TOWNSEND LETTER

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Cocoa and Heart Disease: A New Theory

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We've entered a new chapter in the understanding of chocolate's health benefits, a chapter in which the plot takes a surprising turn.

In the future we may look back on a small study by García-Cordero et al published in May 2023 as marking a turning point in how chocolate affects health and by extension how other foods provide health benefit.[1] The impact of foods may be less about how they affect our cells but rather what effects they have on our microbiome, the cells that live inside of us but that aren't us.

Before we talk about this new study, let's review some of the earlier chapters in the chocolate story and how chocolate was linked to blood pressure and heart disease.

The Story Up to Now

The opening chapters to this chocolate story are now well known and start out with an article by Benjamin Kean published in 1944 that described the Kuna, an indigenous tribe who live off the Caribbean coast of Panama. These people display a curious trait; their blood pressures do not increase with age, a characteristic that for years was assumed to be genetic. In the 1990s Harvard sent Norman Hollenberg and fellow researchers to Panama to study the Kuna, analyze their DNA and identify the responsible genes. These researchers quickly realized that the Kuna's stable blood pressures were not the result of lucky genetics; if individual Kuna moved away from their home islands to an urban area, their blood pressures quickly increased to an age predicted norm.

Meticulous evaluation of their lifestyle and diet revealed that the Kuna living on their home turf routinely consumed large amounts of cocoa as a beverage.[2] A theory was developed that the nearly 2 grams per day of flavanols and polyphenols the Kuna normally consumed from these cold cocoa-like drinks were what provided protection against hypertension.[3] This theory was adopted and assumed true until García-Cordero's new study was published. Now we have good reason to wonder.

During the past decade, the theory that the cardiovascular benefits of cocoa and chocolate were due to cocoa's polyphenol and flavanol content has been taken for granted and subsequent studies focused on what the daily dose of these chemicals should be. (See Sidebar below.) Yet the research has been. frustratingly inconsistent at revealing an answer. Perhaps an alternative explanation was required.

The New Chapter

Let's describe this García-Cordero study. It was a randomized, single-blind, parallel-group study, lasting 12 weeks. Sixty adults aged 45-85 years-old were recruited in Spain to take part. (One participant dropped out so n=59.) Study participants were divided into three groups: Group 1 consumed 5 grams per day of a powdered mixture of "red berries" that contained dried currants, raspberries, and blueberries; group 2 consumed 2.5 g/day of a polyphenol-rich cocoa powder, and group 3 consumed both the cocoa and red berry powders. All these products are made and sold by Salengei®, a Spanish nutraceutical manufacturer, which, before you ask, does not ship to the US.

The researchers tracked cardiovascular biomarkers, including homocysteine, angiotensin-converting enzyme (ACE), nitric oxide (NO), flow-mediated vasodilation (FMD), blood pressure and lipid profile. Additionally, specific metabolites, including secondary bile acids, short-chain fatty acids, and trimethylamine N-oxide (TMAO) were also measured to assess any effect these dietary additions had on the gut microbiome of test subjects.

The participants that consumed cocoa showed significant reductions in TMAO ($p = 0.03$) and uric acid ($p = 0.01$), accompanied by an increase in FMD values ($p = 0.03$) and total serum polyphenols ($p = 0.03$) after the intervention. These values negatively correlated with the TMAO concentration ($R = -0.57$, $p = 0.02$). Additionally, an increase in carbohydrate fermentation was observed in those groups that consumed cocoa ($p = 0.04$) or red berries ($p = 0.04$). These increases were correlated with lower levels of TC/HDL ratio ($p = 0.01$), systolic ($p = 0.01$) and diastolic blood pressure ($p = 0.01$). These results suggest that a modulation of the microbiome from cocoa or berries intake led to improvements in cardiovascular function.

That adding the berry powder didn't increase all the benefits assessed comes as a surprise. If this were a multiple-choice quiz, we would have guessed that it would. In fact, given our general predisposition to believe in synergy of all good things nutritional, we would have assumed that both berry powder and cocoa powder would have shown benefit and the combination would have had a greater impact than predicted by adding effects of each supplement alone, that the combination have had a synergistic effect. But it didn't. The cocoa helped, the berries not as much.

We should reiterate what wasn't seen in the results: the authors write, "…we found no significant differences between groups at the end of the intervention in the concentrations of total serum protein, NO, homocysteine, TMAO and ACE activity corrected by total protein … Between visits, we only observed a statistically significant increase for FMD values, in the cocoa powder group ($p = 0.03$) and a decrease in TMAO levels after the 12-week intervention in the C[ocoa] group ($p = 0.03$). We did not find any other significant change in any of the analyzed parameters between baseline and 12-weeks for any of the diets…." The berries on their own had no significant benefit and their addition to the cocoa appears to have even negated cocoa's effect on FMD and TMAO.

The Newcomer: TMAO

That trimethylamine N-oxide, abbreviated as TMAO, decreased in the cocoa group is the important finding here. TMAO is a small organic compound in the class of amine oxides. It is usually found in marine organisms in particular things that live deep in the ocean at great pressures. It provides numerous benefits to such deep-sea creatures. But in people living on dry ground, TMAO appears to be a problem. "In humans, a positive correlation between elevated plasma levels of TMAO and an increased risk for major adverse cardiovascular events and death is reported. The atherogenic effect of TMAO is attributed to alterations in cholesterol and bile acid metabolism, activation of inflammatory pathways and promotion of foam cell formation. TMAO levels increase with decreasing levels of kidney function and is associated with mortality in patients with chronic kidney disease."[4]

In a 2020 review and meta-analysis of earlier studies on TMAO and CVD, Guasti et al reported that elevated TMAO, "was associated with both major adverse cardiovascular events (RR = 2.05; 95% CI 1.61-2.61) and all-cause mortality (RR = 3.42; 95% CI 2.27-5.15)."[5] Having high TMAO doubles risk of something bad happening to a person's heart and more than triples their risk of dying.

TMAO is formed from trimethylamine (TMA), which is generated by gut bacteria from dietary choline and phosphatidylcholine (lecithin).[6] Another pathway, by which TMAO can be formed is through catabolism of L-carnitine and this may be why red meat, which is high in L-carnitine, raises risk of heart disease. Much about TMAO remains confusing. For example, seafood, which contains substantial amounts of TMAO, still seems to be heart healthy. Lecithin, which is a key component of TMAO, also seems beneficial.

It's not clear why too much TMAO is so bad for humans, but it is clear that high levels are associated with progression of chronic kidney disease, atherosclerosis, heart disease, metabolic syndrome and type 2 diabetes. For a while avoidance of the TMAO's precursor chemicals was encouraged but this proved useless at lowering serum levels. Over the last few years, it has become well established that the disease-causing high levels of TMAO are not due to dietary intake but are due to gut dysbiosis and that the approach to lowering TMAO is through altering the gut microbiome rather than dietary restrictions.[7]

The García-Cordero study stands out as it is the first human clinical trial to show that cocoa consumption will lower TMAO levels. The data from their study clearly suggests that cocoa

does this by altering the fermentation by microbiota in the gut, that cocoa changes the gut microbiome in a way to lower TMAO in the serum. This is a big thing.

This information gives us a new way to understand why chocolate is good for our hearts. It is exciting because using changes in serum TMAO will provide a way to quantify the effectiveness of different chocolates.

The inverse association between cocoa and TMAO is one example of a food that provides benefit through its effect on the gut microbiome. We should expect more examples of such relationships in the coming years. As I wrote earlier, we're entering a new chapter.

Certainly, for those readers only interested in the bottom line of what to tell patients, these results still suggest we encourage adults to consume some cocoa daily. For those who want to understand why foods do what they do when it comes to health, this study raises questions. Dietary polyphenols, flavanols and anthocyanins are still considered the food constituents that play the important roles in good health. We talk about them today the way we once talked about antioxidant vitamins and minerals (vitamins A,C,E, zinc and selenium and the acronym ACES); we assumed that these chemicals were the primary protectors of a healthy body.

Dietary polyphenols are still believed to lessen the effects of ageing, in particular preventing decline of cognitive function[8,9] and the development of CVD.[10-12] A nearly identical study to this chocolate and berry trial was published in 2021, by the same group; the only difference was that instead of CVD markers, the participants were assessed for neurocognitive function. While both the cocoa and red berry groups showed some improvement, the combination of interventions was most effective.[13]

A 2016 meta-analysis conducted by Lin et al. reported that cocoa flavanol intake significantly improved various cardiovascular biomarkers, such as fasting insulin, insulin resistance, triglycerides, HDL-C, c-reactive protein and VCAM-1.[14] A separate meta-analysis by Arab et al. reported that tea consumption (three cups versus lees than one cup per day) reduced the risk of suffering a heart attack by 21%.[15] We have regarded these chemicals as the "new antioxidants," protecting the integrity of cells against oxidative damage. It now looks like there is another, additional, explanation for benefit, one we had not considered previously.

In the last few years our world view has started to shift, and we are emerging into a world where we recognize that multiple body systems are deeply influenced by the body's microbiome. No longer is the question what a food does to the body's cells but also what it does to influence the gut microbiome. We now understand that cocoa shifts the gut microbiome in positive ways.[16,17] It is not necessarily the flavanols that are responsible. Even chocolate's positive effect on mood and emotions is now attributed to shifts in bacterial populations.[18] The results of García-Cordero et al.'s CVD clinical trial serve to further support this line of thinking.[19]

García-Cordero's findings on TMAO stand out as a good example to focus our attention on the gut microbiome. TMAO is formed by gut bacteria from choline-rich foods and TMAO blood levels are strongly linked with CVD risk in large scale clinical studies.[20] In years past, we tried

to lower dietary choline intake to lower TMAO in order to reduce CVD risk. Instead, our focus is now, (or should be) on whether TMAO producing gut bacteria are present or not. Thus, García-Cordero's findings are notable as their report is the first-time cocoa has been demonstrated to lower TMAO levels in humans. They provide evidence for this theory why chocolate reduces heart disease: cocoa's effect on gut bacteria reducing TMAO production brings benefit against CVD. This may be the most important finding of their study.

Cocoa Is a Fermented Food

What the García-Cordero et al. don't mention, yet what seems to be a possible explanation may be the simplest. Cocoa is a fermented food product. A recent analysis of the microorganisms involved in cocoa bean fermentation suggests a complex ecosystem of at least 99 separate organisms that vary by geographical growing region.[21] Kefir, which is produced by the orchestrated fermentation of bacteria and yeast working in a sequential concert, may only require a few dozen different species.[22] Could it be that one or more of those nearly one hundred different microscopic organisms that take part in this cocoa's fermentation create a metabolite that persists into the final processed cocoa powder that alters the consumer's gut biome?

Obviously, bacteria or yeast from the fermentation will not persist through processing, but a chemical they produce might. It could be as simple as an antibiotic substance that limits the growth of TMAO-producing bacteria. It could be a growth-promoting substance that encourages competitors to outgrow the TMAO producers. Or perhaps the exuberant bacterial or fungal growth during cocoa bean fermentation stimulates the beans, even after harvesting, to produce protective substances in response. Recall how grapes make resveratrol as protection against yeast[23] (and how this resveratrol production can be triggered by ultraviolet light). Perhaps even the chemicals we have thought as important players in recent years increase or decrease during fermentation? I can add no citations in support of this idea as I have not come across any publication expressing a similar theory.

We may ask if other fermented foods have a similar benefit through a similar mechanism to chocolate? "Epidemiological studies have shown that the consumption of fermented foods is associated with reduced risks of type 2 diabetes, metabolic syndrome, and heart disease, along with improved weight management."[24] This list is rather reminiscent of the list of diseases associated with elevated TMAO.

Probiotics and Fermented Dairy

Some have suggested that taking probiotics might be helpful in lowering TMAO but the data are confusing. One clinical trial suggests caution. A 2015 paper by Boutagy et al reports treating 19 young men with high dose probiotics (VSL#3) for two weeks and then comparing resultant TMAO levels against those who received placebo. The probiotic did not lower TMAO production. In fact, the VSL#3 was associated with a nearly 90% increase.[25]

A 2018 report does describe a specific lactobacilli strain that lowers TMAO production in mice (*Lactobacillus plantarum ZDY04*).[26] This is a situation where we prefer human data for

confirmation; the overlap between human and mice gut biomes is small, only about 4%, so extrapolation from mice to humans may be unreliable.

Fermented milk products in contrast to VSL#3, may be helpful. In 2020, Burton et al compared the impact of regular milk versus fermented milk products on postprandial TMAO levels and reported that although TMAO increased, it rose less so when fermented milks were consumed.[27] Soluble dietary fiber, in particular fermented fiber, has been reported to lessen production of TMAO in the gut.[28] While there is widespread agreement that lowering TMAO production is desirable, there has been little data suggesting how best this might be done.

A 2020 meta-analysis by Companys et al., examined the influence of fermented dairy products on CVD risk and calculated that "… fermented milk was associated with a 4% reduction in risk of stroke, ischemic heart disease, and cardiovascular mortality [RR (95% CI); 0.96 (0.94, 0.98)]; yogurt intake was associated with a risk reduction of 27% [RR (95% CI); 0.73 (0.70, 0.76)] for type 2 diabetes (T2D) and 20% [RR (95% CI); 0.80 (0.74, 0.87)] for metabolic syndrome development."[29] Consumption of fermented milk products, in contrast to acidified milk, suggest fermented milks are associated with transitory postprandial decreases in TMAO.[30] These examples in which direct consumption of probiotic bacteria don't seem to help but consumption of foods that these bacteria have fermented prompt this writer to wonder whether it is a metabolite left by these bacteria that is the active agent in providing benefit?

Coffee

Coffee is another common food in which the initial fruit undergoes fermentation prior to roasting and consumption. A 2014 meta-analysis on long-term consumption and risk of CVD, reports a 11% decrease in risk in those who consume an average of 1.5 cups of coffee per day.[31] A 2020 in vitro study involving fecal slurries compared the effects of digestion on cocoa, tea and coffee chemistry with TMAO production.

"*Results showed that digestion was required to unlock the …. potential of coffee and cocoa beverages, and that teas did not possess a strong inhibition potential either digested or undigested. By fractionating digested bioactive beverages, we determined that those fractions rich in chlorogenic acid were the most bioactive. Overall, this study suggests that regular cocoa and coffee consumption could be a nutritional strategy able to reduce TMAO levels*."[32]

Wine

When it comes to wine, the posterchild of fermented beverages, we've seen a progression of theories to explain it's possible benefit against CVD. Initially it was the ethanol content, then the polyphenol/resveratrol content that were given credit.[33] At this point, we can't say whether wine drinking impacts TMAO levels (or for tht matter good for one's health). A three week-long trial of adding a glass of wine a day to the diets of 42 men did not alter their TMAO levels even as it did shift the gut microbiomes significantly.[34]

Some of the key bacteria responsible for chocolate fermentation may be proven to have specific value in the future. At this point, we see little hint in the literature that they are being

investigated to prevent CVD. We should remember though how the major chocolate companies appear to perform unpublished proprietary research. For example, both Mars and Callebaut market proprietary chocolate brands, which they claim are manufactured in special ways to enhance heart health. Neither company provides details on why or how they have altered their processes to make these products healthier.

To Be Continued

It's a new world we are entering, not just with chocolate but a wide range of other foods. Where once vitamins and minerals were our focus of concern, and then antioxidants, now we are seeing the world in a new way, through a lens of and how the gut microbiome might be altered to improve health.

SIDEBAR: Cocoa Polyphenols Clinical Study

A recent large scale clinical trial using cocoa polyphenols should be mentioned in this discussion somewhere. Howard Sesso et al conducted a large randomized double blind, placebo-controlled trial of cocoa extract supplementation for prevention of CVD and cancer that involved 21,442 older US adults (12,66 women) between 2015 and 2020. Participants were randomly assigned to cocoa extracts [500 mg flavanols/d, including 80 mg (-)-epicatechin] or placebo.

During a median follow-up of 3.6 y, 410 participants taking cocoa extract and 456 taking placebo had confirmed total cardiovascular events (HR: 0.90; 95% CI: 0.78, 1.02; P = 0.11). For secondary endpoints, HRs were 0.73 (95% CI: 0.54, 0.98) for CVD death, 0.87 (95% CI: 0.66, 1.16) fand or MI, 0.91 (95% CI: 0.70, 1.17).

Although the cocoa extract did not achieve statistical significance in reducing total cardiovascular events among these older adults, it did reduce CVD death by 27%. That should still encourage most adults to consume a bit more cocoa. **[35]**

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