eggs, nuts, and seeds, while removing grains, legumes, added sugars, and dairy products. Irrespective of what people *actually* ate during those times, this incarnation has established itself in research and practical use as “the paleo diet.”

Previous studies have used healthy subjects, as well as people with diabetes, CVD, and obesity. However, up until now, no studies have focused on people with metabolic syndrome. Since many of the previous studies that showed beneficial effects of a paleo diet also included weight loss, it is not clear whether the favorable effects were due to the weight loss itself or to the composition of the diet. The purpose of this pilot study was to compare the metabolic effects

of a paleo diet to a generally healthy reference diet, in the absence of weight loss, in people with metabolic syndrome.

Metabolic syndrome is defined as a cluster of metabolic problems that increase the risk of cardiovascular disease and death. Previous research on "the paleo diet" has not focused on subjects with metabolic syndrome, and had weight loss as a possible confounding factor.

Who and what was studied?

This randomized controlled pilot study was open to men and women ages 18–70 who had at least two of the characteristics of metabolic syndrome. For two weeks, the participants received either a paleo diet (n=18) or a healthy reference diet that was based on the guidelines of the Dutch Health Council (the study took place in the Netherlands; n=14).

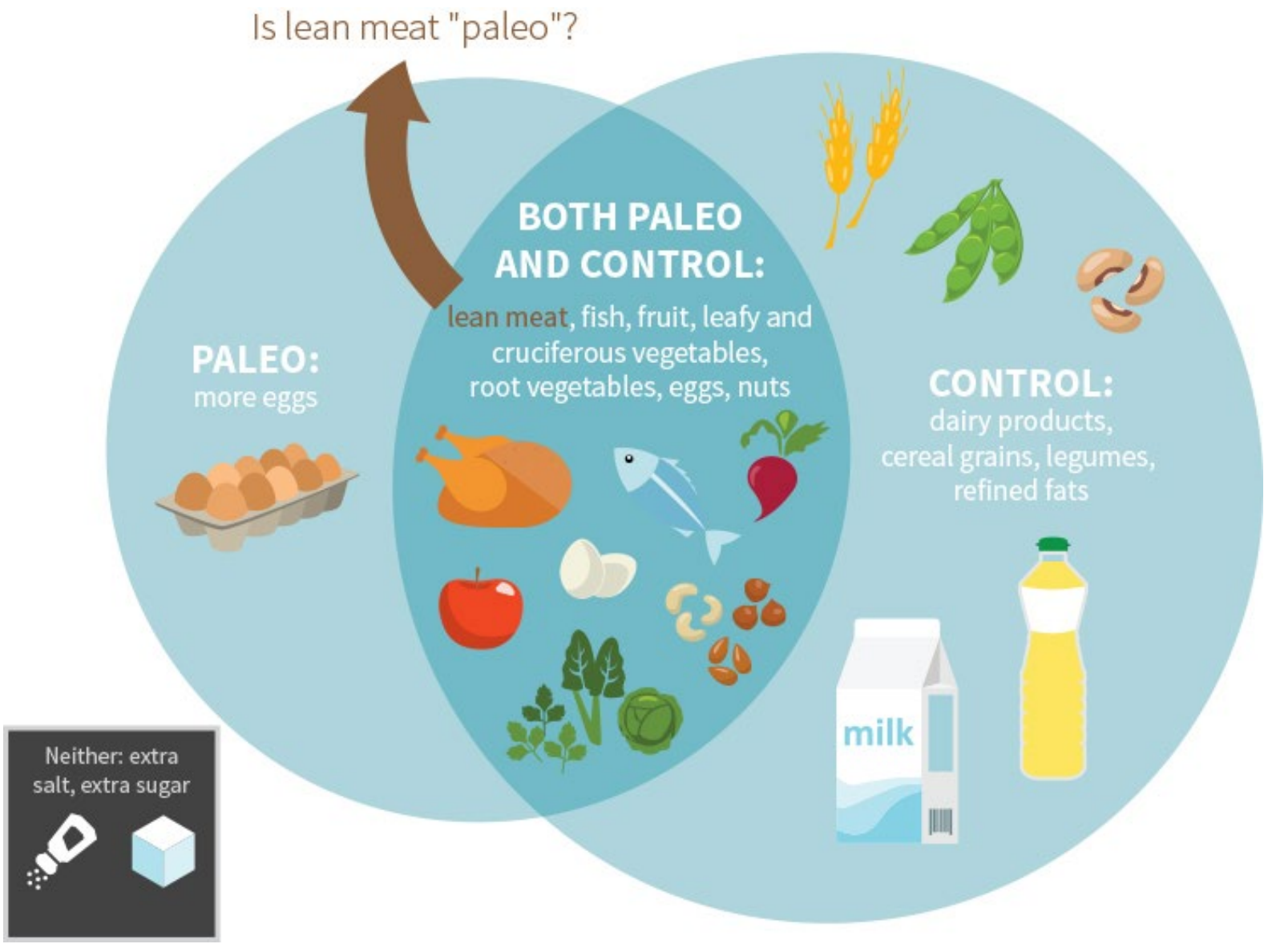
Why use pilot studies?

A pilot study is a small version of a planned larger study, used to test the methods, procedures, and feasibility of the study design and intervention. It allows researchers to work out methodological problems, food delivery, or testing issues on a smaller scale before larger amounts of money, time, and resources are invested.

Another major reason to do a pilot is to get preliminary results that are compelling enough to justify a full-blown study.

As shown in Figure 1, meal composition was similar to that of previous studies using a paleo-type diet, with meals based on lean meat, fish, fruit, leafy and cruciferous vegetables, root vegetables, eggs, and nuts. Dairy products, cereal grains, legumes, refined fats, extra salt, and sugar were not included. One of the most popular questions about paleo is:

Figure 1: Composition of paleo and control diets



"Is [blank] considered paleo?". While many paleo studies use lean meat, it's questionable as to whether our ancestors did, as fatty meat provides more calories.

Both of the study diets provided 2,080kcal/day, and all of the daily meals and snacks were delivered to the participants' homes. Home delivery is very important because it encourages adherence. There were notable differences in the the macronutrient ratios (Figure 2).

The average age of the participants (25 women and nine men) was 53.5 years old. Although the average BMI was 31.8 (greater than 30 is the "obese" category), it is important to note that the researchers put an emphasis on avoiding weight loss during this trial. Body weight was measured every other day, and if it fluctuated by more than two kilograms, a dietician adjusted the caloric content of the diet to compensate. Nine participants (seven in the paleo group vs. two in the reference group, 38% vs. 14%, respectively) required additional snacks because of unintentional weight loss.

The main goal of this study was to determine if a paleo-type diet alters characteristics of metabolic syndrome, independent of weight loss. Characteristics of metabolic syndrome were measured (glucose tolerance, abdominal circumference, blood pressure, glucose, lipids), as well as intestinal permeability, inflammation, and salivary cortisol.

This study was designed to see if a paleo diet could reduce markers of metabolic syndrome in people with the disorder, compared to a healthy reference diet. Researchers added or removed snacks to keep participant body weight constant through the study.

What were the findings?

The findings were very impressive for the paleo diet intervention, which was superior to the reference diet in nearly every category (Figure 3).

Figure 2: Macronutrient composition of the study diets

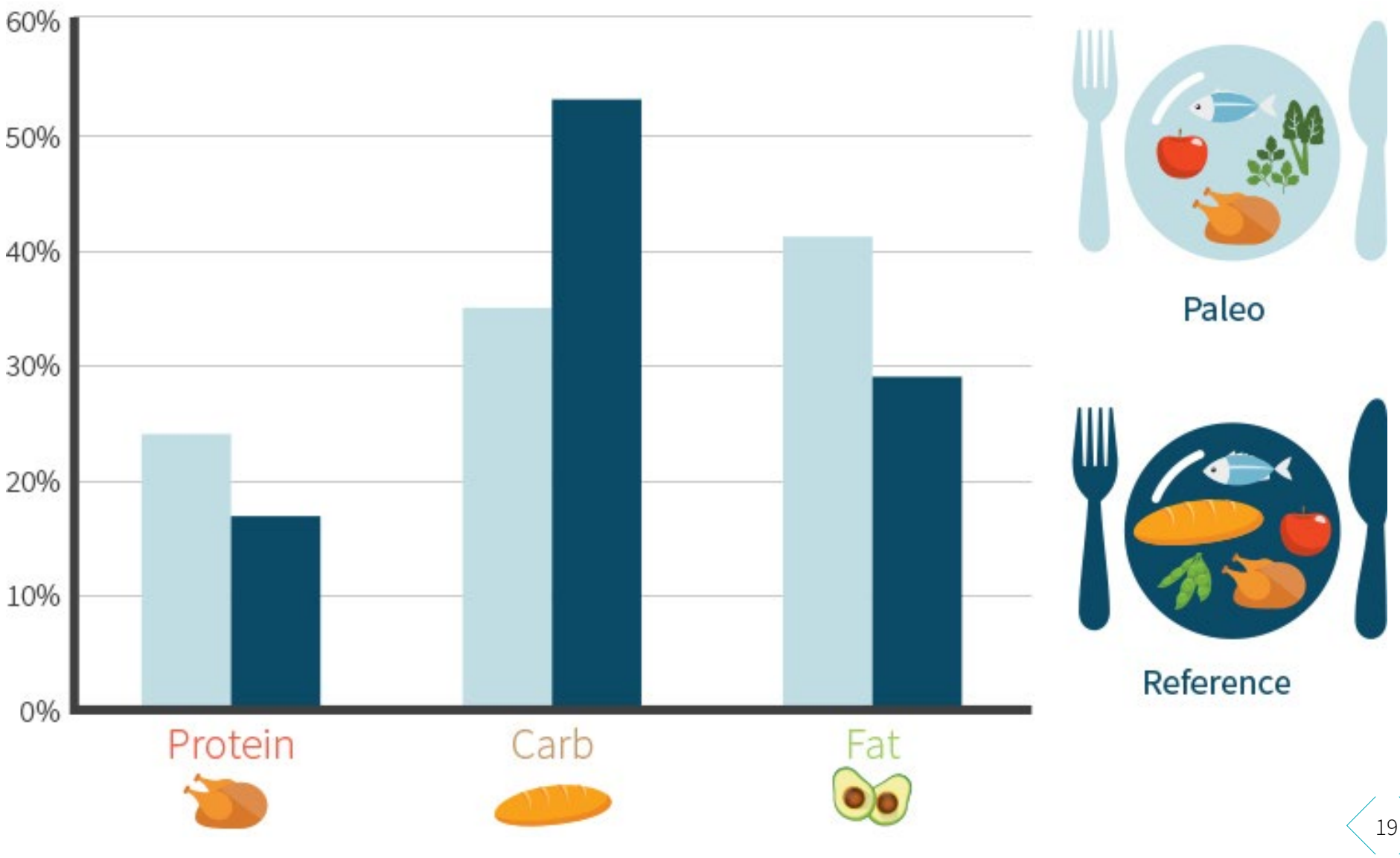
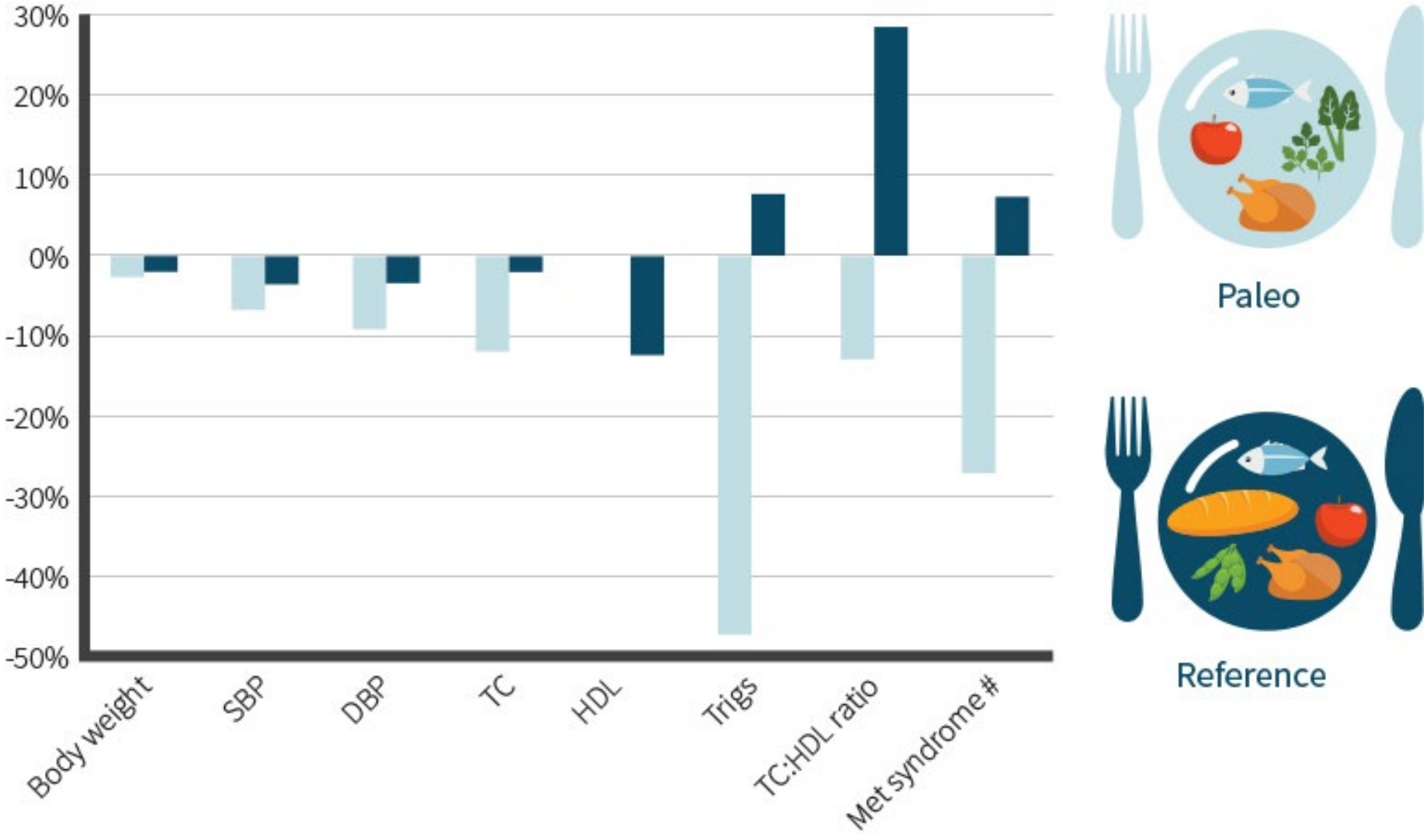


Figure 3: Study Results



The paleo diet resulted in lower systolic and diastolic blood pressure, total cholesterol, and triglycerides, and higher HDL cholesterol, compared to the reference diet. The number of characteristics of the metabolic syndrome decreased with the paleo diet but not with the reference diet. Body weight decreased by an average of three pounds on the paleo diet, despite efforts to prevent weight loss.

Further analysis determined that the favorable effects of the paleo diet remained even after researchers adjusted for weight loss. Both diet groups experienced similar improvements in waist circumference, fasting glucose, and the ratio between urinary sodium and potassium, while fasting plasma insulin and insulin sensitivity improved only in the paleo group. No changes in intestinal permeability, inflammation, or salivary cortisol were observed in either group.

The paleo diet lowered blood pressure, total cholesterol, and triglycerides and raised HDL compared to the healthy control diet. Body weight decreased in the paleo group despite efforts to keep it constant, but the metabolic changes were still significant even after statistically adjusting for the weight loss.

What does the study really tell us?

Although a small pilot trial, this is the first controlled study to examine the influence of a paleo-type diet in people with metabolic syndrome. Substantial changes were found in as little as two weeks, which is even more interesting given that the participants were trying to maintain their weight, and were having a hard time doing so. Observed favorable changes included all aspects of metabolic syndrome: low-

ered blood pressure, improved lipid profile, and improved markers of blood sugar control.

No changes were observed regarding inflammation, gut permeability, and cortisol patterns, possibly due to the short study duration or to the body weight maintenance aspect of the study. These changes are more difficult to measure because they depend on the baseline values. If a participant already had a healthy gut, for example, it wouldn't necessarily improve further on any diet. One of the strengths of this study is that the results may be generalizable to a fairly wide population because the participants included both men and women from a wide age range who had varying characteristics of metabolic syndrome.

> "[...] 89% of the paleo group (and only 64% of the reference group) reported they were still motivated to continue the dietary regimen."

The macronutrient differences between the diets are an important aspect to consider. Although the diets contained the same number of calories, the paleo diet was higher in protein (24% vs. 17% for the reference diet), lower in carbs (35% vs. 53%), and higher in fat (41% vs. 29%). Diets higher in protein are known to increase satiety (leading to a spontaneous reduction in calorie intake), increase thermogenesis (heat production), and promote better maintenance of lean muscle mass.

A recent meta-analysis comparing low-carb (which included anything below 45% of daily energy intake) to low-fat (less than 30% of energy intake) diet studies found that lower carb diets were equally effective as low-fat diets in improving a number of metabolic disease risk factors, but participants on low-carb diets had greater increases in HDL cholesterol and greater decreases in triglycerides compared with people on low-fat diets. The researchers took great care to ensure energy content was the same between the two diets, and that participants did not lose weight. The decision of whether to match the macronutrient distribution is an interesting one. On the one hand, keeping the macros the same would have given a better look at the differences in food types and quality, but on the other hand, these macronutrient ranges are more likely what people would actually be consuming when following either of these diets.

A lack of adequate calcium intake is often suggested to be one of the downsides of a paleo diet. This study diet was no exception, with a 50% lower calcium intake compared to the reference diet (575 mg vs. 1181 mg; the recommended daily allowance (RDA) for calcium is 1000 mg). However, considering that the paleo dieters had lower urinary calcium excretion, calcium homeostasis in the body may not be significantly affected.

Another one of the biggest criticisms against the paleo diet approach is that it is difficult to follow. Adherence and sustainability are key aspects to any diet program. In fact, authors of the A to Z weight-loss study concluded that "strategies to increase adherence may deserve more emphasis than the specific macronutrient composition of the weight loss diet itself in supporting successful weight loss." Even though the current trial was only two weeks long

(and participants had all food provided to them), 89% of the paleo group (and only 64% of the reference group) reported they were still motivated to continue the dietary regimen. Previous research in obese postmenopausal women showed favorable changes in fat mass and waist circumference after six months on a paleo diet that were not sustained after two years. Interestingly, participants in that study decreased their protein intake by nearly 10% during months six through 24.

The study is generalizable because of the diverse demographics of the study participants. While the macronutrient contents of the diets differed considerably, they tended to generally reflect the macros typically found in those diets.

The big picture

"We conclude that consuming a Paleolithic-type diet for two weeks improved several cardiovascular risk factors compared to a healthy reference diet in subjects with metabolic syndrome."

This small pilot study adds to the growing body of research suggesting a paleo-type diet (defined in this study as a diet based on lean meats, fish, eggs, fruits, and vegetables with the exclusion of grains, legumes, and dairy) can be an effective intervention for improving a variety of metabolic disease risk factors. In the attempt to keep body weight stable, which proved to be difficult, some participants had to consume additional snacks. It would be interesting to see if there were even greater effects of the diet if subjects were allowed unlimited snack consumption. It will also be important during a longer trial to see at what point in time the favorable metabolic changes plateau.

> "The vast majority of research on the paleo diet has been favorable."

The vast majority of research on the paleo diet has been favorable. A three-week study in healthy participants led to weight loss and blood pressure improvements. A three-month study on people with diabetes reported improved glycemic control and blood lipids values compared to a traditional diabetes diet. A 12-week study on people with heart disease found improved glucose tolerance, independent of weight loss. A 10-day study on healthy participants resulted in improved blood pressure, glucose tolerance, and lipid profiles, but no weight loss.

Additionally, two studies have found increased satiety when a paleo diet is consumed. Recently, a two-year study in obese postmenopausal women reported improved triglyceride levels compared with a control diet, and better weight loss after six months, which then returned to the same as the control diet after

> “People on a paleo diet can make it high-carb, low-carb, no-carb, high-protein, vegetarian, and more [...]”

24 months. Of note, in the last study, the participants decreased their protein level in months seven through 24. One study in healthy participants found unfavorable changes in lipids on a paleo diet, though these people were also participating in high-intensity exercise training, which introduces a number of other variables. More research is needed, but so far, the paleo diet (i.e., grain-, legume-, dairy-free) has been faring quite well.

Frequently asked questions

How would matching macros have affected the study?

The higher protein/lower carb paleo diet is likely to have produced greater satiety from the same number of calories, which could then improve adherence to the diet. Again, the question arises of whether the study is trying to determine if the types of foods consumed (lack of grains, legumes, and dairy) are causing the changes, in which case the macros should be matched. Contrary to the claims that a paleo diet is low-carb, a diet that excludes grains, legumes, and dairy can actually be macronutrient-agnostic. People on a paleo diet can make it high-carb, low-carb, no-carb, high-protein, vegetarian, and more, so this study could still have been done with a matched macronutrient profile.

But there wasn't one paleo diet...

This is true, but this isn't (or shouldn't be) about historical reenactment. Paleo may be an unfortunate name, but the research studies that have been evaluating a “paleo diet” have generally used something similar to the diet these subjects consumed—moderate carb (lower than most standard recommendations, but not ketogenic), and slightly higher than “standard” amounts of protein (between 24–30% of daily energy intake) and fat (between 27–40% of daily energy intake).

Would the same effects occur in non-obese people?

It is unlikely that such dramatic changes would be seen in such a short time period with already healthy participants. A few studies mentioned above found beneficial changes in healthy participants, though there is a lot less room for improvement when lab values are already in healthy ranges. A controversial study from last year found that an unrestricted paleo diet in healthy people led to unfavorable changes in blood lipids. During the intervention, the participants were simultaneously participating in high intensity CrossFit workouts, which adds a number of confounding variables. There were requests for this study to be retracted, which the journal declined to do.

What I should know?

A paleo-type diet (grain-, legume-, and dairy-free) can be an effective intervention (that is, in the short term, and when the food is provided for the participants) for improving a variety of metabolic disease risk factors in overweight and obese men and women with characteristics of metabolic syndrome.

This study provided food for the participants, which led to very good adherence rates. Results may be more pronounced in obese people or people with some form of metabolic derangement. That being said, it is a small pilot study, and needs to be replicated in a larger and longer trial. ◆

Stripping away the caveman connotation, paleo diets have performed quite well in trials. But arguments still abound … discuss them at the ERD Facebook forum.

Kick the can: how BPA in canned drinks impacts blood pressure

Exposure to bisphenol A from drinking canned beverages increases blood pressure: randomized crossover trial

Introduction

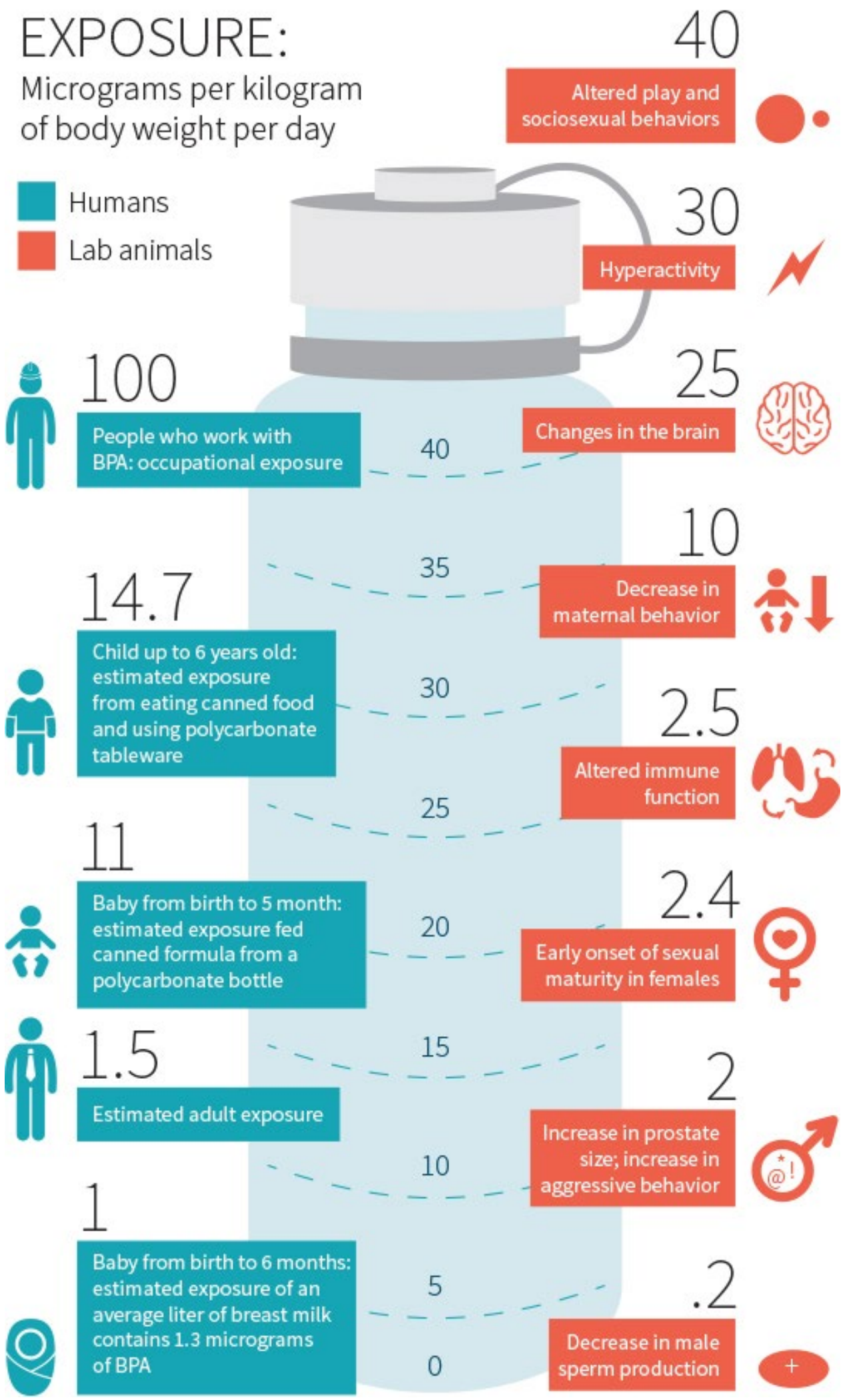
Modern consumers are constantly flooded with warnings of harmful chemicals in everyday products. It's so common for these warnings to be falsified or overblown that finding danger in harmless chemicals has almost become cliche. Unfortunately, these warnings distract from reports on chemicals that could actually cause harm. One of these ubiquitous harmful chemicals is bisphenol A (BPA).

BPA is a critical component of plastic and epoxy manufacturing, which means it's in a variety of products, even those that you wouldn't expect to find any plastic in, like dental fillings and aluminum cans. These cans are lined with a variety of materials, including plastics and epoxies, that prevent the liquid contents from degrading or oxidizing the aluminum of the can.

The BPA in can linings is an estrogen analogue, which means it can interfere with hormonal signaling. Most people normally think of estrogen as a hormone related to secondary sex characteristics and fertility, but it is also involved in many other processes, such as liver function and insulin response. Consequently, researchers have begun to study the effects of BPA on various health parameters. As might be expected, the primary focus of initial research was on the effects of BPA on fertility, especially in light of the fact that environmental BPA contamination generally impairs animal reproduction and development.

As the field matures, however, researchers are starting to assess a variety of other health parameters that could be affected by BPA (as seen in Figure 1), including blood pressure. Some previous studies have shown a correlation between canned beverage consumption or BPA exposure and hypertension, but there are very few studies to assess whether BPA exposure directly causes changes in blood pressure. This study was a follow-up

Figure 1 - BPA in humans, compared to animal trials



Sources: Vandenburg et al., Rev Environ Health. 2013
vom Saal & Hughes, Environ Health Perspect. 2005

on previous work conducted by the authors. It specifically sought to determine whether the BPA exposure from drinking canned beverages could affect blood pressure.

BPA, a component of plastic and epoxy manufacturing, is found in the lining of beverage cans and many other products. It is an estrogen analogue and may cause a variety of health problems. The authors of this study examined whether BPA can affect blood pressure.

Who and what was studied?

The participants in this study were 60 elderly, but otherwise relatively healthy, people. Almost all of the participants were women. About half of them reported medical histories of hypertension or diabetes, and most of the participants that reported these conditions were receiving treatment for them at the time of the study. The researchers specifically selected elderly participants because they are more likely to be affected by environmental chemical exposure than young and middle-aged adults. The participants drank two servings of soy milk from either glass bottles or aluminum cans. The soy milk was sourced from the same manufacturer that offers two different packaging options: a BPA-free glass bottle or a BPA-containing aluminum can.

Participants fasted for eight hours, arrived at the study site, drank two servings of soy milk from a randomly chosen container, and were analyzed two hours later. Because both servings were randomized, the participants either received two bottled servings, two canned servings, or one canned and one bottled serving. Analyses consisted of highly sensitive urine testing for BPA concentration, duplicate resting blood pressure assessments taken about 10 minutes apart, and heart rate variability monitoring.

The researchers performed the same procedure three separate times with a week in between each visit. In each visit, the researchers randomized the participants to another group, so every participant eventually consumed soy milk from all three possible packaging combinations. This crossover design ensured that any demographic differences between the groups canceled each other out because every participant had a data point in every experimental group, which means it takes fewer overall participants to notice any effects. This is a key feature of crossover studies and one of the reasons they are regarded as very reliable.

The researchers also used very stringent statistical analyses to ensure that blood pressure measurements were standardized for all environmental factors, including climate and nonexperimental BPA exposure.

Is there evidence for heart rate variability?

Heart rate variability (HRV) has recently gained more attention in popular media, but it has shown associations with health outcomes for many years. Most of the original research on HRV was done in the context of heart attack risk and mortality.

More recent studies have focused on the interaction between HRV and other parameters beyond cardiac functions. Associations between HRV and a variety of processes, including factors like concentration, have been noted, and the field is rapidly expanding. HRV monitoring has also become a popular tool for athletes to assess whether or not they are over-reaching, because it is believed that HRV can be used as a barometer for overall bodily stress. But so far, the scientific evidence behind this claim isn't strong.

The experiment consisted of giving healthy, elderly participants soy milk to drink either from two bottles, two cans, or one bottle and one can in a crossover design, where each participant participated in each condition. Researchers measured the blood pressure and urinary BPA concentration of each participant.

What were the findings?

Urinary BPA concentration increased significantly only in participants who consumed soy milk from aluminum cans. After accounting for environmental factors, blood pressure increased linearly with urinary BPA. Participants who consumed two servings from the cans showed roughly a five mmHg increase in systolic blood pressure compared to those who consumed two servings from bottles. The change in systolic blood pressure was significant between groups both with and without extensive statistical adjustment, whereas there was no significant change in diastolic blood pressure.

Despite the significant changes in blood pressure, heart rate variability did not change. This is in contrast to the researchers' previous study, which found that heart rate variability significantly decreased (and blood pressure increased) in a much larger cohort of patients. Decreased heart rate variability is associated with a variety of negative health outcomes, including mortality after heart attacks.

BPA was associated with an increase in systolic blood pressure, but not with changes in heart rate variability.

What does the study really tell us?

BPA exposure acutely increases blood pressure in elderly women. One canned drink serving is sufficient to significantly increase BPA concentrations in the body, while two canned drink servings causes a transient, measurable blood pressure effect.

It can be implied that a substantial amount of BPA leached from the can liners and into the canned beverages. This suggests that the consumption of canned beverages (depending on the BPA content of the packaging) will not only increase the BPA concentration in the body, but also have measurable, negative effects on blood pressure. Drinking the same beverage from a BPA-free glass bottle does not result in this response.

The researchers used soy milk rather than water. They note that soy milk hasn't been found to increase blood pressure and is widely commercially available, and is hence an ideal study beverage. While they cited longitudinal and trial evidence of soy milk actually decreasing blood pressure, that evidence doesn't really apply to the current study because it doesn't look at acute blood pressure changes, occurring right after beverage consumption.

Given that soy phytoestrogens have some estrogenic activity and that soy milk contains fat (which could theoretically increase the extraction and solubility of fat-soluble compounds such as BPA from the liner), soy might not actually be the ideal study beverage. Interestingly, even the soy milk in the glass bottles had a bit of BPA in it (with levels at 0.31 and 8.2 μg/L, in bottles and cans respectively.) As shown in Figure 2, heat exposure and age of packaging can affect BPA levels, so there are other factors to consider outside of simply the beverage and type of packaging material. It would be worth replicating this trial with water, soda, and other common beverages, to see if the results are similar.

The big picture

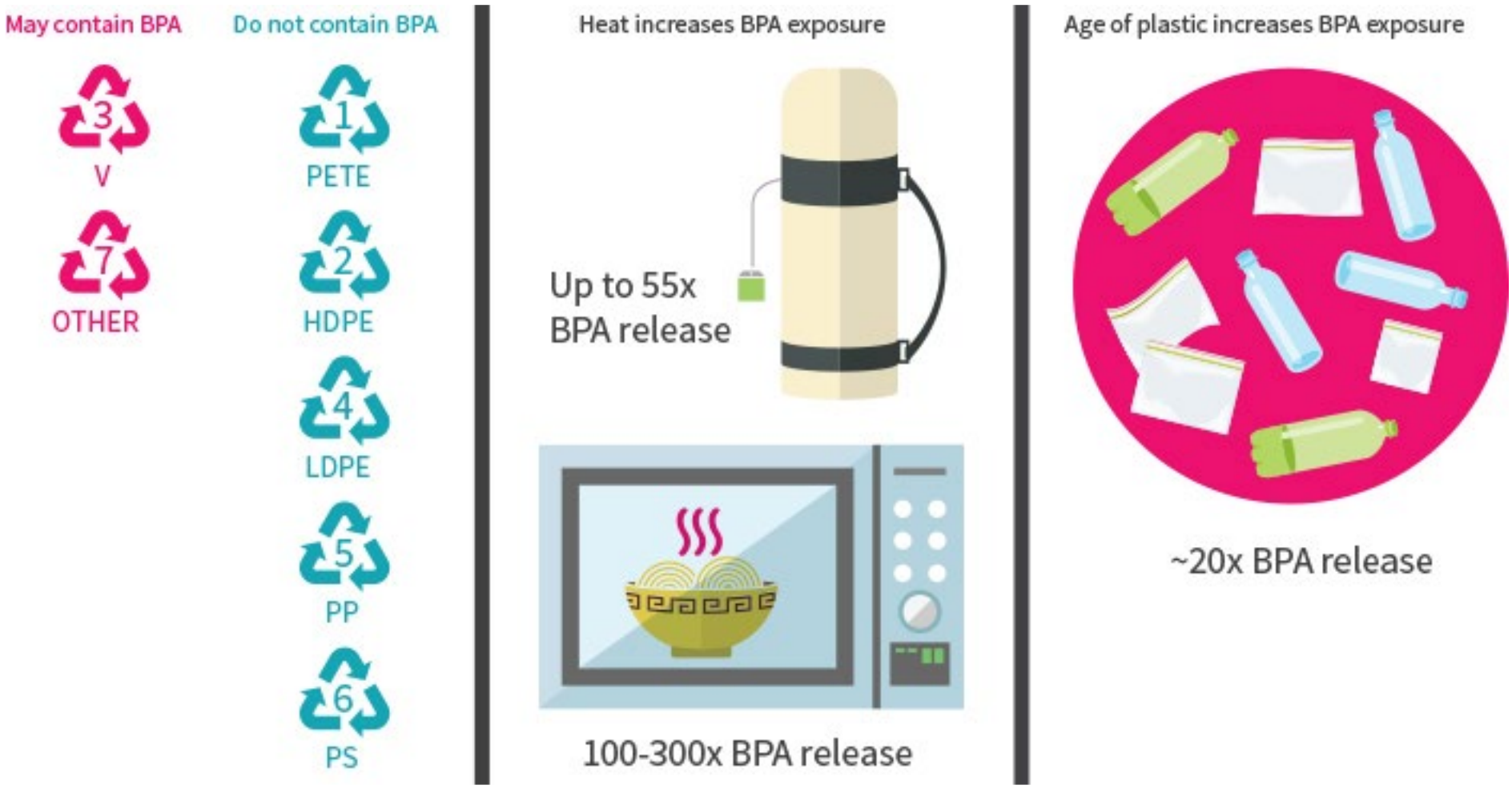
This study is in line with previous studies that found significant biological changes in response to acute BPA exposure. Some of these previous studies were conducted by the same group that conducted this study. This study is still important, however, because it confirmed many pre-

vious correlative findings from a variety of groups. More importantly, it also suggests a direct causative role for BPA increasing systolic blood pressure: participants experienced a small, but significant, increase in blood pressure after consuming a canned beverage, and this blood pressure increase appeared to correlate with an increase in urinary BPA.

Despite the fact that this study showed that BPA exposure can cause acute blood pressure changes, it is still not clear how chronic BPA consumption affects health outcomes. It is important to avoid extrapolating biological changes to disease states. This is one of the most common errors in science journalism. Practically speaking, this means that a small transient increase in blood pressure may not necessarily cause hypertension or other chronic diseases in the long run. BPA exposure might have a clinically relevant effect on blood pressure in some cases (for example, increasing the blood pressure of a prehypertensive person into the hypertensive range for a few hours), but it is not clear how long the response to BPA lasts. Much more work needs to be done before can-based exposure to BPA can definitively be linked to a disease state like hypertension or cardiovascular disease. If this link is eventually found, it would be a major finding, but it would also make it difficult to conduct further studies on BPA because ethical review boards are unlikely to approve studies that involve exposing participants to chemicals with known harmful effects.

Another factor to keep in mind is that many of the participants in this study were already being treated for hypertension. There is always a risk for confounding factors when a large subset of a study population is known to have aberrations in a measured variable. Consequently, a similar study in a different population could have different results. The findings of this study are likely real, but they may be population-specific. For example, younger participants with normal blood pressure values could respond very differently to BPA, and BPA may interact with anti-hypertensive medication in some unknown way. Therefore, other studies to specifically assess the mechanisms linking BPA and blood pressure are needed to conclusively say how BPA causes these effects.

Figure 2: BPA in everyday life



Sources:Tan & Mustafa, Asia Pac J Public Health. 2003 Munguia-Lopez & Soto-Valdez, J Agric Food Chem. 2001

Even in this study, the researchers were uncertain as to how exactly BPA increased blood pressure. The effect may have been due to estrogen receptors (which can play a role in blood vessel repair, although that may more of a role in longer term blood pressure impacts), thyroid hormone effects, or mechanisms that haven't yet been well-elucidated.

Frequently Asked Questions

If BPA is ubiquitous, what's the point in avoiding it?

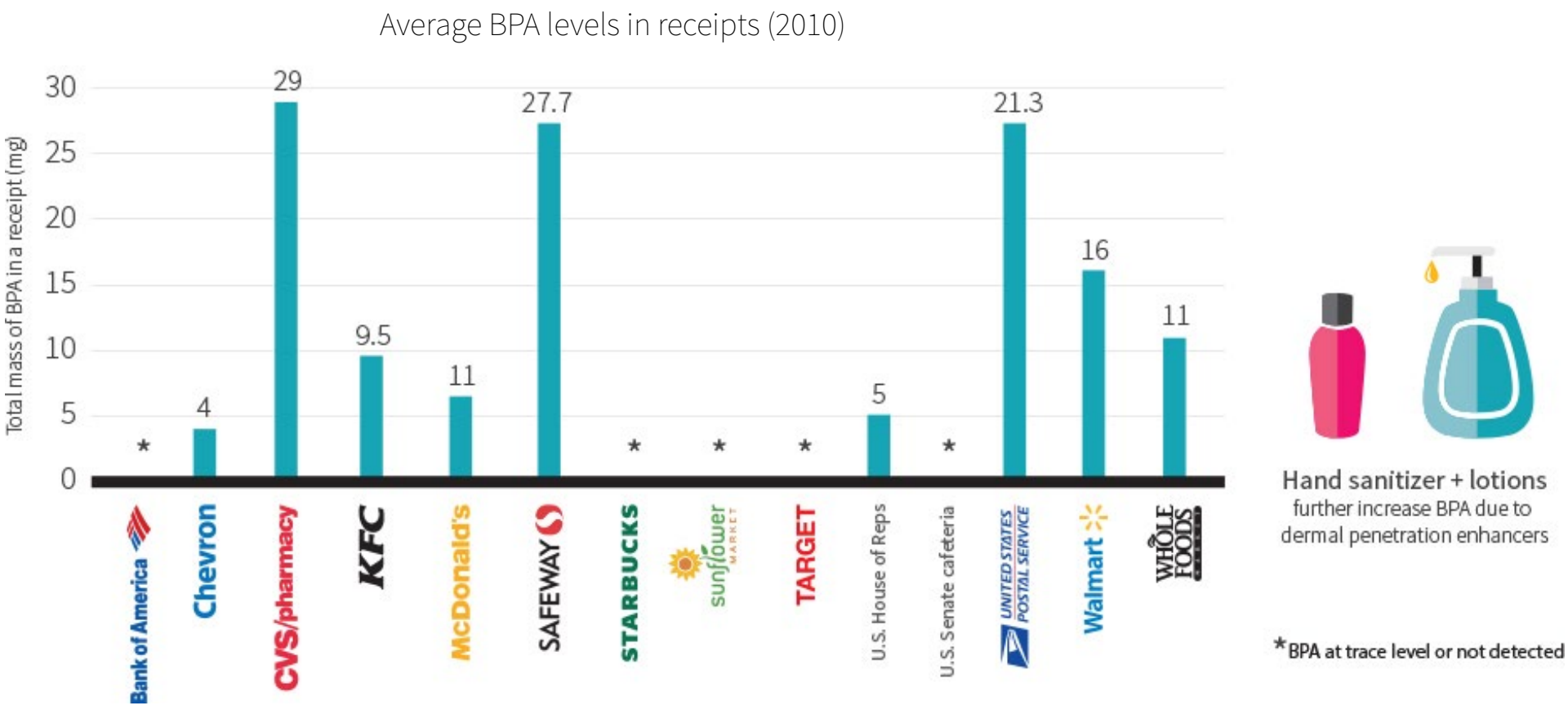
Because BPA seems to have dose-dependent effects, it's still likely useful to minimize BPA exposure whenever possible. Many correlative studies, some of which used extremely large data sets, have found associations between BPA exposure and a variety of cardiovascular disease states, including heart attacks.

However, there isn't much evidence for direct interactions between BPA exposure and health risks in humans. There is, however, a large body of evidence studying the effects of BPA exposure in a variety of animals, and which typically show harm. At this point, minimizing BPA exposure is a good idea, since a policy-level intervention like banning BPA from all food products isn't so likely, at least in the US (in contrast, France has much stricter policies, with regulations starting in 2015). This may be due to huge economic ramifications and lack of feasible alternatives for all of BPA's many uses.

Why don't manufacturers move away from BPA if there's so much data suggesting against its use?

It would be very expensive to convert machinery and processes from standard procedures to BPA-free alternatives. More importantly though, such a conversion likely wouldn't increase sales enough to offset the expense. Because there's no government regulation and no definitive proof that BPA causes medically relevant harm, it's far easier and more economical for large companies to maintain their current manufacturing and packaging processes. One example is store receipts. As seen in Figure 3, many receipts contain fairly large amounts of BPA. This may be a concern for those who repeatedly handle store receipts (such as clerks or shopaholics), and the effect is amplified when hand sanitizer or certain lotions are worn. Despite the public outcry in 2010, when the data was released, few stores have turned to alternatives.

Figure 3 - BPA exposure from receipts



Source: Environmental Working Group, 2010

However, many niche and small manufacturers, especially those in the health and wellness community, have moved to BPA-free processes. Therefore, these BPA-free products may be good alternatives as the growing body of research on BPA's negative effects pushes consumers toward BPA-free products

Why is there so much research on BPA?

BPA is an estrogen analogue, which means it can bind to estrogen receptors in the body. Estrogen is a nearly ubiquitous signaling hormone in the animal kingdom, and it is responsible for many things beyond the secondary sexual characteristics it is normally associated with. Estrogen receptors are found on nearly every major tissue type in the human body, and they are key regulators of processes including bowel motility, fluid balance, blood coagulation, and metabolic health.

It is also similarly important in most animals, some of which are far more sensitive to endocrine disruption than humans. Fish and other aquatic species are especially susceptible to BPA exposure, and because it causes a variety of birth defects in these species, it is believed (with a growing body of supporting evidence) that it may also affect human cellular and metabolic signaling.

What should I know?

BPA is nearly ubiquitous, but it should still be avoided when possible, which means avoiding canned beverages and trying to find containers that are BPA-free.

The findings in this study are mostly applicable to elderly women, but the entire body of research on BPA exposure seems to indicate that BPA offers no known health benefits while being associated with a variety of potential health risks. Unfortunately, BPA is everywhere, which makes avoiding it very difficult. However, it is also important to realize that although there are many correlations between BPA and disease states, as well as some direct evidence that BPA causes acute biological and metabolic changes, there still isn't enough evidence to say whether or not BPA actually directly contributes to disease. If it does, it's nearly impossible to quantify the exact contribution to disease, as it's one single part of a multifactorial disease process. Since the evidence for health impacts will never be ideal, it's a personal decision as to how much you want to focus on BPA-reduction in everyday life. ◆

How much BPA is in your kitchen? Does your keyboard have BPA in it? Don't fret, you can head over to the Facebook ERD forum, which is both BPA-free and a great place to talk about the evidence on this important issue.

> "[...] a policy-level intervention like banning BPA from all food products isn't so likely, at least in the US (in contrast, France has much stricter policies, with regulations starting in 2015)."

The gut microbiome's role in type I diabetes

The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes

Introduction

There is a lot of evidence to suggest the microscopic bacteria in our gut, known as the microbiome, plays a large role in health. The ERD has discussed some of these roles in past issues (see: "Of mice and guts (and exercise performance)" and "Gut bugs and fiber" in ERD #2, and "A mouse's microbiome may cause its brain to leak" in ERD #3). Some of the diseases in which the microbiome may be involved include irritable bowel disease, rheumatoid arthritis, and even obesity. Recent evidence suggests that type I diabetes may be added to the list.

The types of diabetes

The term "diabetes" comes from an ancient Greek work meaning "to go through" or "to siphon." The disorder was named for one of its cardinal symptoms: frequent urination. When diabetics drink liquids, they tend to "go right through them."

There are actually several types of diabetes. Diabetes insipidus is a disorder characterised by frequent urination that can be traced back to how the body responds to a hormone known as antidiuretic hormone. However, this disorder has little in common with what most people refer to when they say "diabetes," which is formally known as diabetes mellitus.

"Mellitus" comes from the Latin word for "honey," which refers to the fact that people with diabetes mellitus have urine with sugar in it. There are two types, both of which have to do with insulin (which helps the body deal with sugar, hence the "sweet" urine). Type I diabetes mellitus, previously known as insulin-dependent diabetes or juvenile diabetes since patients often develop it early in life and require insulin injections, is an autoimmune disorder characterized by the body's inability to produce insulin. Type II diabetes mellitus, previously known as adult-onset or insulin-independent diabetes since patients often develop it in adulthood and do not usually need insulin injections to live, is primarily characterized by insulin resistance, where the body can usually produce insulin but has trouble responding to it.

Type I diabetes is an autoimmune disorder, meaning that a person's immune system attacks their own body. In the case of type I diabetes, the immune system specifically attacks the insulin-producing beta cells of the pancreas, which reduces or eliminates the person's ability to regulate their own blood sugar. The typical symptoms of type I diabetes include excessive thirst, frequent urination, and excessive hunger. The first and second occur because of high blood sugar, which pulls water from out of the body and into urine as it's filtered by the kidneys. The third symptom is due to varying factors, such as body tissue's lack of ability to use sugar in the blood as fuel (since insulin is no longer being produced in sufficient quantities), in addition to shrinking fat stores caused by the condition leading to declining leptin, which provokes hunger. This condition can lead to death if not managed through insulin, and its long-term complications include kidney, heart, eye, and circulation problems.

Most people (70%) who develop type I diabetes tend of have certain kinds of human leukocyte antigen (HLA) genes, which are major proteins that help regulate immune function. At first glance, type I diabetes looks largely like a genetic disorder. The weird thing, though, is that only about 5% of children who have these HLA genotypes which put them at risk actually develop type I diabetes. This strongly suggests that more than one gene impacts this condition, and/or something beyond genetics influences the development and progression of the disease. But what could that something be?

Animal studies point to the gut microbiome as a possible culprit. Studies conducted in diabetes-susceptible mice show that completely knocking out both copies of a gene important for immune system microbe recognition results in the prevention of diabetes development. Furthermore, mice with only one of the two copies of the gene knocked out, which usually go on to develop diabetes, are protected from developing the disease if their guts are colonized by healthy microbes. This indicates that an immune response to certain species of gut microbiota may play a role in the development of type I diabetes, at least in mice.

But what about humans? There is evidence to suggest that the composition of the gut microbiome differs between children who have anti-pancreatic antibodies and those without. This doesn't say much about whether the microbiome changed in response to the diabetes, or whether it developed before the diabetes, though. To answer that question, a prospective study of how the gut microbiome changes through time was conducted.

Type I diabetes is a partially genetic disease, but has a strong non-genetic component. This study was designed to determine whether the gut microbiome may play a role in the disease's development.

Who and what was studied?

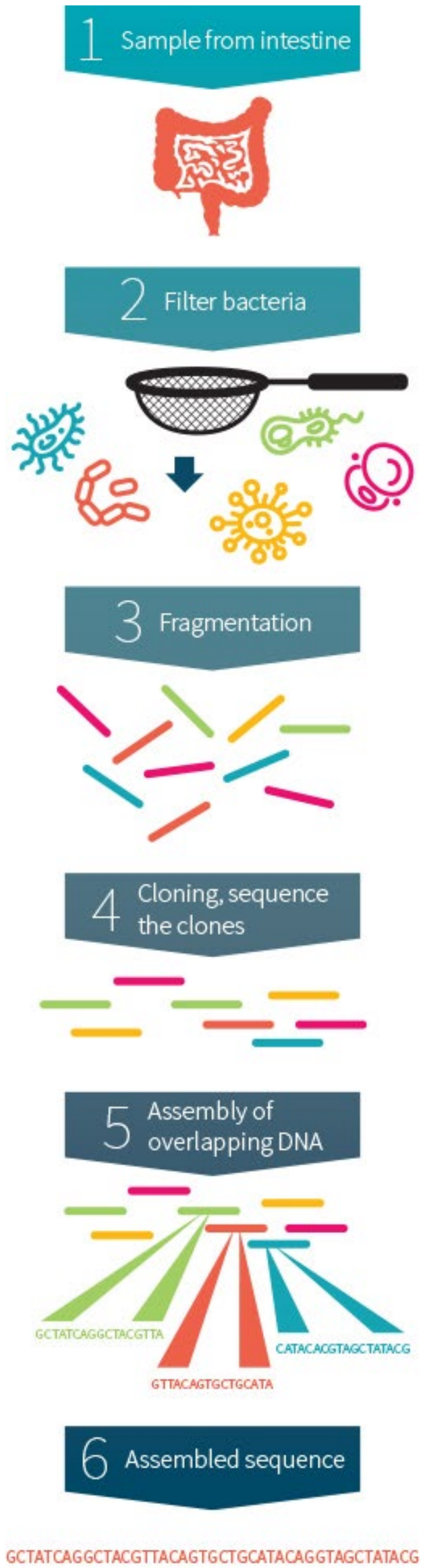
This study involved a group of infants from Estonia and Finland, recruited at birth. All of them were very likely to develop type I diabetes early in life based on their HLA genotype. Stool samples from the infants were collected monthly by the parents and sent to the researchers for approximately three years. That's a lot of poop, but out of volumes of poop came volumes of data about what was going on inside the guts of the infants as some of them went on to develop type 1 diabetes.

There are many different species of bacteria in stool, all doing different things biochemically, and producing different metabolites. The researchers were able to examine this big and complicated picture using metabolomics and shotgun metagenomics. The various "-omics" are the study of large and complex samples of biological molecules. Metabolomics was described in the ERD #5 article "Can you go too nutty over pistachios?" but basically involves identifying specific biochemicals in a sample. Shotgun metagenomics (shown in Figure 1) allows for identification of unique segments of the DNA from a population of different organisms, which allows for identification of both the organism and their unique biochemical profiles. Thus, shotgun metagenomics can tell us both about what organisms are in the sample as well as what they can do biochemically.

By applying these techniques to stool samples throughout the three year study period, a very detailed picture of how the infants' gut microbiome changed through time emerged. In addition, serum samples were taken and metabolomics were performed on these as well. Dietary and environmental variables were also measured to see what other factors may have contributed to changes in the infants' microbiome.

During the course of the study, some of the infants developed antibodies against their own pancreas, which was expected since all the infants were at risk for type I diabetes. Stool and blood samples from infants who developed antibodies against their own pancreas during the course of the

Figure 1: Shotgun metagenomics



study (called "seroconverters," since their serum converted to carrying these antibodies) were matched with the samples of non-seroconverting infants. This allowed the researchers to compare the microbiomes of seroconverted infants with those who did not seroconvert to see what differences, if any, there were between the two groups.

Infants at risk for type I diabetes were followed from birth for three years to see how their gut microbiomes changed as autoimmune antibodies and type I diabetes developed.

What were the findings?

The authors were able to get quite a high-resolution view of how the microbiome of these infants developed over time, and found two general trends concerning how the microbiome developed over time in all the infants. First, the diversity of the microbiome increased exponentially over time, peaking at the end of the study. However, those species which were abundant at birth tended to remain present and constant throughout each infant's development. Second, even though the bacteria themselves grew in diversity over time, the metabolites that the bacteria produced in the gut and the biochemistry those bacteria were capable of remained remarkably stable throughout the course of the study. Thus, although the bacteria became more diverse with time, they were doing similar things biochemically.

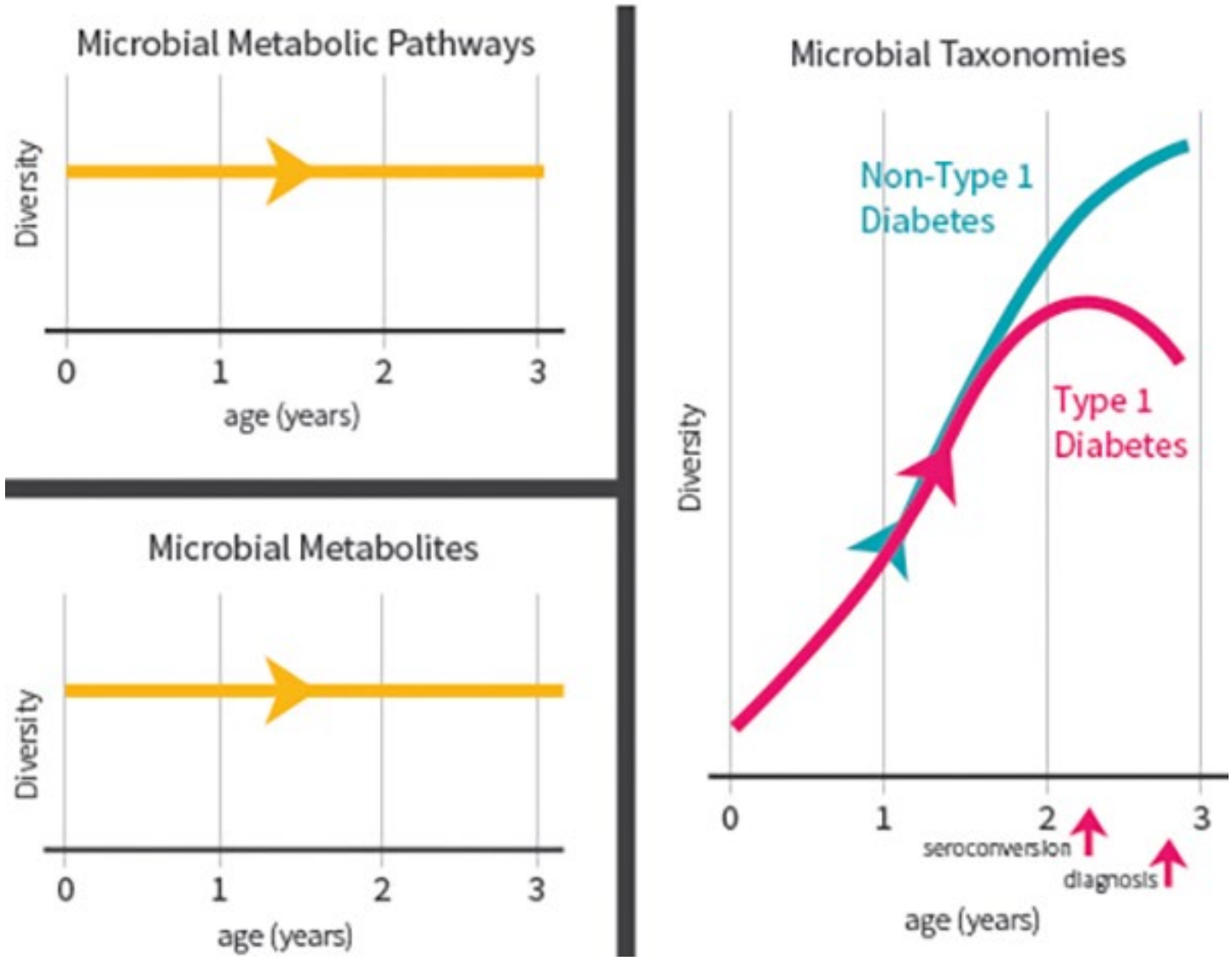
> "[...] although the bacteria became more diverse with time, they were doing similar things biochemically."

When subgroups of the infants were examined, some interesting patterns also emerged. Breastfeeding infants led to increased levels of *Bifidobacterium* and *Lactobacillus* species, some of which are being investigated for their probiotic properties. Also, the Estonian infants had higher levels of Bacteroides and Streptococcus species, some of which may be pathogenic. This suggests that nationality is correlated with the microbiome. Ethnicity, specific geographic location, and related factors (such as differing diet by locale) may also be correlated with the microbiome, and should be taken into consideration when interpreting observational genetics-based studies such as this.

Diet also seemed to affect the microbiome, but mildy. Finally, there was no difference seen in the microbiome of infants who were delivered vaginally versus cesarean section, although the sample of infants who were delivered via C-section was small, so perhaps larger samples would reveal a difference.

But what about the microbiome's relation to type I diabetes? Out of the 33 infants, 11 seroconverted. Out of the seroconverted infants, four developed type I diabetes during the course of the study. As seen in Figure 2, the researchers found that there was a drop in diversity of the microbiome in those infants who went on to develop diabetes during the study period. This drop preceded the diagnosis itself, but occurred after these infants seroconverted. Curiously, both seroconverters who didn't develop diabetes during the study period and nonconverters experienced the exponential increase in microbial diversity described earlier. This drop

Figure 2: Microbial diversity in guts of study infants



Adapated from Kostic et al., Cell Host & Microbe, 2015

in diversity was accounted for by an increase in species from Ruminococcus and Streptococcus, both of which are possibly pathogenic, along with a decrease in certain species which tend to be depleted in inflammatory states.

The metabolic pathways used by the bacteria were also correlated with type I diabetes. Bacteria in the infants who developed the disease tended to make less nutrients for themselves and increased their passive absorption of nutrients. This suggests an inflammatory state in the gut, where the bacteria were able to feed off of the nutrients from inflamed, dying tissue. Increased human beta-defensin 2, a marker of gut inflammation, was also seen in those infants who went on to develop diabetes, which also suggests that inflammation is playing a role here. An increased amount of triglyceride and branched-chain amino acid production was also observed in diabetic infants, which were correlated with increased amounts of the microbes *Blautia* and *Ruminococcus*. This is bad news, since high triglycerides are associated with kidney problems and poor glycemic control in type I diabetes patients, and branched-chain amino acids are associated with worse diabetic control. Thus, it's possible that changes in the microbiome may hasten the development of type I diabetes in these infants.

The microbiome generally tends to increase in diversity with age, but becomes less diverse in those infants who develop type I diabetes. This change precedes diagnosis of the disease, but occurs after seroconversion.

What does the study really tell us?

This study gives an extremely detailed, unprecedented look at changes to the microbiome of infants predisposed to type

I diabetes. It found that microbiome diversity decreased prior to type I diabetes, while inflammation and possibly pathogenic species increased, and that these changes only occurred only in infants who went on to develop the disease during the course of the study. In both infants who developed autoimmune antibodies against the pancreas but didn't develop diabetes and infants who didn't make any autoimmune antibodies, no such decrease in diversity occurred; instead, their microbiomes kept on increasing in diversity.

But behind all of the "-omics" and impressive techniques used, this is still an observational study. That means it's hard to determine causality. Did the increase in pathogenic species in the microbiome cause diabetes in the infants, or did the infants' inflammation during progression to diabetes simply create an opportunity for these pathogenic species to thrive? We can't tell from this study alone. The fact that the decrease in microbiome diversity and increase in potentially pathogenic species occurred before diabetes developed, and not in those who didn't, is at least consistent with causality, but there's no sure-fire way to tell. The applicability of this study to infants in general may be limited, as the sample consisted of infants in Finland and Estonia, and as such isn't likely to be very diverse genetically.

However, this study definitely opens the door to test some hypotheses further down the road. Probiotics are being investigated in newborns and children, and perhaps interventional studies in infants predisposed to type I diabetes may yield promising results. Also, antibiotics have been shown to partially prevent type I diabetes in mice. Since this study helps identify some of the specific species associated with the development of diabetes, it opens the door for testing targeted antibiotic therapy to prevent or delay diabetes onset in susceptible infants.

The microbiome changes in the gut preceding type I diabetes in susceptible infants are consistent with causality, but causality cannot be proven here since this is an observational study.

The big picture

Colonization of the gut by the microbiome actually begins while the fetus is still in the womb, and is strongly impact-

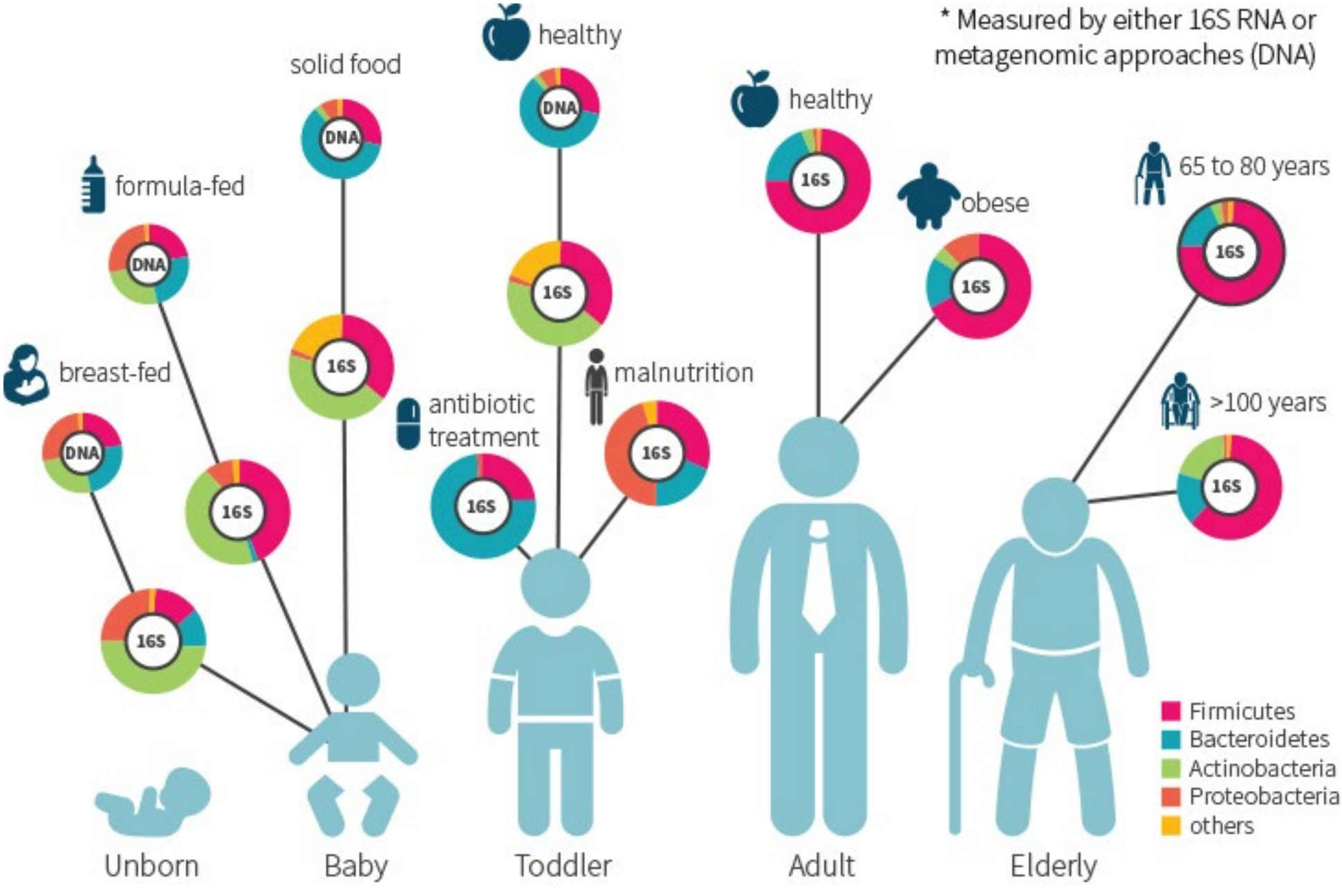
"The applicability of this study to infants in general may be limited, as the sample consisted of infants in Finland and Estonia, and as such isn't likely to be very diverse genetically. However, this study definitely opens the door to test some hypotheses further down the road."

ed by microbial exposure during birth, as seen in Figure 3. This study showed that the normal microbiome generally increases in diversity as an infant ages, and that a decrease in diversity precedes the development of type I diabetes. This pattern of decreasing microbial diversity being associated with disease has also been noted in other diseases, including *Clostridium difficile*-associated diarrhea, Crohn's disease and ulcerative colitis, and obesity. It is looking more and more likely that diversity of the microbiome is important for, or at least associated with, health.

In the case of type I diabetes, there is a large variability in onset of diabetes after seroconversion, ranging from weeks to decades. It is unclear why this is the case. This study hints that the gut microbiome could play a role in onset, although it can't be said for certain given the study's observational nature. And it may not be the only factor. One model for the development of type I diabetes suggests that a "perfect storm" of three factors need to coincide for diabetes to develop in genetically susceptible individuals: an altered microbiome, a leaky and permeable gut, and an aberrant immune response. So, while this study provided some very detailed insights into one of these factors, there's still a lot left to be learned.

Decreased diversity of the microbiome has been observed in other diseases in addition to the onset of type I diabetes. And while the microbiome may be important in the development of diabetes, there are other pieces of the puzzle still left to explore.

Figure 3: Human gut microbiome changes, from birth to old age



Source: Ottman et al., Front Cell Infect Microbiol, 2012

Frequently asked questions

Is breastfeeding good for microbial diversity?

The research is still young on this, but one study found that exclusively breastfed infants actually exhibited a lower microbiome diversity than infants who were not exclusively breastfed. However, exclusively breastfed infants did have lower levels of possibly pathogenic bacteria, and may have been able to transition to solid foods more easily.

What about antibiotics? How do they affect infant microbial diversity?

Antibiotic therapy reduces microbial diversity, with more intensive therapy having a larger impact. The longer-term consequences of this impact are not completely understood, however. Antibiotic use in the first year of life has been linked to inflammatory gastrointestinal conditions in later childhood, although the evidence here is also observational and can't prove causation.

Is there any clinical evidence that probiotic supplementation could help type I diabetes?

There are case reports suggesting efficacy, but these are not strong evidence since confounding factors are present and there was a lack of controls. A clinical trial is in the works, but the results have not yet been released.

What about type II diabetes? Any clinical evidence there?

There's not much evidence, but it is a little stronger. One double-blind, placebo-controlled trial found that probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* over a six week period reduced fasting plasma glucose and hemoglobin A1C in people with type II diabetes. Another study found that insulin sensitivity was preserved in men with type II diabetes supplementing *Lactobacillus acidophilus*, compared to a control group over four weeks.

What should I know?

Gut microbiome diversity steadily increases from birth onward, although major species in the microbiome tend to establish themselves early and stick around as the infant ages. However, diversity sharply decreases, and potentially pathogenic organisms increase, prior to the development of type I diabetes in infants genetically predisposed to the disease. This is consistent with the hypothesis that the gut microbiome may partially cause type I diabetes in susceptible infants. However, causality cannot be strictly demonstrated here, since this was an observational study. ◆

Contribute to the increasing diversity of opinions about this article over at the ERD private Facebook forum!

> "Antibiotic use in the first year of life has been linked to inflammatory gastrointestinal conditions in later childhood, although the evidence here is also observational and can't prove causation."

Curry… brain food?

Curcumin boosts DHA in the brain: Implications for the prevention of anxiety disorders

Introduction

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are one of the most well-studied dietary supplements, usually in the form of fish oil. Examine.com's fish oil page boasts one of the highest citation counts out of all the entries in the database, with over 700 studies cited. Two of the main n-3 PUFAs of interest are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

DHA is considered essential for brain function. Deficiency is linked to various maladies, including Alzheimer's disease, schizophrenia, psychosis, and anxiety.

Vegetarians have lower circulating DHA, compared to omnivores. This is not surprising, as DHA is not often found in plant-based food. However, alpha linolenic acid (ALA) is a readily available plant-based n-3 PUFA. The caveat is that humans must convert it to DHA before it can be utilized, as shown in Figure 1. This conversion rate may not be ample enough to provide a person with enough to satisfy the recommended amount, though.

It is, however, entirely possible to thrive on a vegetarian diet. Part of this may be due to dairy and eggs being a possible source of DHA. But there is also a question as to whether or not something in the vegetarian diet increases the conversion efficiency of ALA to DHA.

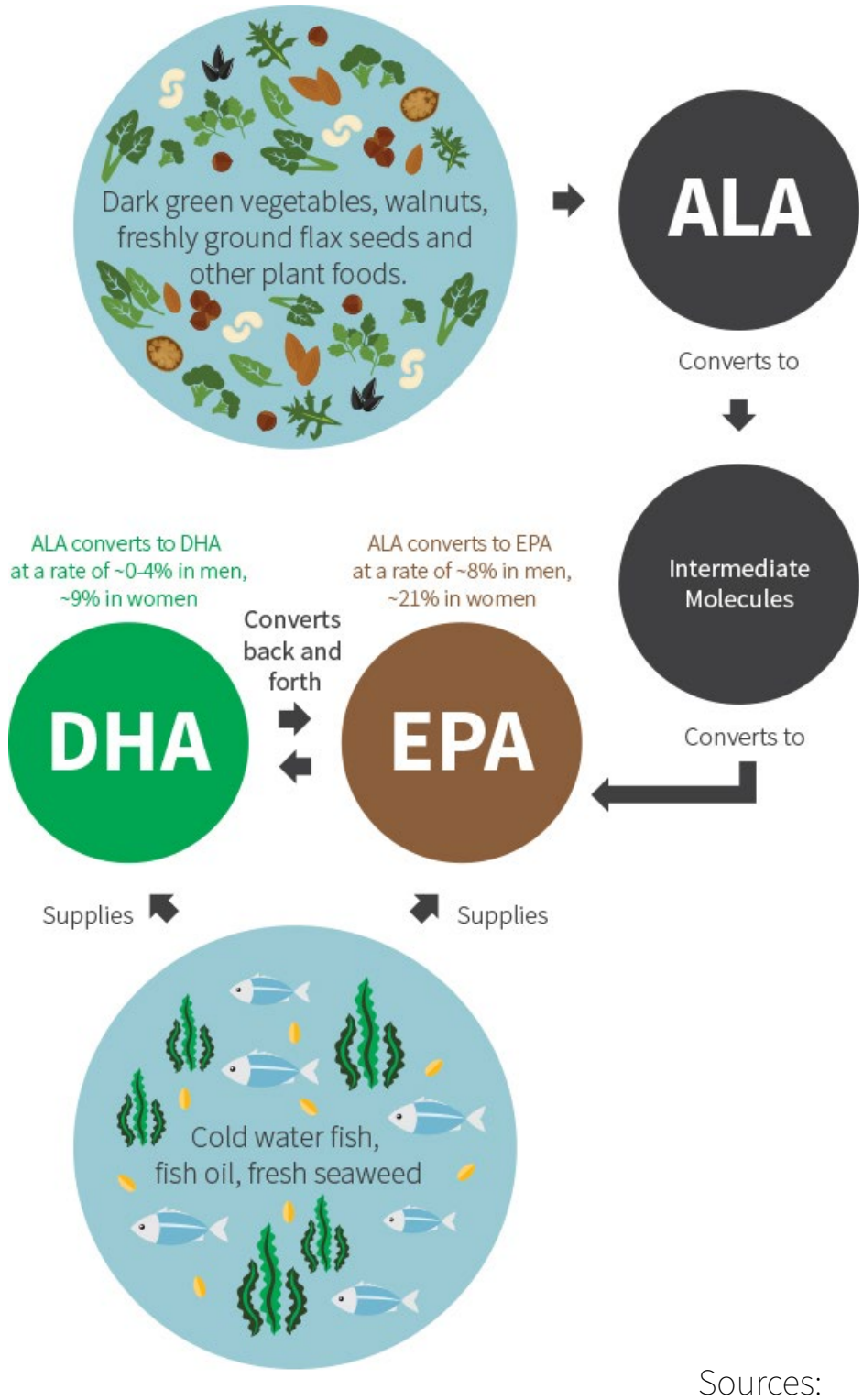
As it turns out, curcumin is a component of turmeric, a frequently used spice in Indian populations, many of whom are vegetarians. Since these populations do not experience noticeable declines in cognitive abilities, a couple of research groups in California teamed up to determine if curcumin consumption could be one of the factors that increases the DHA conversion rate. Curcumin is a heavily researched dietary supplement on its own and boasts a plethora of potential benefits. Examine.com has over 200 cited publications on their curcumin page. Combining both biochemical assays and cognitive testing, rats were studied to determine if curcumin affected DHA levels (and DHA-converting enzymes), and if there was any significant impact on perceived anxiety.

While vegetarians have a lower intake of DHA due to its relative absence in non-animal based foods, they do not suffer from many of the problems linked to low DHA levels. Researchers investigated whether something in their diet attenuates the signs of DHA deficiency.

Who and what was studied?

Rats were split into five groups, with five to six rats in each group, and were fed one of five diets. Either a control diet (with no added ALA or curcumin), an ALA diet where ALA comprised 2.7% of the total fat content, a curcumin diet (CUR) where curcumin was added at 500 parts per million (ppm, or milligrams/kilogram) to the chow, and two ALA+CUR diets which contained curcumin at either 250 ppm or 500 ppm alongside ALA.

Figure 1: ALA to DHA conversion



Sources:
Burdge, Curr Opin Clin Nutr Metab Care, 2004
Burdge, Wootton, Br J Nutr, 2002

The rats were fed this diet ad libitum for 3.5 weeks before they were euthanized. The brains were further examined. Half of the brain tissue was harvested for protein assays, and the other half was stored for lipid assays.

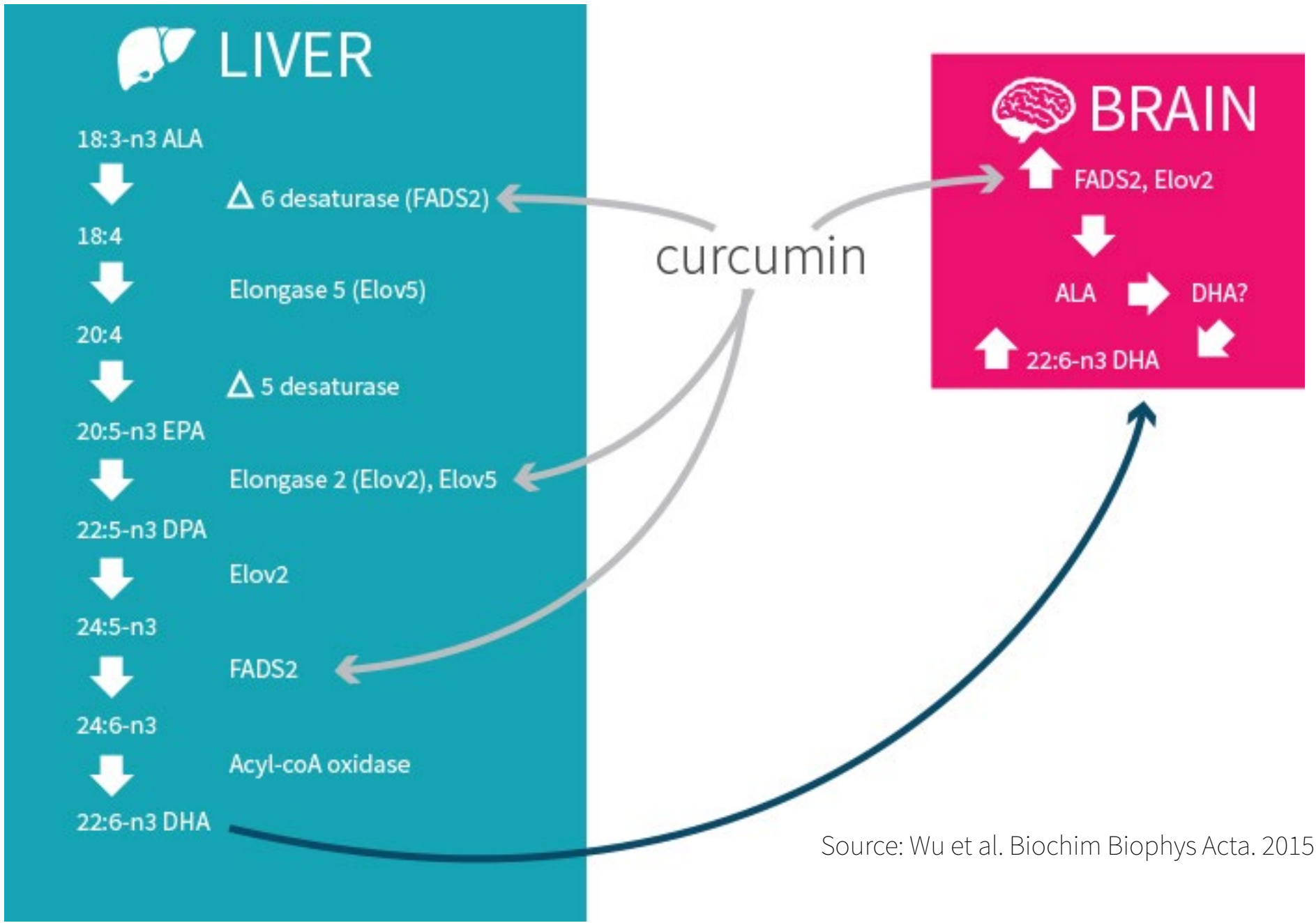
Before decapitation, the rats were subjected to an elevated plus maze where they were placed on a cross-shaped platform. Two of the platform's arms (in line with each other) are walled up and the other two arms are not. Since rats normally don't like open spaces, an anxious rat will spend less time in the open arms compared to the closed arm, as it is more likely to stay in areas which it is more comfortable. Anxiety level of the rat is calculated by total time spent in respective portions of the platform in a five minute period.

The amount of certain proteins in the rat hippocampus was also measured, using a technique called immunoblotting. This technique works through antibodies, which bind to unique sites within proteins. Once a protein has been blotted for, a secondary antibody specific to the primary antibody can be used to quantify the protein through the use of luminescence detection, which is proportional to the amount of secondary antibody. In other words, the amount of protein can be relatively quantified through a series of steps that ultimately lead to light production. In this study, two enzymes involved with DHA synthesis (FADS2 and Elov2, whose roles are shown in Figure 2) and a marker of byproduct of lipid oxidation metabolism (4-HNE) were assessed.

Lipid analysis was done by sticking what was left of the rat brains in a blender and extracting the lipids with a mixture of organic solvents (chloroform and methanol). This mixture is evaporated to dryness and prepared for gas chromatography to determine the composition of the extracted fraction.

Lastly, *in vitro* experiments were carried out in cultured human liver cells to determine if curcumin influenced conversion of DPA to DHA. DPA is a few conversion

Figure 2: Impact of curcumin on DHA synthesis pathway



Source: Wu et al. Biochim Biophys Acta. 2015

steps more mature than ALA, and acts as a precursor and rate-limiting step in the conversion to DHA. These experiments were done by incubating the liver cells with DPA + CUR for the experimental group and DPA + CUR + DPA converting enzyme inhibitor for the negative control. Similar protein and lipid assays were performed on the liver cells as compared to the rat brains.

Rats were fed a diet containing ALA alone, CUR alone, or a mix of ALA and CUR. Their anxiety was measured and biochemical tests were performed on their brains after they were sacrificed. Liver cells were also incubated with CUR and analyzed biochemically to see if curcumin affected their ability to synthesize n-3 PUFAs.

What were the findings?

Anxiety experiments showed that rats with a combination of DHA and CUR spent more time in the open portion of the elevated plus maze. Longer times spent in the open portion of the maze means less rodent anxiety, which correlated with higher levels of DHA in the brain.

ALA alone and CUR alone did not increase DHA. A combination of ALA and CUR was required to see the increase of DHA in the brain. Interestingly, higher levels of CUR were related to slightly less levels of accumulated DHA (162% of control at 250 ppm and 152% at 500 ppm).

When it came to measuring enzymes involved in DHA conversion, CUR alone increased the levels of FADS2 by 126% of control and further increased to 150% with the addition of ALA. There was no change with ALA alone. CUR alone also increased levels of Elov2 to 114% of control and 132% when combined with ALA.

Levels of 4-HNE were not affected by CUR alone, but when combined with ALA the levels of 4-HNE decreased. This decrease in 4-HNE is indicative of decreased oxidative

> “The study of the human liver cells revealed that when much of the DHA is synthesized, ALA alone was enough to increase DHA content to 136% of control while CUR alone had no effect.”

stress. CUR alone is not enough to decrease lipid oxidation in the brain, but it is unclear why more accumulated DHA would lead to less oxidative byproducts and why the addition of an antioxidant would not decrease it when compared to control. Unregulated radical chemistry must not be the culprit for this oxidative process. Gas chromatography showed increases in DHA precursors that correlated with increases in their respective conversion enzymes.

In short, curcumin upregulates the enzymes used to convert ALA to DHA, but if no ALA is present then there is no net accumulation of DHA.

The study of the human liver cells revealed that when much of the DHA is synthesized, ALA alone was enough

to increase DHA content to 136% of control while CUR alone had no effect. When ALA and CUR are combined, DHA rises to 179% of control. Similar to brain tissue, CUR alone increased FADS2 and Elov2, but this did not lead to increased DHA. Also similar to brain tissue, gas chromatography data correlated this enzyme upregulation with increases in respective DHA precursors associated with those enzymes.

> "[...] curcumin alone is not enough to enhance DHA production, it must be combined with dietary ALA."

ALA + CUR did not increase DHA in the liver but DPA + CUR did. This implies that the conversion of DPA to DHA is the rate-limiting step of DHA synthesis in the liver. If an FADS2 inhibitor is added to the DPA+CUR culture, the conversion of DHA is decreased 69%. This indicates that CUR enhances the FADS2-dependent portion of DHA conversion in human liver cells.

Curcumin increased the synthesis of DHA from ALA, which correlated with decreased anxiety in rats. Increased DHA synthesis seemed to be due to curcumin's ability to upregulate two key enzymes in the DHA synthesis pathway called FADS2 and Elov2.

What does the study really tell us?

Not only does curcumin act as an anti-inflammatory and antioxidant, it may also act to increase DHA synthesis as well. However, curcumin alone is not enough to enhance DHA production, it must be combined with dietary ALA.

Since vegetarians aren't a population any more prone to neurological disorders than fish-eaters, the data on DHA's role in rat anxiety has ambiguous practical applications. Low intake of DHA is associated with negative effects on cognition in rodents, but this can be reversed through diet alone.

The authors are confident that curcumin is pivotal in preventing disease states that aren't correlated with vegetarians to begin with. Although vegetarians may have lower circulating DHA, that may not reflect the DHA levels in the brain, where neurological pathology is likely to originate. In fact, vegetarian diets are associated with "healthy mood states". The discrepancy with the animal model leads to more questions regarding DHA accumulation in diets that are otherwise lacking in it.

It would be interesting to extend this study to humans, perhaps measuring circulating DHA content in a turmeric-consuming vegetarian population before and after cessation of turmeric consumption.

Since human vegetarians have low circulating DHA levels but not higher levels of anxiety, it's questionable how much the results of this experiment apply to humans.

The big picture

Unfortunately, current farming practices are slowly decreasing the amount of DHA content in food. However, ALA is a readily available n-3 PUFA in commonly produced food-

stuffs like canola oil. Including turmeric as part of a diet rich in ALA may mitigate the effects of modern farming on DHA content in the brain.

That being said, there is no indication that this would be a necessary additive to maintain neurocognitive stability in a DHA-deficient diet. Yet this combination may play a beneficial role in certain people, such as those with diminished capacity to biosynthesize DHA and people with neurocognitive disorder(s) such as Alzheimer's.

Lastly, while the idea for this study stemmed from observations in vegetarians, there is no reason that omnivores can't also eat (or supplement with) curcumin in order to potentially boost DHA levels. This study provides a starting point for further research in human trials, which don't always show the same results as rodent studies.

Frequently asked questions

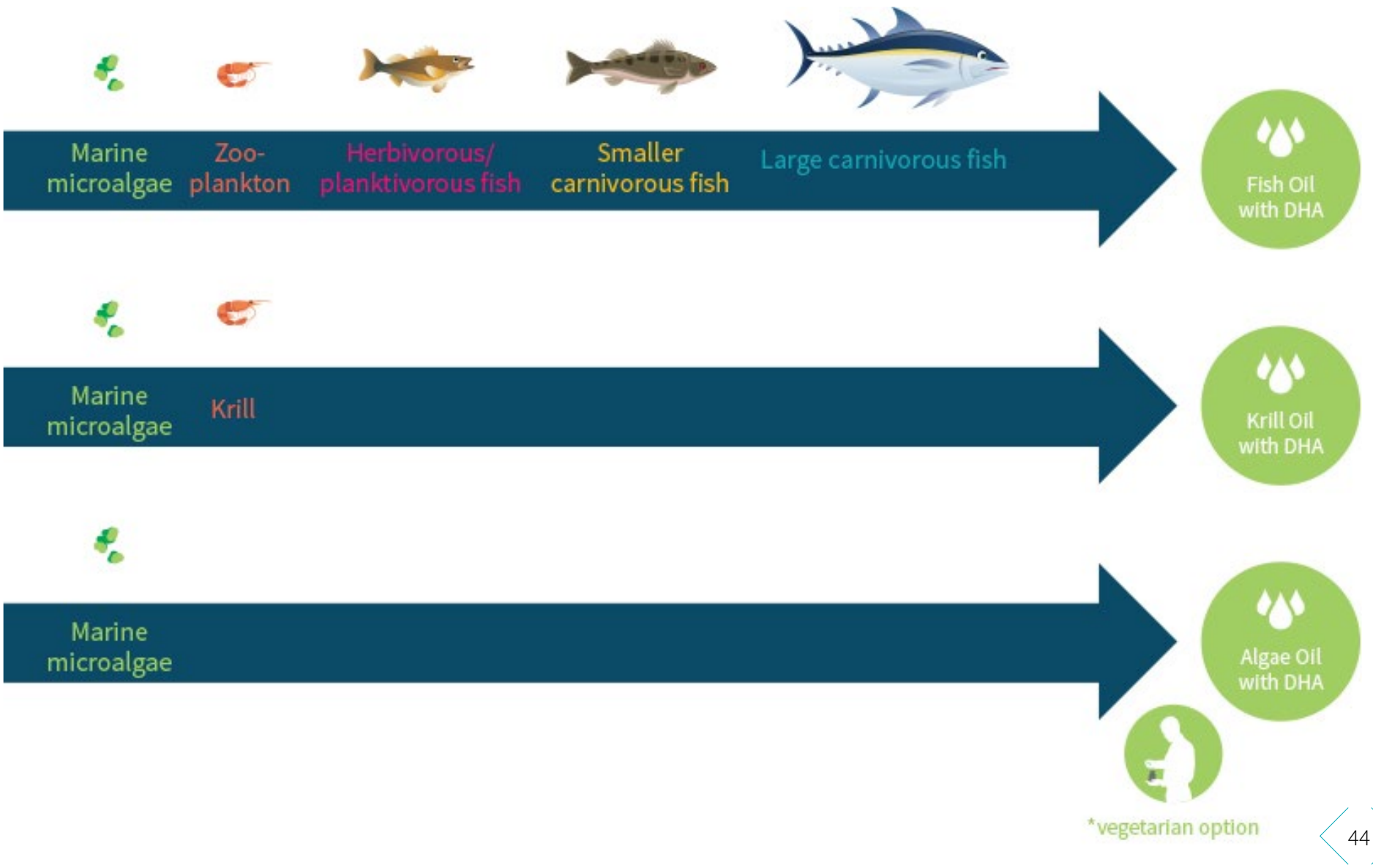
Are there vegan sources of DHA?

Micro-algae oil contains DHA, and there is limited evidence to suggest that these oils can be absorbed into the plasma and integrated into red blood cell membranes. Three popular DHA sources, and how they fit into the food chain, are shown in Figure 3.

Will a vegetarian diet impair my mood or cognition?

Plasma DHA is lower in vegetarians and vegans than in meat-eaters, and circulating DHA levels are also directly related to better cognitive outcomes during aging. This sounds like it may imply that vegetarian diets could have a negative impact on cognition. However, evidence is scarce regarding the effects of a vegetarian diet on cognition, and some epidemiological evidence suggests that a vegetarian diet may actually be protective against dementia. Observational evidence suggests that some vegetarians might have higher levels of anxiety and other mood disorders, but there are many confounding factors and a cause-effect relationship can't be determined.

Figure 3: DHA throughout the food chain



Why can't I just eat a lot more ALA in order to get my DHA levels up?

In short, because too much ALA can interfere with DHA synthesis. That's because the FADS2 enzyme is needed for two steps in the pathway from ALA to DHA: first along the pathway converting ALA to EPA, and again along the pathway that converts DPA to DHA. If there's a lot of ALA, FADS2 becomes overwhelmed working on ALA, at the expense of converting DPA to DHA. In other words, ALA inhibits DHA production by competitively inhibiting FADS2. Partially because of this, ALA is much less potent when it comes to raising long-chain n-3 fatty acids levels. One study found that over 30 times the amount of ALA was needed to raise long-chain n-3 fatty acid levels, compared to EPA and DHA.

Is there any human evidence concerning anxiolytic effects of curcumin?

Yes. One gram of curcumin a day was recently shown to reduce anxiety when supplemented by obese people, compared to placebo in a cross-over study. Depression was also measured, but there was no significant effect.

Could curcumin's effect on DHA levels account for any of its purported health effects?

The authors of this study mention that this is at least a possibility, and bring up the example of Alzheimer's disease. Curcumin has shown some promise in preliminary studies, although a direct test of its efficacy in humans has not yet been done. A human study has shown that Alzheimer's patients have trouble synthesizing DHA, and that this may contribute to some factors of the disease. The authors of the current study speculate that curcumin may be able to affect Alzheimer's disease by increasing DHA synthesis. It should be emphasized that this is theoretical speculation at this point in time.

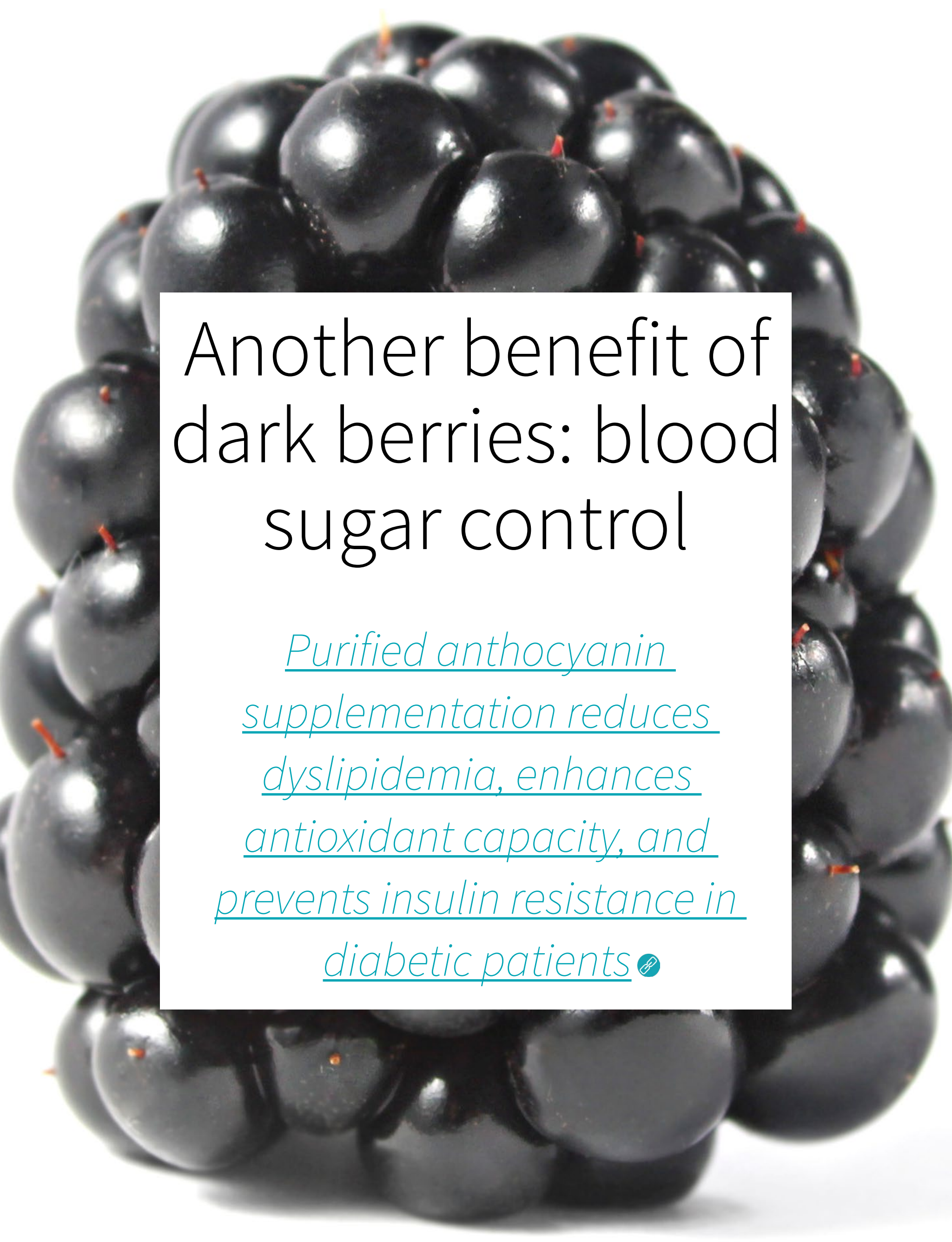
What should I know?

DHA is an essential fatty acid that is a major component of brain tissue with implicated roles in healthy brain function. ALA is a vegetarian precursor to DHA but may convert at too slow a rate to support the needs of a healthy human's cognition. Yet vegetarians have lower blood levels of DHA and do not seem to suffer cognitively.

Curcumin is a component of the widely available spice turmeric. In rats, it was found that curcumin increases the synthesis of DHA from ALA by stimulating key enzymes in this synthesis pathway. This was accompanied by decreased anxiety in the rats.

Supplementing with curcumin may provide a host of benefits outside of what is discussed in this article. Head over to Examine.com's curcumin page to read more, and check out the ERD Facebook forum to talk about this powerful compound.

"One gram of curcumin a day was recently shown to reduce anxiety when supplemented by obese people, compared to placebo in a cross-over study."



Another benefit of dark berries: blood sugar control

Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients

Introduction

The term "superfood" is one of the most popular health buzzwords in the media. The word doesn't even have a well-defined meaning, but is usually used to refer to foods deemed 'better' for you than other options, even though 'better' isn't defined either.

Many of these foods, like the acai berry, which became popular due to its antioxidant capacity, spend a short time in the spotlight before follow-up studies reveal that the food in question didn't actually provide any unique health benefits. Other foods, like pomegranate juice, which can play a useful role in prostate health, provide specific benefits but don't have nearly enough evidence to deem them 'superfoods'.

Still, some foods really are better for you than others. Blueberries are a great example, as are other dark berries, as they can provide benefits for people with metabolic disorders, as well as healthy people.

These benefits stem from the pigments in the berries, a class of molecules known as anthocyanins, which are also present in some other plant foods. Berries are the most commonly cited source of anthocyanins since they are common in the diet. Other options include purple rice (which is heavily researched, but not commonly eaten) and eggplant, which contain relatively few anthocyanins since they are only present in the skin, not the body of the vegetable. Dark berries hold promise for lifestyle-related diseases not only due to their effects, which are notable at reasonable dietary doses, but also for their palatability. Delicious fruit goes down far easier than medication.

So far, anthocyanins (either alone or in the form of berry products with specified anthocyanin content) have been linked to cognitive benefits in the elderly, and reductions in blood pressure in high risk individuals with cardiovascular disease. Their antioxidant effects have been linked to reductions in DNA damage. Dark berries may also influence glucose metabolism, and previous research has noted that people with insulin resistance experience benefits after dark berry consumption.

The study under review was specifically designed to assess the effects of anthocyanins in people with type II diabetes. Other factors were also tracked alongside insulin sensitivity to better determine if anthocyanins could play an adjunct therapy role, since it would be a low cost and low risk treatment.

"Dark berries hold promise for lifestyle-related diseases not only due to their effects, which are notable at reasonable dietary doses, but also for their palatability. Delicious fruit goes down far easier than medication."

Anthocyanins are found in dark berries and some other fruits and vegetables, and may have a positive impact on several aspects of health, including insulin resistance and blood pressure. The study under review was designed to examine the impact of anthocyanins as an add-on treatment for people with type II diabetes.

Who and what was studied?

This was a double-blind study that lasted for 24 weeks and investigated the effects of anthocyanin supplements on Chinese adults with type II diabetes. After excluding other chronic disease states and pregnant women, 58 adults with type II diabetes between the ages of 56 to 67 were divided into either the anthocyanin group or the placebo group. Many of the participants took medication to control their diabetes and other health problems. This is appropriate for a study that intends to investigate a supplement as an adjunct treatment.

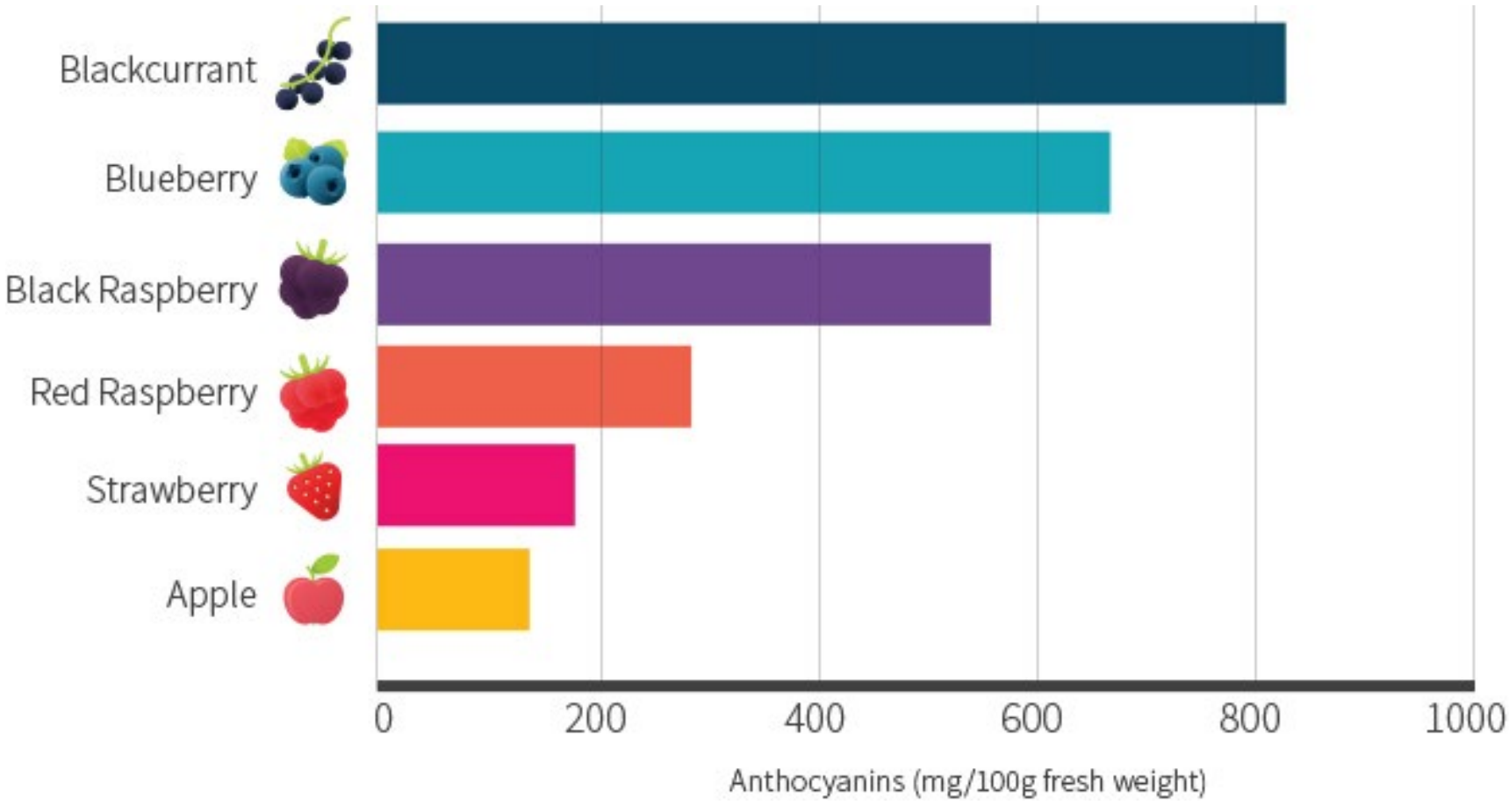
Anthocyanin supplementation was provided by capsules containing 80 milligrams of anthocyanins derived from bilberry and blackcurrant. Each was a mixture of anthocyanins (17 total types) rather than a single anthocyanin, but the majority compound was cyanidin glycoside. Study participants took two of these capsules, or placebos, twice a day. The active group ingested 320 milligrams of total anthocyanins each day, shortly after meals. As seen in Figure 1, this amount would be fairly easy to obtain from eating dark berries.

The study dose of anthocyanins is thought to be attainable by eating a dose of 100 grams of blueberries (less than a cup) or similar dark berries. This is for the fresh weight, which includes the water content of fruit, rather than the dehydrated weight. The results are thought to also apply to fresh berry consumption, as previous studies have noted equivalence between fruit and supplement, after anthocyanins have been standardized.

When talking about doses of a bioactive in a food, pay attention to the water content. Most fruits have upwards of 80% water content, so if this variable is not accounted for you may miscalculate your dose five-fold.

Researchers conducted a three-day dietary recall to approximate participant diets. Study participants also filled out a food frequency questionnaire (FFQ) to provide more

Figure 1: Fruits high in anthocyanins



information on the differences between anthocyanin and flavonoid intakes.

The researchers took various blood measurements related to diabetes (fasting glucose, insulin, and glycated haemoglobin) or oxidative biomarkers. Finally, the researchers assessed serum adiponectin and plasma anthocyanins. The latter was measured to determine if the treatment increased the body's exposure to anthocyanins and the former was measured because this adipokine plays a role in type II diabetes pathology.

Chinese adults with type II diabetes took 320 milligrams of anthocyanins daily for 24 weeks. Researchers took measurements related to diabetes.

What were the findings?

Compliance in both groups was very high (98.1% and 98.5% of the pills were consumed in the placebo and anthocyanin groups, respectively), and no dropouts or adverse effects related to treatment were reported.

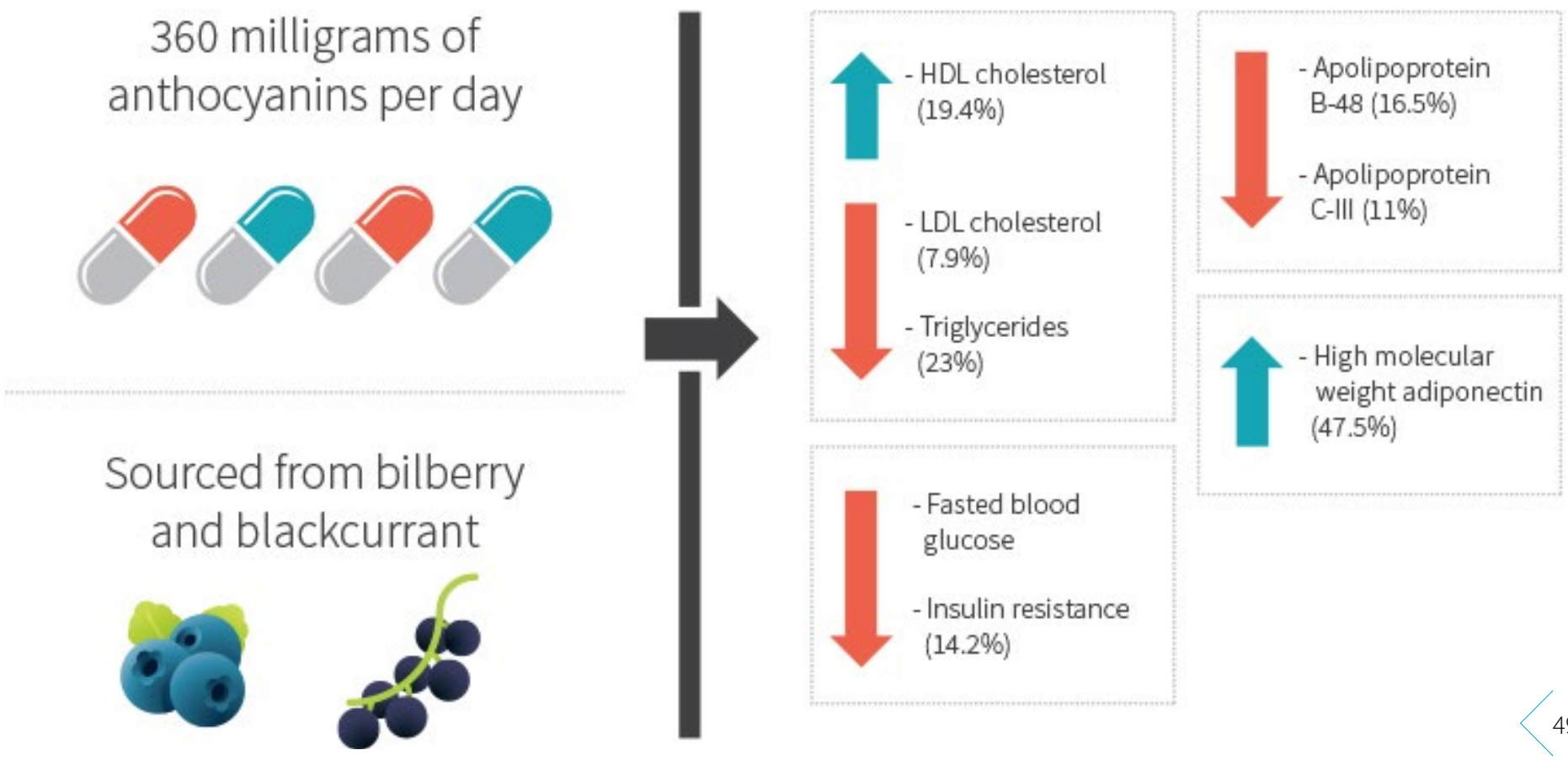
Supplementation of 360 milligrams of purified anthocyanins shortly after meals increased anthocyanin concentrations in the blood of the treatment group. The variance (difference from one subject to the next) appeared to be relatively low and all participants experienced some manner of increase in circulating anthocyanins.

This increase of circulating anthocyanins in the treatment group appeared to coincide with a number of benefits in the treatment group as well, as summarized in Figure 2.

Participants in the anthocyanin-supplement group experienced an increase in HDL cholesterol (19.4%) and decreases of both LDL cholesterol (7.9%) and triglycerides (23%). Two other cardiovascular parameters also improved, including a reduction in apolipoprotein B-48 (16.5%) and apolipoprotein C-III (11%), while two other apolipoprotein levels (A-I and B-100) and free fatty acids were unchanged between groups. There did not appear to be clinically relevant changes in systolic or diastolic blood pressure related to treatment.

The benefits to type II diabetes related pathology appeared to be somewhat selective. Though there was a reduction in blood glucose in the fasted state (8.5%) and insulin resistance (14.2%) there were no changes in either fasting insulin

Figure 2: Study results



concentrations or glycated haemoglobin. Adiponectin appeared to be greatly influenced in people with type II diabetes, though total adiponectin increased 23.6% and the high molecular weight adiponectin (thought to be the form more related to the health benefits of adiponectin) increased 47.5% when compared to baseline. These changes were not observed in the placebo group.

> “ [...] studies testing blueberries in similar populations have found no change in triglycerides, which casts just a bit of doubt on this study’s results. ”

Overall body weight and fat mass of the participants was not influenced by treatment.

Anthocyanins improved the lipid profile of people with diabetes while also reducing fasting blood glucose and insulin resistance. However, blood pressure, fasting insulin levels, and glycated haemoglobin were unaffected, compared to placebo.

What does this study really tell us?

There are a few parameters affected by anthocyanin supplementation that are thought to be highly relevant to the progression of type II diabetes. Specifically, the large increase in adiponectin (particularly the high weight molecular form) and the improvement in insulin sensitivity may provide benefits for people with type II diabetes. Unfortunately, anthocyanins are not a cure-all. While HbA1c is a good proxy measurement for overall bodily glycation and ideally drops during successful diabetes treatment, anthocyanin supplementation failed to influence it.

Since supplementing anthocyanins did not result in any side effects while improving health parameters, it appears to be fairly safe. The dosage used (which is thought to be comparable to 100 grams of fresh dark berries) suggests that both supplementation and food can both be used to achieve these beneficial effects.

It’s also important to note that insulin resistance was measured indirectly (using HOMA-IR), and hence should be taken with a grain of salt, since other physiological changes other than improved insulin resistance could have affected the HOMA-IR calculation.

The study results are also almost too good to believe. While they may very well be true, a triglyceride reduction of almost a quarter from such a simple intervention is simply bananas. Or berries, in this case. Some research has found a small improvement in triglycerides from bilberries, but others have found no change when using a mix of bilberry and blackcurrant, similar to that used in this study. And studies testing blueberries (also rich in anthocyanins) in similar populations have found no change in triglycerides, which casts just a bit of doubt on this study’s results.

The big picture

This study was relatively well constructed, but its results should still be interpreted cautiously. In this study, a specif-

ic supplement was assessed in the context of its effects on blood parameters. Assessing the magnitude of these changes is an important step to future, larger studies focused on pathology. Despite the promise of anthocyanins as a supplement, and by plausible extension dark berries, this study did not actually assess the development of complications from the disease state or the quality of life of the participants.

This study only included people with type II diabetes, excluding even people who were diagnosed too recently. By design, this study cannot be used to determine preventative or therapeutic effects, though it does provide enough evidence to lay the foundation for future studies.

If the lipid effects of this study are true, it would be a big deal in the management of complications from type II diabetes. The primary cause of death of diabetic people is cardiovascular events, since diabetes promotes CVD. In fact, the study authors theorize that the lipid changes seen in this study could translate to a roughly 25% reduction in heart disease risk. The medical and research community is always looking for ways to reduce CVD events in this population. Thus, it's particularly important to replicate these findings if possible.

> “ [...] the study authors theorize that the lipid changes seen in this study could translate to a roughly 25% reduction in heart disease risk. ”

The amount of anthocyanins used in this study is comparable to a realistic dose ingested from fresh berries, which means the effects observed in this study can be plausibly recreated through the regular consumption of berries. The observed effects, while positive, did not extend to all important measures of diabetes health, so further research is needed to determine how anthocyanins may impact the progression of the disease and the quality of life of people who suffer from it.

Frequently asked questions

Are supplements and berries interchangeable?

Anthocyanin supplements are considered somewhat interchangeable with dark berries for a few reasons. Past studies have found benefits stemming from both dark berries and supplements. The unique benefits of dark berries have been narrowed down to their anthocyanin content, and there is consistency between studies using berries and supplements, assuming the anthocyanin content is similar.

The dose equivalence may not be perfect, since an anthocyanin supplement can be standardized to contain a certain quantity of anthocyanins, but food anthocyanin content varies based on growing conditions, preservation methods, and packaging. Blueberries are the standard when discussing dark berry anthocyanins, due to their popularity. But darker berries like bilberry, mulberry, and cloudberry have even higher anthocyanin concentrations.

Why 'dark berry' rather than berry?

Anthocyanins are a class of pigments that share a similar structure, somewhat similar to the flavonoids found in most fruit and vegetables, but with a polar oxygen molecule in their backbone. The spectrum of phytonutrients found in plants is shown in Figure 3. Anthocyanins, as pigments, aren't always dark and sometimes cover the red-purple spectrum. But red pigments can be of different types. For

example, betalains in beets are also red, but are different in structure from anthocyanins.

Dark berries are specified in this study because both of the berries the supplement was derived from (bilberry and blackcurrant) and blueberries contain blue-purple anthocyanin sources, which have different properties than other color anthocyanin sources. While most anthocyanins are of the blue-purple coloration, the one found in strawberries responsible for their red color, pelargonidin, is not yet confirmed to have the same actions as other well-researched anthocyanins.

Is there a major difference between taking a supplement 'shortly after meals' and with meals?

The researchers conducting this study advised the participants to take their supplement 30 minutes after the first and last meal of the day. Does this differ much from taking the supplement with food? Not especially.

Sometimes medication needs to be taken on an empty stomach if something in food may interfere with absorption.

Supplements that don't need to be taken on an empty stomach can survive the acidic environment of the stomach, and food won't interfere with the absorption of the relevant compounds. This is the case with dark berry anthocyanins, which can easily be taken with food.

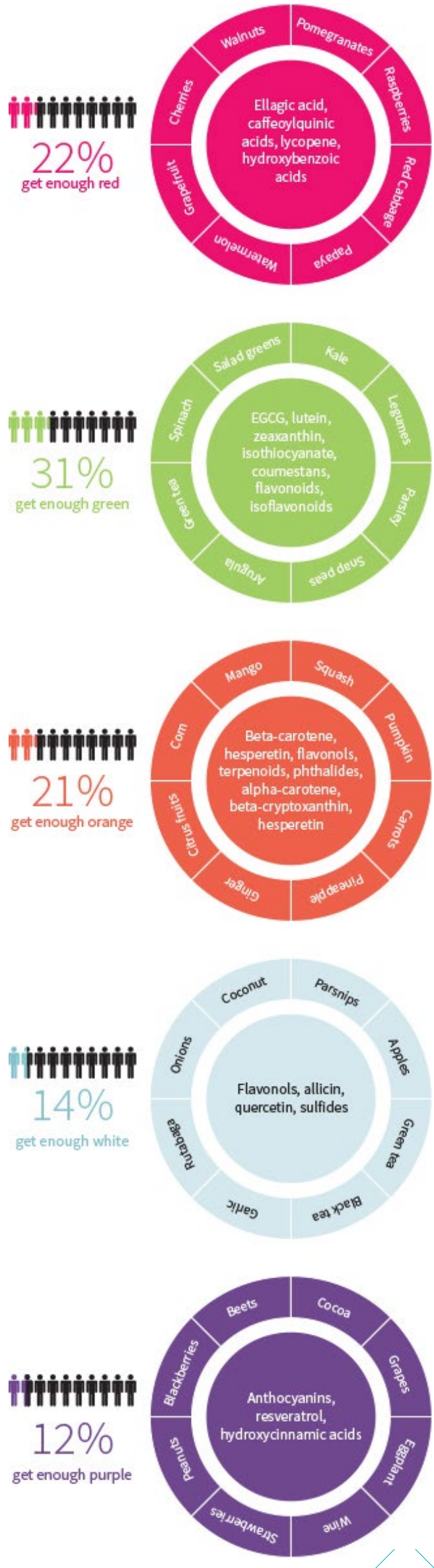
What should I know?

A variety of plant foods contain anthocyanins, but the most widely available concentrated source are dark berries. There appears to be little-to-no downside to using these types of berries an adjunct treatment for blood sugar control in people with type 2 diabetes.

While this trial showed an improvement in parameters related to type 2 diabetes pathology, there was no change in the longer-term parameter of glycated hemoglobin. Future trials exploring the effects of anthocyanins on disease complications can help to shed light on what role this compound can play for people battling type 2 diabetes. ◆

With results like these, why aren't more people with blood sugar issues using anthocyanins? Is this trial believable? Discuss it over at the [Facebook ERD forum](#).

Figure 3: Common phytonutrients in plants



Can fiber change your emotions?

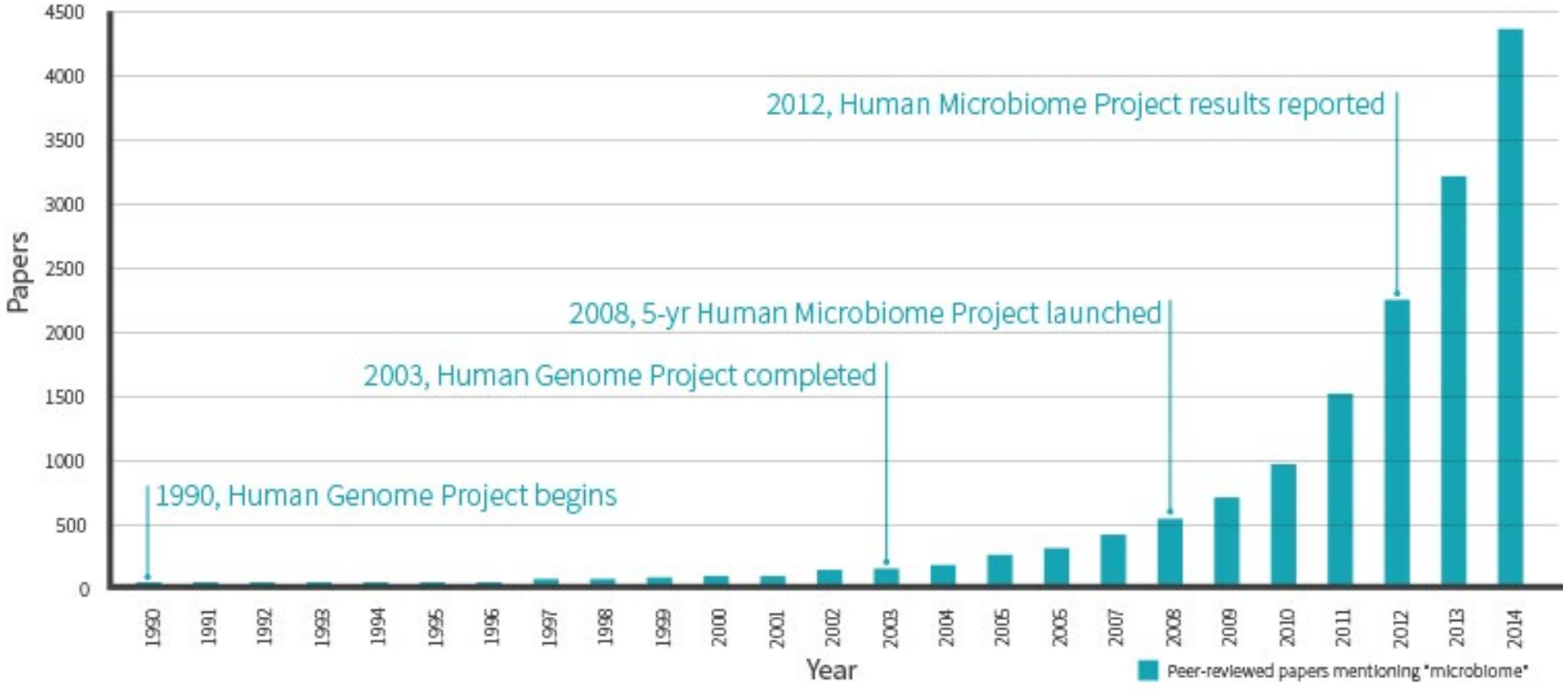
Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers

Introduction

With the exception of the March issue, each ERD so far has included at least one study that involves the gut microbiome. This is by no means surprising. After all, science is slowly discovering the complexity of the gut microbiome (as evidenced by Figure 1) and its potent, far-reaching health effects. One area the ERD has yet to discuss is the microbiome's possible role in stress.

The stress response is regulated by a network called the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is ultimately responsible for the release of cortisol, and its effects spread to every corner of the body, affecting digestion, immune system function, energy storage and expenditure, and emotions. For instance, it has been suggested that HPA-axis communication is disrupted in people with depression and that greater cortisol dysregulation predicts increased symptom severity.

Figure 1: Microbiome is so hot right now



Our understanding of the interactions between the microbiome and the stress response is in its infancy. The "gut-brain" axis is a relatively recent focus of research, and the health consequences of its dysregulation may range from visceral pain, obesity, and cardiovascular disease to autism spectrum disorders, anxiety, depression, and multiple sclerosis. It has been previously demonstrated in rodents that the lack of a microbiome exacerbates the neuroendocrine and behavioral responses to stress, possibly because cortisol can alter gut permeability and barrier function, and that recolonization of the gut bacteria reverses these effects.

Identifying the beneficial and harmful bacteria responsible for these effects is a long way off, however. This should come as no surprise considering that our intestinal tract is home to upward of one hundred trillion microorganisms—more than ten times the number of human cells in our bodies—that contain 150 times as many genes as our genome. Rather than using probiotics, which would supplement only a handful of species, the current study explored the effects of two types of common prebiotic fibers, which may help multiple healthy species flourish. Researchers investigated the processing of emotional information and HPA-axis activity in healthy human volunteers.

The brain and gut microbiome communicate through multiple pathways. The HPA axis that regulates stress and how we respond to it is one such pathway that may be affected, for better or worse, by changes in the microbiome.

Who and what was studied?

Forty-five men and women ages 18–45 years with a normal-weight BMI participated in this study. None had a history of neurological, psychiatric, gastrointestinal, or endocrine disorders, and none had used antibiotics, prebiotic, or probiotic supplements in the three months prior to the study. This was necessary to help ensure that the participants had a relatively normal gut microbiome before the study began.

For three weeks, the participants were randomized (double-blind) to one of three groups—those taking fructooligosaccharides (FOS), those taking galactooligosaccharides (GOS), or those taking a maltodextrin placebo. Each group took 5.5 grams a day of their assigned supplement in powder form with breakfast, and were asked to maintain their usual diets and to avoid taking any additional supplements.

HPA-axis activity was assessed the day before the study (day 0) and the last day of the study (day 21) through five cortisol measurements taken upon waking and every 15 minutes for one hour thereafter. Additionally, several questionnaires that evaluated anxiety, perceived stress, and mood were also completed before and after the intervention period. Oddly enough, emotional processing (one of the study's main outcomes) was evaluated with a battery of computerized tests only after the intervention, not at baseline. This is problematic because we have no idea what the baseline differences between the groups were. It is completely possible that any differences showing up after the intervention were due to differences between the participants at the start rather than to changes due to the modulation of their microbiome from the fiber. This is a major limitation of this study.

This study randomized healthy men and women to consume 5.5 grams of fiber or a placebo with breakfast daily for three weeks. Researchers investigated the effects of the intervention on emotional processing and HPA-axis activity.

What were the findings?

None of the groups had any significant differences in anxiety, stress, cognitive status, or cortisol at baseline. The average age of the participants was 23–24 years. Also, compliance was very high, with 41 of the 45 participants taking the supplements every day as intended.

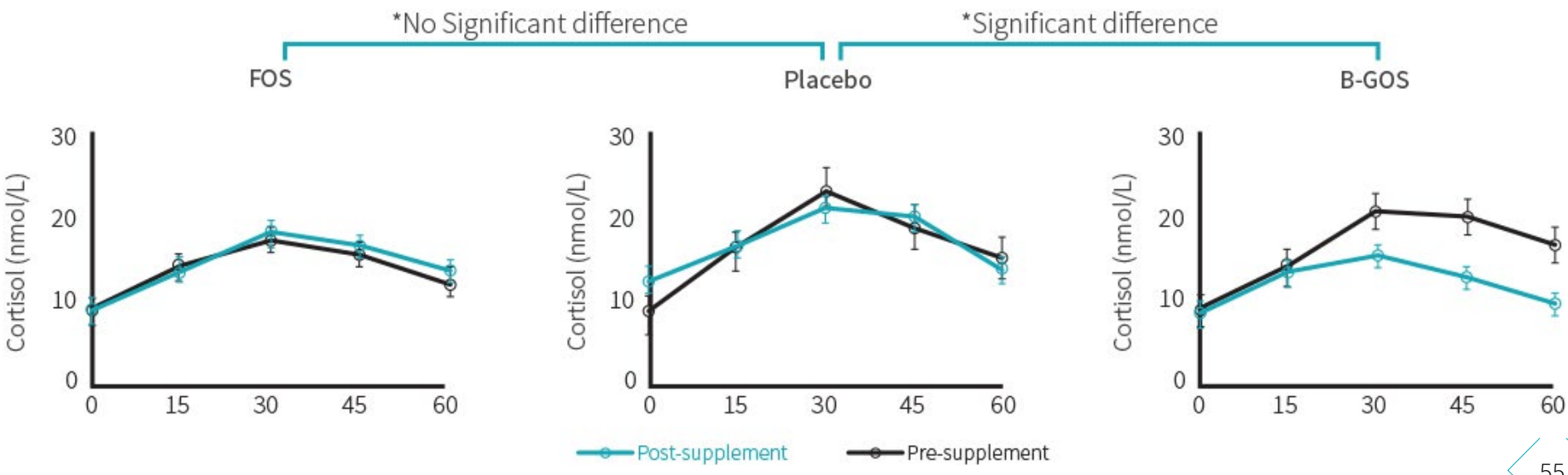
Did contraceptives or menstruation affect the results?

Of the 23 female participants, 13 used hormonal contraceptives, and a subgroup analysis between women who used contraceptives and those who did not showed no significant differences in the total cortisol response within the hour after waking.

Additionally, 16 women reported their particular menstruation stage at the times of testing. This did not differ between groups, but a lack of statistical power precluded subgroup analysis to see how menstruation affects cortisol.

The salivary cortisol measurements showed a significant rise in cortisol for the first 30 minutes after awakening during both pre- and post-intervention, at which point cortisol levels were greatest. A decline followed this peak. The only significant difference was between the placebo and GOS groups post-intervention (as seen in Figure 2), with the GOS group demonstrating a markedly reduced peak at 30 minutes, as well as an overall cortisol response over the hour that was about 29% lower than the placebo group. This clearly indicates a reduced stress response in the morning.

Figure 2: Cortisol awakening response



There were no differences observed between groups in terms of emotional processing, as measured by their ability to accurately perceive facial expression of basic emotions (happy, sad, surprise, fear, etc.), nor were there any differences in emotional categorization, recall, and recognition. In fact, the only emotional difference between the groups was in attentional vigilance, with the GOS group showing significantly increased vigilance toward positive stimuli compared to the placebo group. However, correlational analysis showed this was not associated with cortisol levels.

In the modern world, vigilance toward positive and negative emotional stimuli works a bit differently than with our ancestors. Constant vigilance isn't typically needed to avoid physical threats and help with finding food and shelter. Rather, consistently being on the lookout for negative aspects of life can be part of depressive symptoms, while more temporary/situational hyper-attention is linked to anxiety. Conversely, sustained awareness of positive stimuli is indicative of a non-depressed state.

What does this study really tell us?

This study set out to explore the neuroendocrine and emotional effects of two similar types of prebiotic fibers in healthy individuals. Researchers found that GOS supplementation was associated with reduced waking cortisol and altered attentional bias compared to placebo, but that these two changes were not statistically related to one another. Out of all the different mood-related variables tested, only one was found to be significant, plus the study did not mention statistically adjusting for testing multiple outcomes. This casts doubt as to whether the one positive emotion-related result they found means much.

The lack of a significant difference for the FOS group could be due to their lower baseline cortisol levels. Although there was no significant difference from the other groups in this regard, it is noteworthy that over the hour after waking, baseline cortisol levels in the FOS group were about 20% lower than they were in the other two groups. Based on this information, it is hardly surprising that the post-intervention levels in the FOS and GOS group did not differ significantly. The GOS supplement may simply have brought down the cortisol levels of the subjects in the GOS group to the same low level the subjects in the FOS group exhibited in the first place. Or maybe FOS targets difference microorganisms in the gut, leading to different effects.

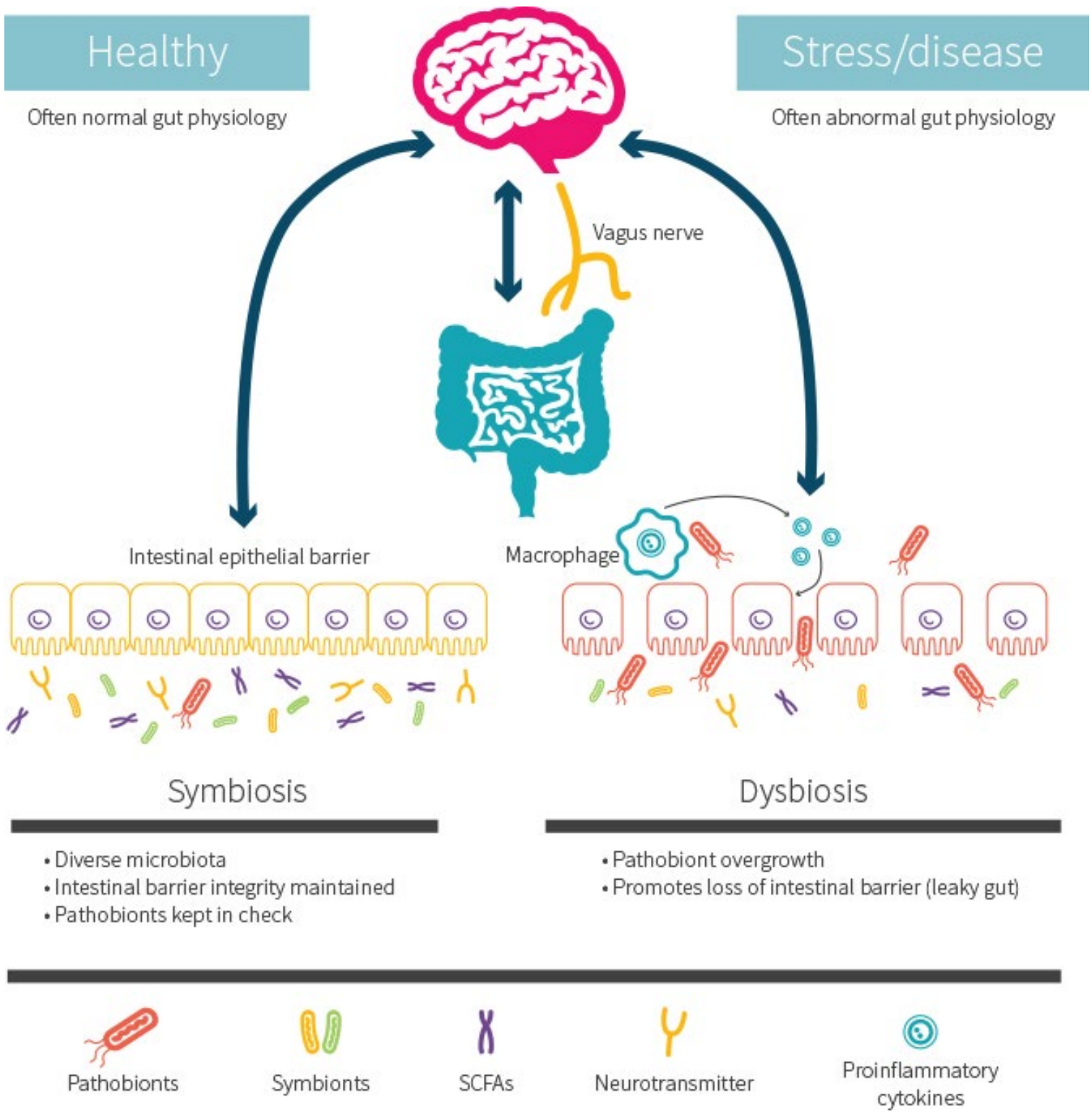
The differences for attentional vigilance are more difficult to explain. Increased vigilance toward positive stimuli, or, in other words, an attention bias towards positive vs. negative emotions, have been observed with the selective serotonin reuptake inhibitor citalopram, which has also been shown to make people pay less attention to threatening stimuli. Importantly, increased vigilance toward fearful and negative stimuli is significantly greater in anxious individuals when compared to healthy controls with low anxiety levels. Threat processing is believed to play a central role in the symptomatology of anxiety-related disorders. This is also in line with previous research that showed that a fermented milk product elicited changes in brain activity, supporting the idea of a gut-brain axis.

“ Out of all the different mood-related variables tested, only one was found to be significant ”

None of these factors explain why the FOS group showed no significant changes though, and it is unfortunate that the researchers did not characterize the gut microbiota of the participants. Research in rats has shown GOS to elicit significantly greater elevations in *bifidobacteria* compared to FOS, but even FOS led to greater elevations than the placebo. Perhaps there is a threshold of these bacteria that needs to be reached for effects to be seen. Or perhaps GOS induces changes in other types of bacteria that are not yet known or measured. Finally, a direct effect of GOS cannot be ruled out.

Since there were no changes in perceived stress or subclinical anxiety, it is also questionable how clinically relevant the changes the scientists did observe are. Something we can say for sure; however, is that the effects were not brought about by cortisol, as analysis showed no associations between emotional processing and the waking cortisol response. Overall, the study at hand adds more evidence to the already strong foundation suggesting a role for the microbiome in HPA-axis regulation. As for the exact mechanism(s), we are still left in the dark, although anti-inflammatory effects and increased structural integrity of the mucosal membrane have been suggested (as shown in Figure 3).

Figure 3: Interplay between gut bacteria and the brain



Source: Adapted from Lyte, PLoS Pathog. 2013

This study tells us that GOS supplementation (5.5 grams a day) in healthy young adults attenuates the waking cortisol response and increases attentional bias toward more positive (happy) stimuli. These changes are unrelated to one another and may be the result of changes in the gut microbiome, but more research is needed to confirm this effect. These changes did not translate to changes in anxiety or stress.

The big picture

The gut microbiome is most studied for its role in gastrointestinal disorders and chronic disease, but increasing attention is being paid to its influence on the brain. This "gut-brain axis" is a bidirectional communication pathway mediated in part by the HPA axis and cortisol.

This point is best illustrated by a study conducted in 2004 that showed germ-free mice exposed to a mild stressor exhibited an exaggerated release of corticosterone (the rodent equivalent of human cortisol) and ACTH (the hormone that stimulates cortisol release) compared to normal mice. This difference was partially reversed upon colonization of the germ-free mice with fecal matter from the normal mice, but only when colonization occurred during the early stages of life. These findings not only clearly demonstrate the importance of a healthy microbiome for the proper development of the stress response, but also that there is a critical window of opportunity early in life where colonization must occur to ensure normal development of the HPA axis. Since the germ-free mice also exhibited reduced levels of brain-derived neurotrophic factor (BNDF), a key regulator of normal neuronal growth and survival, it becomes clear how important for our future health the early colonization of our guts with "good bacteria" is.

The idea of a gut-brain axis is further supported by numerous studies administering probiotic supplements, with the most consistent data being in relation to anxiety. For instance, the administration of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 in rats and humans has demonstrated anxiolytic-like activity, and a 28-day treatment of mice with *Lactobacillus rhamnosus* (JB-1) reduced stress-induced corticosterone and anxiety- and depression-related behavior. This latter study also demonstrated that mice with their vagus nerve removed did not show the same behavioral effects, implicating the vagus nerve in the direct communication between the gut and the brain; an interaction that may also explain why specific *Lactobacillus* strains exert pain-reducing effects similar to morphine.

> "What these studies collectively demonstrate is that the microbiome influences the HPA axis and any behaviors associated with it."

What these studies collectively demonstrate is that the microbiome influences the HPA axis and any behaviors associated with it. An interesting trend is that most probiotic studies use strains of *Lactobacillus*, *Bifidobacterium*, or both. Research in rats has shown GOS to elicit significantly greater elevations in bifidobacteria than FOS, which may explain why only the GOS group in the current study elicited changes in emotional vigilance and cortisol. Regardless, the previous research also uses specific strains of these bacteria, which may explain why the magnitude of effect in the current study was not as pronounced.

A normal cortisol response is thought to be a sharp 50% increase within the first 30 minutes after waking, with about 75% of healthy adults showing this response, although there is wide variation. Women, for example, display a peak similar to men but have a significantly delayed decrease back to pre-waking levels. However, age, the use of oral contraceptives, smoking, time of awakening, sleep duration, and mode of awakening (alarm compared to no alarm) have a considerable impact on cortisol levels after waking. Although this awakening response is considered a good measure of HPA axis activity, some researchers argue that the cortisol response is linked specifically to the event of awakening and is independent of circadian variation in HPA-axis activity.

Despite the widespread belief that the morning cortisol rise is a means to prepare the body for the anticipated stress of daily living, its specific function is unknown. One meta-analysis concluded that the overall rise is greater with increased job and life stress, and lower with greater fatigue, burnout, and exhaustion. Another study found the awakening response to be blunted in psychotherapy inpatients suffering from depression, an observation that would support its importance for our overall psychological health.

The current study found no association between the blunted cortisol response and emotional vigilance; however, previous studies have indicated that both play an important role in the etiology of depression. In the study at hand, at baseline, none of the participants were depressed, but this questionnaire was not repeated after the intervention. Thus, we can only speculate about the effects the fiber supplements may have had on symptoms of depression. Future studies should clarify if prebiotic fiber intake can lead to clinically meaningful changes in mood states other than anxiety.

There is little doubt that the gut-brain axis exists, and it appears to be mediated at least in part through the vagus nerve. Research with specific strains of Lactobacillus and Bifidobacterium have shown promise in reducing anxiety-like symptoms and behaviors, but research on prebiotic fibers is lacking. The waking cortisol response is directly influenced by the daily stressors of life and may be linked to the development and severity of depression.

Frequently Asked Questions

What factors influence the awakening cortisol response?

Quite a few factors can influence the awakening cortisol response. For instance, waking up earlier in the morning and waking to light rather than darkness have been shown to increase the response, while sleeping to low-frequency noise (recorded ventilation noise) and chronic use of aspirin (300 milligrams a day) have been shown to suppress the cortisol response. Additionally, there does not appear to be a response to awakening from naps, suggesting that waking up by itself is insufficient for a subsequent cortisol increase. Rather, the authors of this study propose that awakening time is an important modulating factor for the subsequent cortisol response.

Other non-sleep-related factors include one's chronotype; morning "larks" show a greater awakening response than night "owls." Being fatigued or in pain also blunt the morning rise. Finally, stress plays a central role. For instance, waking up to a work day compared to a work-free weekend day and experiencing chronic stress and worry result in a

larger cortisol response, as does job strain. People of a lower socioeconomic status tend to exhibit an increased response, possibly due to greater stress.

Recall from the end of the "big picture" section that one study actually found the awakening response to be blunted in psychotherapy inpatients suffering from depression. It is interesting that sleeping to noise, chronic use of aspirin, being a night owl, and being stressed, fatigued, and in pain are all related to a blunted response as well.

If the gut-brain axis is bidirectional, does that mean that the brain can influence the gut?

Most definitely—haven't you ever experienced that gut-wrenching nausea after an emotionally painful event, like being rejected by your high-school crush with everybody watching? But seriously, it has long been known that stress and the associated activity of the HPA axis can influence the composition of the gut microbiome. For example, a study in which mice were exposed to a chronic restraint stressor shifted the population of microbes to favor *Clostridium* over *Bacteroides*. In addition to altering the gut microbes directly, chronic stress can also disrupt the intestinal barrier, leading to leaky gut.

What exactly are FOS and GOS, and where can I find them?

If we break down the name fructo-oligosaccharide (FOS) and galacto-oligosaccharide (GOS) it becomes clear that we are dealing with oligosaccharide compounds, which are short chains of simple sugars. Specifically, oligosaccharides contain anywhere from three to 10 simple sugars, while, for example, polysaccharides such as starch, inulin, β-glucan, and cellulose may contain hundreds to thousands of simple sugars. The first portion of the name tells us what sugars the chains are made from: FOS is a few fructose molecules linked together and GOS is made of galactose molecules.

Both FOS and GOS are made commercially and can be purchased as a supplemental fiber or found added to foods. The main difference is where they are naturally occurring. FOS is very common in plants. The best sources include Jerusalem artichoke (58.4 mg/g), shallot (8.5 mg/g), wheat germ (4.6 mg/g), chicory root (3.9 mg/g), garlic (3.9 mg/g), rye (3.8 mg/g), onion (1.4-3.1 mg/g), and bananas (1.4 mg/g). On the other hand, GOS is found primarily in human milk and, to a less extent, the milk of domestic mammals such as cows. Overall, the total oligosaccharide content of human milk is about 7-12 g/L while animal milk has 10 to 100 times less.

What should I know?

The gut-brain axis definitely exists, and the implications of such a connection are vast. Studies have demonstrated that the connection works both ways (bidirectional), from the brain to the gut and vice-versa. The current study goes beyond much of the research focusing on probiotic microbes to show that supplementation of their food, in this case 5.5 grams of galactooligosaccharides, can also have downstream effects on emotion and the HPA axis. Specifically, the fiber resulted in a depressed morning awakening cortisol response and increased attentional vigilance toward positive stimuli. What remains to be seen, however, is how practically and/or clinically relevant these changes are, especially since only one out of the many emotion-related variables tested was affected by a prebiotic. Assuming that prebiotic fibers could be used to "treat" anxiety or depression is a premature conclusion. ◆

It might be premature to advocate for prebiotics impacting mood and emotions. Have you noticed any interesting changes from eating specific prebiotics? Let us know at the Facebook ERD forum.

One pro of probiotic drinks: mitigating harm from overeating

Probiotic supplementation prevents high-fat, overfeeding-induced insulin resistance in human subjects

Introduction

Probiotics are microorganisms that can integrate into the microbiota of our gut in a beneficial way. They are living organisms that can colonize the gastrointestinal (GI) tract, and potentially crowd out some harmful microbiotic species.

Gut microbiota interact with their host in a number of ways. One is by contributing to intestinal metabolism through excretion of their own enzymes into the GI lumen, or through their own metabolism, particularly the fermentation of some types of fiber into short-chain fatty acids (SCFAs). These SCFAs support uptake of positively charged ions, also known as cations (e.g., Ca, Mg, K, Na), and can be taken up and metabolized further in the body. Microbiota are also involved in folate and biotin biosynthesis.

Since our food contains hundreds of thousands, maybe millions, of biochemical compounds, and since microbiota changes metabolism and uptake of nutrients, different microbiota can induce different changes in the host body. The purported effects range from changes in feeding efficiency (i.e., how much food is taken up) in animals, levels of gastric upset, and immune function under stress to reducing antibiotic-induced diarrhea and improving resistance to opportunistic infections. Figure 1 shows some of the ways

that an overly-permeable gut lining, influenced by microbiota balance, can impact conditions such as type II diabetes.

Previous studies have shown that distinctive microbiological profiles are associated with poor metabolic function in people and animals with chronic conditions. A couple examples are shown in Figure 2. Furthermore, probiotics may help ameliorate metabolic dysfunction in the host. However, whether probiotic administration can prevent the impairment of glucose homeostasis induced by overfeeding in humans has not been studied. The purpose of this study was to answer that question.

Gut microbiota play a large role in health, including potentially in metabolic disorders associated with diabetes and obesity. The goal of this study was to determine whether probiotics that influence the microbiota could help prevent glucose impairment induced by overfeeding in humans.

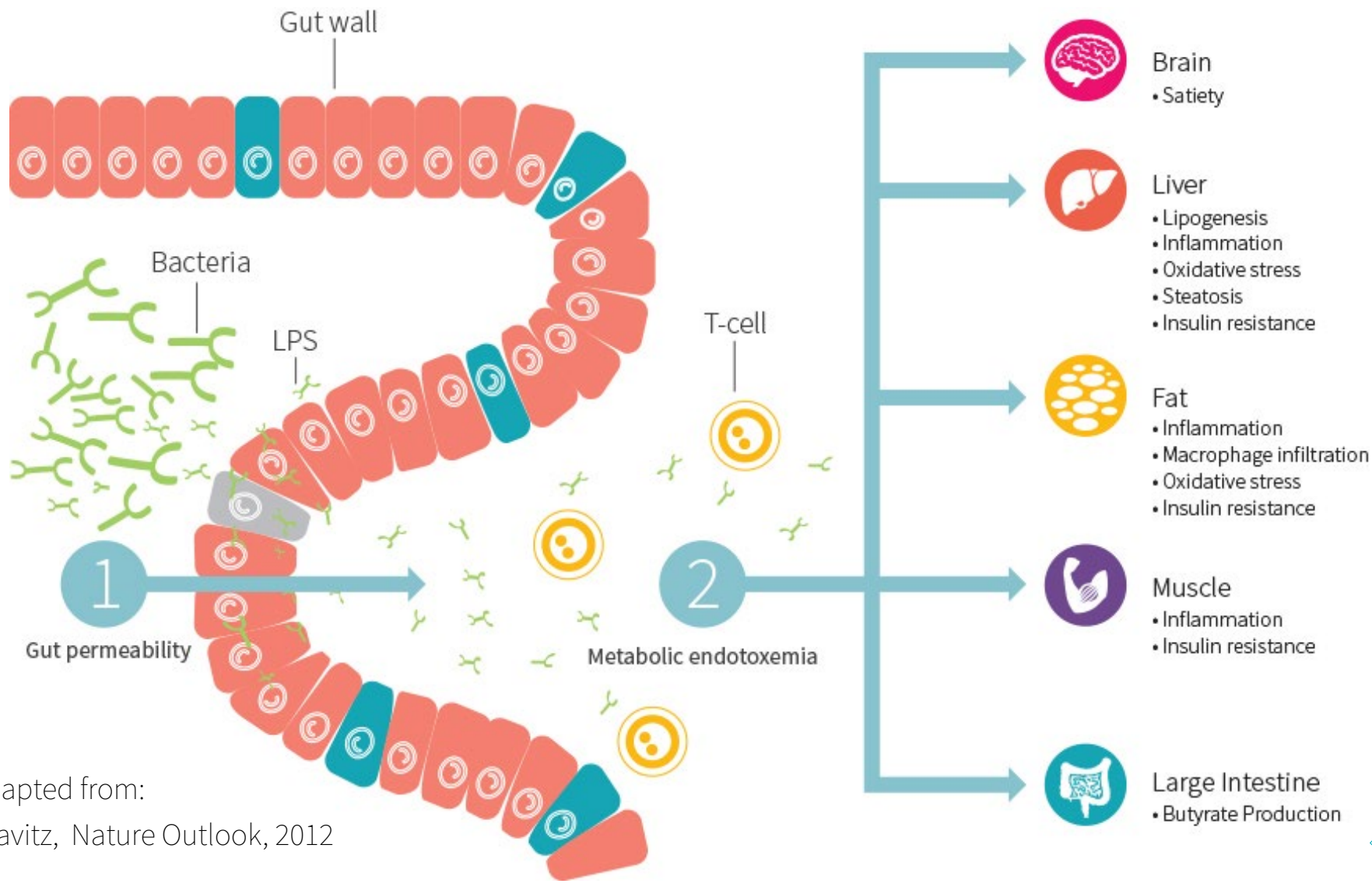
Who and what was studied?

In this study, 17 young, healthy participants of both genders were assigned to ingest either control or a yogurt containing *Lactobacillus casei* Shirota (LcS) for three weeks followed by one week of high-fat overfeeding.

The participants were at a normal weight (BMI of 18.5–24.9) and exercised at least three times per week for more than 30 minutes at a time. Their weight had been stable for at least six months, they exhibited good cardiovascular and metabolic health, and did not take any medication. Also, participants were required to not have used any probiotics for at least three months prior to the study and to not follow a vegan or vegetarian diet.

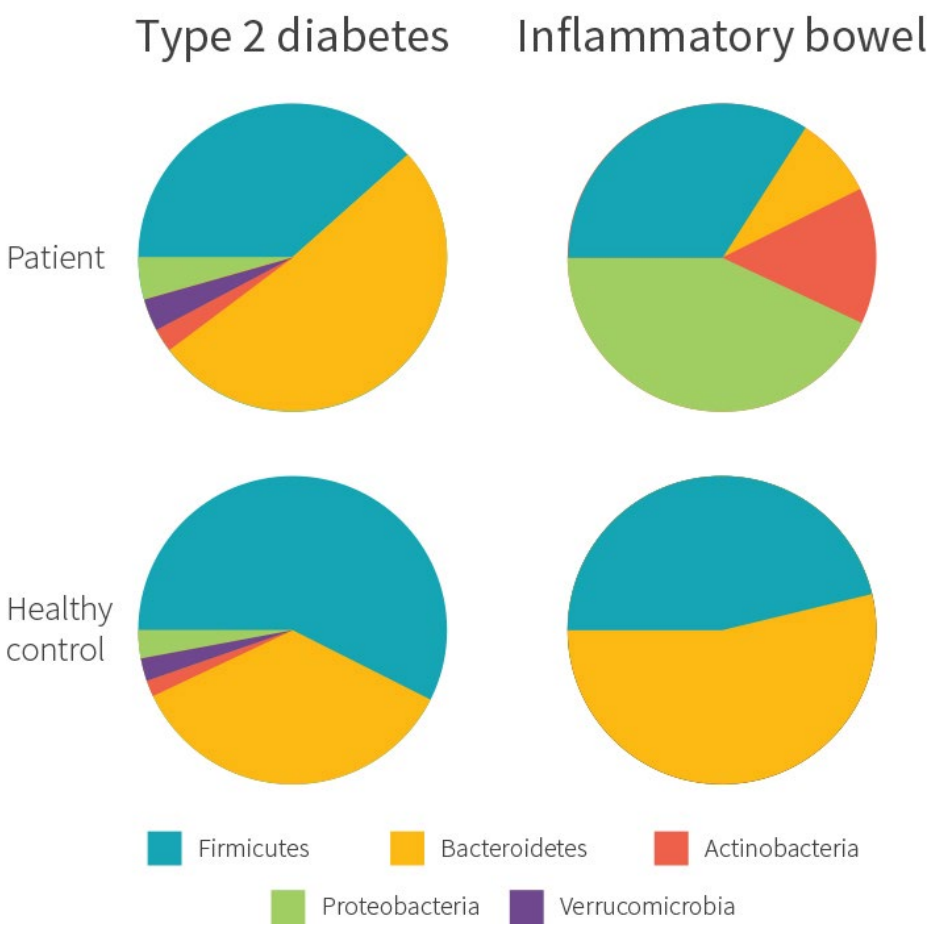
Before the onset of the intervention period, participants were weighed and had their fasting insulin and glucose measured. Next, study participants were randomly assigned to either the the probiotic group (n=8, seven males and one female), drinking a LcS-containing fermented skimmed

Figure 1: Mechanisms for gut bacteria influencing metabolic disorder



Adapted from:
Gravitz, Nature Outlook, 2012

Figure 2: Gut dysbiosis and chronic disease



milk product (Yakult Light) or the control group (n=9, seven males, two females), drinking nothing, not even a placebo.

The study itself consisted of three weeks of habitual food intake supplemented with 65 mL of the probiotic twice daily (only in the probiotic group), followed by one week of overfeeding. During the habitual food intake period, participants were required to keep detailed food logs, which included weighing food items, for three days per week. This was analyzed to estimate daily energy expenditure. After the habitual intake period of 21 days, the "real" part of the study began, i.e. assessing the effect of the probiotics on overfeeding-induced changes in glucose clearance. On day 22, all of the study participants were subjected to an oral glucose tolerance test (OGTT) and had fasting insulin, glucose and triglycerides measured. After that, the overfeeding phase began. Following one week of overfeeding, participants did another OGTT and fasting insulin/glucose/triglycerides sampling.

Overfeeding was intended to provide a 50% caloric surplus at a 65% fat intake for one week. Fat intake from the food logs was 90–100 grams a day during the habitual intake, while in the overfeeding period, fat intake was bumped up to 260–280 grams a day! Protein intake increased by approximately 20 grams a day, while carbohydrate intake decreased by approximately 80 grams a day.

Young, healthy people of normal weight either supplemented with a probiotic drink or were given nothing as a control for three weeks. They were all then given a 50% caloric surplus for a week. Glucose clearance, fasting insulin and glucose were measured before and after overfeeding.

What were the findings?

Weight gain and BMI

All of the study participants gained weight. The control group gained 0.6 kilograms, which was a statistically significant change, whereas the probiotic group gained 0.3 kilograms, which was not a significant change. The study did not report whether the between-group difference was significant, but given the sample size and variation, it most likely was not.

Fasting insulin and glucose

Fasting insulin was unchanged at 86 nmol/L in the control group and decreased slightly in the probiotic group, albeit not significantly so. Fasting triglycerides decreased in both groups (from 1.4 to 0.9–1.0 mmol/L). Fasting glucose increased from 5.3 to 5.6 mmol/L in the control group but remained steady at 5.8 mmol/l in the probiotic group.

OGTT results

The OGTT data consists of repeated measurements of insulin and glucose after ingestion of a glucose challenge. A common way to report this is as an area under the curve (AUC) which is, in essence, a summation of the data points. Overfeeding in the control group led to a significant increase in the glucose AUC by about 10%, with no change

Matsuda Insulin Sensitivity Index (ISI)

The Matsuda Insulin Sensitivity Index allows comparison between the results from an OGTT and those from clamp studies, which is considered the gold standard technique for directly measuring glucose clearance and insulin sensitivity.

The ISI is a common method used by researchers, with almost 3,000 articles citing the original article that describes the index. While modifications of ISI have been used for increased accuracy, neither ISI or the widely used HOMA-IR may correlate that well with other estimates of insulin sensitivity in certain populations.

in the probiotic group. A similar pattern could be observed for the insulin AUC data, but this was not significant, probably owing to greater variation in the insulin data.

Also, the mean OGTT insulin and glucose were combined with the fasting insulin and glucose in the calculation of the Matsuda Insulin Sensitivity Index (ISI). The ISI fell significantly from 5.3 to 3.9 (27%) in the control group only.

Probiotic supplementation prevented fasting glucose and glucose tolerance from increasing after overfeeding.

What does the study really tell us?

The study shows that the loss of glucose-clearing ability in (normal BMI, fairly young) humans induced by overfeeding can be prevented by a beverage containing LcS-containing probiotic—at least in the short term. The study was financially supported by the makers of Yakult, which doesn't necessarily mean anything with regards to the results, but should be noted.

One important problem with this study lies in the baseline fasting glucose levels. The fasting glucose in the control group was 5.3 +/- 0.2 (across nine participants) and in the probiotic group, it was 5.6 +/- 0.1 (across seven participants). However, looking at the numbers, the fasting glucose was higher in the probiotic group than in the control group before the intervention started. Whether this skews the data is hard to tell. On the one hand, this could mean that the subjects in the probiotic group were a little worse off to begin with (higher glucose) and therefore less likely to show a response to overfeeding (higher glucose again). On the other hand, it could also be argued that being slightly worse off, metabolically speaking, would reinforce the negative overfeeding effects as less metabolically fit people have poorer metabolic flexibility and adaptative resources. The first scenario may be the most likely, because the blood glucose values are within healthy ranges and the subjects are otherwise young and fairly fit. Therefore, a higher initial blood glucose level could make increases from overfeeding less likely to appear with overfeeding, thereby inflating the effects of the intervention.

The OGTT glucose and insulin curves definitely tell a story by themselves—overfeeding makes the control subjects much less efficient at removing glucose from the blood. But the fasting data and OGTT data were used to calculate Matsuda ISI and when we revisit those, we see once again that the baseline-calculated insulin sensitivity in the probiotic group was actually closer to the control overfed value than the control baseline value! Again, when insulin sensitivity is low, it may reduce the potential efficacy of an overfeeding period that should reduce insulin sensitivity, thereby helping to create an image that the intervention (probiotic administration) prevented a drop in insulin sensitivity.

Whenever a study can be shown to contain flaws that seemingly reinforce the effect of the intervention, and particularly when this flaw or error falls to the benefit of an entity pro-

viding financial support to the study, some red flags should be raised. Although this probably is just an unfortunate randomization coincidence, the authors could have taken away any doubt by commenting on it, but they did not.

Furthermore, the authors actually had access to fasting insulin and glucose measurements from the pre-intervention period that could have been used to randomize the participants (i.e., distributing the subjects so that their average fasting insulin and glucose were comparable across groups), but this was not done. While this does not invalidate the findings of the study, it should raise some doubt about the magnitude of the reported effects.

Due to the pretreatment differences between the probiotic and control groups, there is a chance that the study effect sizes may have been exaggerated.

The big picture

Another study has shown that administration of probiotics for people with type II diabetes reduced fasting glucose and glycosylated hemoglobin (a marker of accumulated blood glucose levels), as well as several oxidative markers. The present study plays nicely into previous evidence, although the effects here may be exaggerated for reasons described earlier.

One limitation of this study was that the control group was not administered any kind of placebo, so it's hard to tell to what extent other components of the yogurt besides the bacteria may have affected the outcomes. Benefits might have come from the dairy, the probiotics, or both. Perhaps the researchers should have used a skim milk control if they wanted to isolate the effect of probiotics.

Sequencing gut microbiomes is all the rage in nutrition and metabolism science these days. Sequencing of ribosomal RNA can be used to identify which species are present in the GI tract and shotgun sequencing can be used to identify the genes present in the genomes, as an indirect way to describe the types of microorganisms, but also a way to get closer to knowing what metabolic pathways are influenced by which microbial profile. Also, assessment of SCFA content in stool and starch/fiber fermentation products can help elucidate how any observed metabolic changes in the study came about.

A recent study very elegantly used these techniques to show that saccharin produced long-lasting changes in the

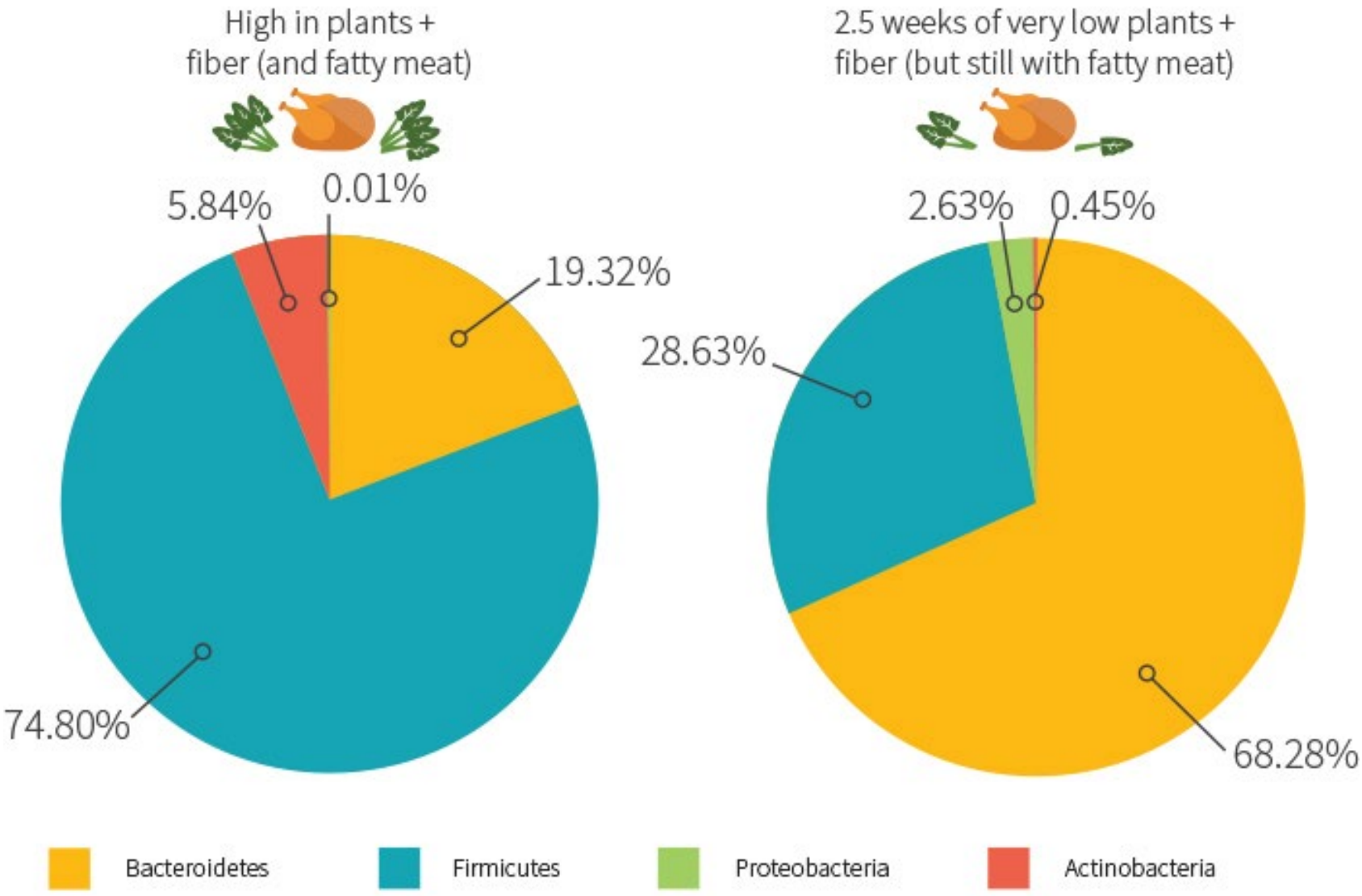
“One limitation of this study was that the control group was not administered any kind of placebo, so it's hard to tell to what extent other components of the yogurt besides the bacteria may have affected the outcomes.”

gut microbiota that resulted in impaired glucose tolerance in mice, with weaker data in humans. This effect could be transferred to saccharin-naive animals through fecal transplants, in mice only. Without sequencing technology, it would have been impossible to claim that the intervention resulted in lasting changes in microbiota composition. A study like the current one would benefit immensely from these techniques (although trial costs could also skyrocket) because they would provide a peek into the composition and functional changes induced by the ingested probiotic. This would help determine if and to whom probiotics would be of benefit.

What's the real-world implication from this study? Although the study findings can't necessarily be attributed to the probiotic (because the control group didn't ingest anything at all), it's quite possible that the probiotic used in this study improved glucose tolerance after overfeeding. This matches well with several large cross-sectional studies (e.g., here, here and here) in which intake of cheese and fermented milk products is associated with lower all-cause and several disease-specific mortalities. Beneficial effects from probiotics might not necessitate supplementing directly with probiotics, however. Figure 3 shows a substantial change in gut microbiota after just 2-3 weeks of considerably altered plant intake, in an n=1 experiment from the founder of the Human Food Project (which focuses on the impact of microbiota on human health). While probiotics may introduce billions of bacteria to the gut, prebiotics from plant-eating may be able to feed quite a larger number and wider variety of beneficial bacteria that already colonize the intestine.

In the context of public health, the model of acute overfeeding isn't always a very realistic one. Some overweight individuals do not have large daily caloric surpluses, but rather sustained, small ones. Others go through cycles of overeating and undereating. One group of people who routinely expose themselves to acute overfeeding are fitness

Figure 3: A shift in plant intake quickly changes gut bacteria



*n=1 experiment by founder of Human Food Project

buffs during bulking phases intending to boost muscle growth. While the findings reported here could in principle extend to that type of situation and benefit insulin sensitivity, more research is needed to determine if that kind of overfeeding is even associated with impaired insulin sensitivity.

While this study did not confirm whether the probiotic supplement led to changes in the gut, other animal studies have demonstrated such an effect. Also, the nature of this study makes it difficult to generalize because it involved healthy subjects with large caloric surpluses over a short period of time, which isn't always how people gain weight.

Frequently asked questions?

What is the oral glucose tolerance test (OGTT)?

An OGTT measures your ability to control blood glucose after glucose ingestion. This involves removing glucose from the bloodstream, but it also involves suppressing endogenous glucose production by the liver. Both make important contributions to glucose control during an OGTT. It is normally performed through ingestion of 75 grams of glucose in the morning following an overnight fast. After the ingestion, blood can be sampled once at the two hour timepoint for glucose, or for research studies every 15 to 30 minutes for both glucose and insulin (in order to calculate indices such as ISI).

What does the "Shirota" mean with regards to Lactobacillus casei?

Lactobacillus casei is a species of bacteria with many subtypes, several of which are considered probiotics. One of the subtypes is the Shirota type described in this paper. Shirota lactobacilli were discovered/isolated by the Japanese scientist Minoru Shirota in 1930. Five years later, he commercialized his discovery by making it into one of the first probiotic fermented skimmed milk products, Yakult. There are other fermented milk/yogurt products in which the main probiotic microorganism is *Lactobacilli*, like Actimel.

What should I know?

Supplementation with a probiotic-fermented dairy beverage may prevent glucose impairment induced by overfeeding. However, because there were pretreatment differences between the studied groups, it is possible that the study's effects may have been exaggerated.

Also, the nature of the study limits its generalizability—people often gain weight as a result of small daily caloric surpluses, and not always as a result of periods of overfeeding. Future research in different overfeeding situations with different population types, possibly with a control group also ingesting a dairy beverage rather than nothing, can help confirm the present results. ◆

This study showed promise, yet had some important flaws. Does it make a strong enough case for these probiotics to be used in real-life situations? Discuss probiotics more at the Facebook ERD forum.

> "One group of people who routinely expose themselves to acute overfeeding are fitness buffs during bulking phases intending to boost muscle growth."

Ask the Researcher

Mike Ormsbee, Ph.D.

Michael Ormsbee, Ph.D., CSCS is an Assistant Professor in the Department of Nutrition, Food, and Exercise Science at The Florida State University (FSU) and an Honorary Research Fellow at the University of KwaZulu Natal in Durban, South Africa. He is a faculty member at the Center for Advancing Exercise and Nutrition Research on Aging and the Institute of Sport Science and Medicine at FSU. His research and expertise involves the interaction of exercise training and nutrition to achieve optimal body composition and human performance in both athletes and clinical populations.

Possibly the most ingrained piece of health advice from our parents' generation is "Don't eat right before bed." You've done a good deal of research on this topic, including pretty specific clinical trials. What can our readers learn from research on night-time feeding?

Much of the advice that we all were told about was from studies that dealt with just two populations, 1) those with Night Eating Syndrome (eat a large percent of total daily calories in the late evening or upon waking in the middle of the night) or 2) shift works (those working through the evening). Of the research that did look at eating before bed, most studies provided pretty large, mixed meals. So, until recently, not much was done with smaller, single-macronutrient "meals" or, in reality, shakes or drinks. The latest work in humans tends to give about 150 kcals of whey protein or casein protein (or even carbohydrate in some cases) and some benefits have been shown in populations including young fit men, older men, and overweight/obese women.

These benefits include an increase in muscle protein synthesis while sleeping, increased morning metabolism, improved blood pressure and less hunger/improved satiety. Now, these studies are mostly short term (the longest to date that we have done is only four weeks). Looking at it another way, eating before bed also did not harm health or alter body weight either acutely or

over weeks. Only one negative finding has been shown with insulin sensitivity, however, this was entirely removed when exercise training was incorporated into the protocol.

In sum, eating small (150kcal) single-macronutrient drinks that are primarily whey or casein protein is likely to improve muscle protein synthesis and metabolism and not harm overall health. Work is now being done to look into performance outcomes and metabolic changes that occur while sleeping. The next target we are looking into will be whole foods like milk, cottage, cheese, and eggs.

> "I have always eaten like this before bed, even before we started researching this in 2010."

Did you change any of your eating habits based on research you've done or seen?

I have always eaten like this before bed, even before we started researching this in 2010. So, I didn't change how I ate but it is nice to know that I wasn't too far off track. Anecdotally, we know that many physique competitors choose to eat before bed or upon waking in the middle of the night. So, as often happens in sports nutrition, the competitors are ahead of the research.

We covered a recent meta-analysis on meal frequency and its impact on body composition. You've done some research on normal versus more frequent protein feedings. What's your take on different meal frequencies, for bulking, cutting, and other common situations?

My take is that people try to make things too darn complicated. In my opinion, if you like to eat frequently, then do it. If you like to fast intermittently, then do it. I find that it is best to keep things as simple as possible. I know people that are "stage ready" most times of the year that follow extremely different eating patterns. Just like for weight loss, for body composition change, I also think that adherence is probably the best goal to aim for. My thought on frequency is that most work has given frequent mixed-meals as opposed to frequent higher-protein meals. In our study that was lead by Dr. Paul Arciero from Skidmore College, the aim was to look at high-protein feeding frequently and we reported some significant metabolic changes that could be beneficial for metabolism and body composition. So, if you like to eat frequently, it might be a good idea to have some protein with each of those meals.

Lifters often look for ways to reduce DOMS, but most studies haven't seemed to be very fruitful thus far. And taking something like vitamin C post-workout may harm more than it helps. Do you have any thoughts on balancing "helpful" muscle damage with excessive damage, through supplements or other methods?

Well, we looked into this with a multi-ingredient performance supplement that we just published in JISSN. The supplement had some of the key ingredients like protein, creatine, etc. but we did not see any benefit to loading with the supplement prior to damaging downhill running in well-trained male runners. So, this approach did not help with DOMS or performance after the downhill run. What I've found is, again, back to the simple approach: eating a good combination of carbs, fats, and proteins, managing post-workout nutrition, and periodizing workouts so that excessive damage is mitigated. Supplements like fish oil, creatine, BCAAs, etc., have shown some benefits but they are not consistent as you pointed out. So, if these are part of your lifestyle they may help in a number of different areas but nothing beats smart training and smart eating (all the time) for balance helpful training and harmful training.

Related to post-exercise antioxidants: I haven't seen any evidence on consuming antioxidant rich foods post work-out, such as acai juice. Do you know if harms attributed to isolated antioxidants might apply to foods as well?
As far as I know, this has not been shown. However, my lab has not investigated this directly.

Florida State has a pretty decent athletic program, last I checked. Do you have any idea about the food and supplementation strategies they employ? If you had a say, what advice would you give them?
Yes, the 'Noles have had some excellent teams over the years for sure. Some of my Sports Nutrition graduate students also work with the Head Team Sports Nutritionist, Katy Meassick. They have some excellent programming to have athletes incorporate more food groups, work on hydration, and try to refuel and recover properly. Outside of that, and the sponsors that FSU has like Muscle Milk and Chocolate Milk, I know Katy is really doing great things with the teams. If I worked in that capacity, I'm sure I'd focus on many of the same things like coaching nutrition to the level of the athletes knowledge, recovery, and taking care of your body at that age (in general) is far more important than these athletes typically understand. Even with high-level athletes, often times the very basic things are still needed first.

When competing for grant money, where do potential studies on healthy athletic populations fall in terms of priority? On Internet weightlifting forums, I'd often see people bemoaning the lack of applicable studies to their population. But I'm not so sure micromanaging supplement strategies for people looking to get that elusive six/eight/twelve pack is as important as helping sick people, not that they're mutually exclusive goals. Thoughts?
It all depends on what sort of grants you are pursing. For Federal funding (i.e. NIH, NSF, USDA, DOD) the goal is certainly more about disease states than healthy fit people. Unfortunately, there are a lot of unhealthy people that need lots of attention from researchers. So getting grants from these organizations is pretty difficulty in general and even more difficult if trying to study sport or athletic performance

> "My take is that people try to make things too darn complicated. In my opinion, if you like to eat frequently, then do it. If you like to fast intermittently, then do it. I find that it is best to keep things as simple as possible."

of any sort. There are a few ways to work these populations into large funding but you would need to be extremely creative and lucky. Now, other funding avenues exist from corporate sponsors, private donors, and some major nutrition and exercise organizations. For these organizations, healthy and fit populations can be studied if you ask the right question and come up with a very good study design.

Luckily, my lab focuses on human performance. We define this as not just athletic/sport performance but improving the performance of everybody—it could be in their work, relationships, or general health. If you improve your body composition, health, or performance, you will generally be better off. Because our goals in human performance are targeted at both the clinical populations and at athletes, we have a number of avenues to pursue grant funding. What many people don't understand is that if a company financially supports research by a university, this does not corrupt the data. For example, many universities will not enter into any agreements with companies unless all rights belong to the university—meaning they can publish whatever they find, good or bad, positive or negative.

Without the grant dollars, research does not move forward, and what fun is that. Even private donations from anyone interested in this sort of research: Night feeding, metabolic flexibility, supplementation and strength training, endurance performance, etc can be extremely helpful. ◆

Michael Ormsbee, Ph.D., is a certified strength and conditioning specialist and a fellow of the International Society of Sports Nutrition. He is also regularly featured on TV and radio as the health, nutrition, and fitness expert. Dr. Ormsbee now competes in triathlon at the Sprint, Olympic, and 70.3 Ironman distances, is an avid lifter, and attempts to call himself an athlete.

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“ [...] if a company financially supports research by a university, this does not corrupt the data. [...] many universities will not enter into any agreements with companies unless all rights belong to the university ”

Ask the Researcher

Duane Mellor, Ph.D.

An experienced clinical dietitian, turned research and now lecturer, Dr. Mellor has a keen interest and passion for food, health and education. He has worked at the University of Hull and University of Chester initially as a research dietitian and then Chester as a Senior Lecturer. Currently he is a Lecturer in Dietetics at The University of Nottingham.

You've worked in diabetes research pretty extensively and helped develop the Diabetes UK guidelines. With so much vitriol over low carb versus medium or higher carb for people with type 2 diabetes, what's your stance on the importance of carbohydrate level?

I am not sure focusing on a single nutrient and pronouncing innocence or guilt is helpful. We probably need to consider whole dietary patterns to consider the best ways of eating. However, to provide a direct answer to the question, there is not one single ratio of macronutrients which is ideal for type 2 diabetes. Yes, low carbohydrate diets work, they seem to be great for some individuals but they are not for everyone and their effects are likely to via an energy restriction reducing insulin resistance, not via any ketone mediated mechanism. If I had to favor one it would be medium, the current evidence appears to suggest over 50% energy from carbohydrate may be too high for many (especially if they were overweight and not losing weight), so if I had to suggest around 35-40% of energy from carbohydrate appears to be best according to the evidence for both type 2 diabetes and weight management.

One specific area you've done research in is flavonoids. How can flavonoids help people with and without diabetes?

This is a complex question. The test tube answer would be as antioxidants, but over the past 10 years this has largely be confined to the test tube. These are amazing compounds found in plants, which have functions ranging from acting as plant sunscreen through discouraging animals from eating them and to the rainbow of colours we see in our fruit and vegetables. I don't think

beyond helping to preserve our foods that the antioxidant potential seen in the lab has a direct effect on our health. So, how can they help people with or without diabetes? There are many potential ways. They help blood vessels to relax by helping to induce endothelial production of nitric oxide, help improve the balance of lipoprotein to improve cholesterol ratios and may even improve insulin signalling. It is likely that the effects seen are due to an accumulative effect of lots of pathways being very modestly altered by flavonoids and their metabolites.

“There is interesting work on plant based diets emerging from interventions which may suggest a role, but it is more likely to be eat more vegetables and fruit rather than abandon meat altogether.”

I saw that you're vegetarian. Although I do eat meat, I grew up in a vegetarian family, and was surprised to see how much animosity there was toward vegetarians from some meat-eaters. What nutrition/ethical/etc. issues do you think about when it comes to animal products?

For me, it was initially it was not a health or ethical choice, it just happened. I don't have a problem with different dietary choices, the evidence for many points can be argued either way. I would say from a nutrition perspective there is some evidence that a vegetarian diet can be associated with lower levels of chronic disease, however as the level of education and social status of vegetarians can often be higher, it is more association and may be biased these and other factors. There is interesting work on plant based diets emerging from interventions which may suggest a role, but it is more likely to be eat more vegetables and fruit rather than abandon meat altogether.

From a sustainability perspective, especially when considering global food security, the arguments for vegetarianism become stronger. This ranges from land use, water use, and greenhouse gas production per calorie. However, we need to remember acceptability as part of food security, so again compromise is necessary. A final thought is cost, for me that is a continuing motivator, our household food bills are approximately half the UK national average, something that is often overlooked!

You teach nutrition at the university level. What are some challenges you face when informing both students and the general public about health and nutrition?

The sheer volume of information has to be the number one challenge. As it is so great it can be hard for students and especially the general public to be able to filter the material in terms of quality, relevance, and meaning. This leads to a range of interests coming into play, these may be financial, an unwillingness to move with emerging evidence or even a natural scientific desire to challenge the current world view. This then leads to debates, which are often confrontational, which to the general public could be seen as a lack of agreement or even personal attacks and bullying. The truth is that this is how scientists, including in nutrition which is a highly complex field, form consensus. So, transparency is

needed to allow objective debates to emerge.

I think we also need to be open and honest by saying nutrition is an area where there are many variables. The three main macronutrients for example: change the amount of one and it will have an effect on the amounts of the others. However each macronutrient in turn, is composed of a number of subtypes (many different fatty acids, sugars and amino acids) meaning that each macronutrient can form many thousands of different molecules. This is even before considering food, which is a mixture of these nutrients and other compounds in varying amounts. Hopefully framing the debate in terms of food would lead to better communication of risks and benefits with the general public. Then, finally, we need to think how we communicate health messages to the public, and whether the way research is framed might potentially impact current guidance, so not to cause confusion and potential harm.

You did your Ph.D. on chocolate. Did you have to eat a lot of chocolate for research purposes? What did you learn about chocolate that you didn't know before?

I would not say for research purposes exactly, though I had to sign a disclaimer that I biologically disposed of left-over product without selling it. So, you can guess what that meant! Chocolate is fascinating. In Europe we have strict laws and regulation governing what can be called chocolate, many consumers would say that could be why European chocolate tastes quite different to American products. One of the things I learned was that the flavonoid content of chocolate can be stable for up to two years, which was very useful when trying to recruit participants for a clinical trial. Also, the flavonoid content is not necessary related to the percentage of cocoa solids, as the cocoa solids are made up of two parts: the cocoa butter, which is low in flavonoids, and the fat free cocoa mass, which is often rich in them. Finally, the culture and history of chocolate and cocoa, from the use in sacrificial ritual and warfare by the Mayans to the interesting flavours of drinking chocolate in Italy.

I was happy to see that you've published on standing desks and blood sugar, a study that also showed an extra 174 kcal burn from standing. Do you ever stand while working at your computer? Is it something you'd recommend to people with diabetes? I know there are some risks if you go overboard, depending on conditions one might have.

I have built a standing desk from old box files which is working well and allows me to vary my working position, which is important to increase activity levels and also helps, I personally find, with respect to creativity. As for recommending for people with diabetes, as with other groups, health issues need be considered. Back pain is one problem

“I have built a standing desk from old box files which is working well and allows me to vary my working position, which is important to increase activity levels and also helps, I personally find, with respect to creativity.”

that is commonly cited, along with issues with hip and ankle joints, as many consider standing at a workstation to be stationary rather than constant small movements.

For people with diabetes, a lot would depend on whether they had any complications such as loss of sensation in their feet (neuropathy) and of cause if they were treated with insulin or sulphonylureas the extra activity may increase the risk of hypoglycaemia. So, as with many changes in lifestyle anybody with a long term health condition should probably consider chatting to their healthcare team first before investing in a standing desk.

Last question. The UK depends on evidence in a different way than the US does, with national health insurance requiring some pretty rigorous systematic reviews. In your opinion, what are some things the UK does right in this regard, and what are some things it can improve on?
I think on the whole the systems we have in the UK NICE – National Institute of Health and Clinical Excellence do a great job, often the lack of robust evidence means they have to rely on consensus and expert views which is not ideal. Additionally in Scotland, we have SIGN – Scottish Intercollegiate Guideline Network who do some fantastic reviews and clearly rate the quality of evidence available too. So, when you ask about the UK, it is probably best to say we have four separate healthcare systems all free at point of care in England, Scotland, Northern Ireland and Wales, all act slightly differently with slightly different priorities.

Also in England, public health is managed by local area councils who deliver services to a few hundred thousand people, the idea was to make this more responsive to local needs. I think in the UK the NHS is amazing, it could be improved by linking more closely with public health and other forms of care. The insurance system such as in the US and parts of Europe may be better at encouraging prevention as the aim is to reduce future costs, in the UK we probably have not placed the same level of value on this including nutritional education to prevent disease than might be optimal? ◆

Dr. Mellor has experience in designing and running clinical trials, both using CTiMPs and nutritional interventions (mainly chocolate). This has been further assisted by his involvement in the ethical approval of research, both as a member of an NHS research ethics committee and chair of the departmental ethics committee at Chester.

His research and clinical interests have been linked to diabetes and weight management, with over 10 peer reviewed papers and in excess of 30 conference proceedings since 2008. Dr. Mellor still practices as a dietitian, over the past couple of years this has been as a dietitian volunteering on Diabetes UK Children's and Young Persons holidays. The focus of his clinical work included group education, having previously been a DAFNE educator and a special interest in diabetes in pregnancy and the effects of obesity on pregnancy.

ERD

Until Next Issue...

The next issue of ERD will come out the first week of May. In the meantime:

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