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David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014 Jan 23;505(7484):559-63.

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

The microbiome is a headspinning topic with associations seemingly related to all manner of chronic health conditions. It is thought that the microbiota is relatively stable after the first 3yrs of life, and that the composition of the microbiota is most influenced within those first 3yrs from factors like birth delivery method, infant feeding practices, and then early whole-foods diet [1,2].

It is also known that the microbiota of populations consuming diets rich in indigestible carbohydrates display greater microbial diversity than Western diets, which corresponds to the diversity of complex carbohydrate structures, including resistant starch, prebiotic fibres and other non-starch polysaccharides, which are associated with host health [2,3].

Conversly, the evidence also points to negative impacts on the microbiota and microbiome from 'Western' diets high in animal fat, protein, and sugar, and low in fibre [4,5]. This dietary pattern is consitently associated with increased pro-inflammatory bacteria, secondary bile acids [which are carcinogenic in the colon], and increased levels of pathogenic bacteria which tolerate bile acids [4,5].

We currently live in a very strange time for diets in the general population, defined by increasing severity of exclusion. Nowhere is reality further detached than in the form of 'Carnivore' diets. I chose today' s paper because it is the closest study to answering, in intricate detail, the likely effect on the gut microbiome of consuming a diet that has zero fibre, zero plant foods, and is predominantly based on animal meats and other foods.

The Study

10 participants [6 male, 4 female] with an average age of 28yrs [BMI of 22.7] underwent two distinct dietary interventions:

- Animal-based diet [ABD]: Consisted of only meats, cheese, and eggs [predominantly meats]. Snacks provided were cured meats, pork rinds, and string cheese. Macronutrient composition on this diet was ~70% fat, 0.6% carbohydrate [0g fibre per 1,000kcal], and ~30% protein.
- Plant-based diet [PBD]: Consisted of cereals for breakfast, and lunch and dinner consisted of rice, butternut squash, lentils, and spices. Snacks provided were fruits and banana chips. Macronutrient composition on this diet was ~22% fat, 69% carbohydrate [25g fibre per 1,000kcal], and ~10% protein.

Participants consumed their habitual diets for 4-days leading into both dietary interventions. The ABD and PBD were both consumed ad *libitum* [i.e., with no restrictions on energy intake] for 5-days. Participants were then observed for a further 6-days by the investigators to assess whether the microbiota recovered.

Both diet arms were separated by 1-month. The study utilised a range of analytical techniques to study the composition of bacteria, genes associated with bacteria, bile acids, short-chain fatty acids [SCFA], and RNA-sequencing.

*Geek Box: Microbiome Terminology

Many terms of often used, sometimes interchangeable, when reading about this topic. So it can be helpful to define some terms.

- *"Microbiome" is the term for the 'extended genome' provided by the bacteria in the human gut, i.e. what genes are expressed and functions they exert.*
- *"Microbiota" is the term for the different bacteria in the gut, i.e. what bacteria are present, and in what proportions.*
- *"Bacteria": single-cell organisms that are highly adaptable.*
- *"Dysbiosis": the term for disturbances in the composition of the microbiota, influencing disease states.*

Related to the term microbiota, the term "microbial", i.e., 'microbial diversity', refers to bacterial populations. And sometimes this is just plainly stated as "gut bacteria"!

In general, the most important distinction to bear in mind is between microbiome [i.e., the functions of the genome in the gut] and microbiota [i.e., the composition of bacteria in the gut].

Results: Of the subjects who completed the intervention, 10 were omnivorous and 1 was a lifelong vegetarian. Overall, the ABP had a greater impact on the gut microbiota than the PBD. 22 clusters of bacterial species changed significantly on the ABD, compared to 3 which changed signficantly on the PBD.

- ***Bacterial Diversity*:** The ABD resulted in a significant increase in bacterial diversity, effects which did not occur to the same extent on the PBD. The shift in bacterial diversity was evident after 1-day on the diet.

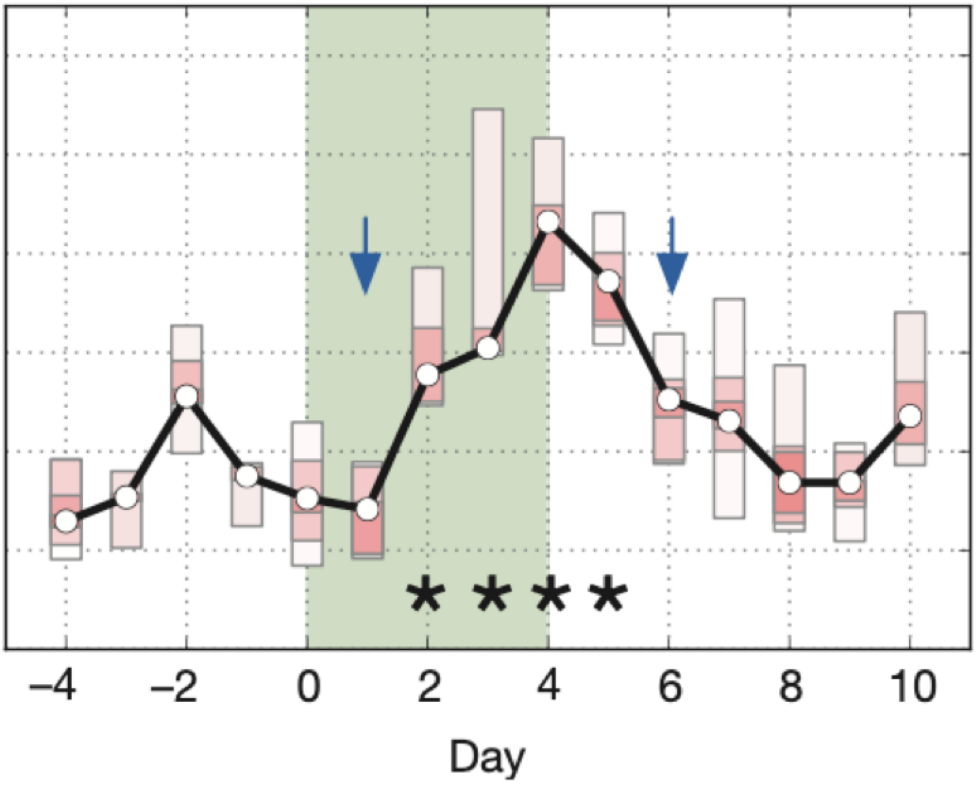

***Figure** rom the paper illustrating [shaded in green] the 5-days on the animal-based diet. By Day 2, the significant increase in diversity was observed, and sustained over the course of the diet. However, as you can see by Day 6, i.e., around two days after the dietary intervention, this had largely returned to baseline. Now, generally you would probably think that "diversity = good" for the microbiota. Or you could be thinking that Diversity was an old, old wooden ship that was used during the Civil War era. But in the case of the microbiota, whether diversity is "good" depends on what bacteria have been diversified! When "diversity = good", it generally means increased abundance of fibre-degrading bacterial species.*

- ***Composition of Bacterial Diversity*:** The composition of the bacteria which incresed on the ABD to the most abundant levels included *B. wadsworthia, A. putredinis,* and *Bacteroides*. These are all bile-resistant bacteria, reflecting the high dietary fat intake on the ABD resulting in increased bile acid secretion during digestion.
- ***SCFA and Bile Acids*:** SCFA levels were significantly lower during the ABD compared to the PBD. The ABD displayed signficiantly higher levels of the byproducts of protein fermentation, and lower byproducts of carbohydrate fermentation. During the ABD, bile acids increased significantly, including specific secondary bile acids associated with intestinal inflammation and liver cancer.

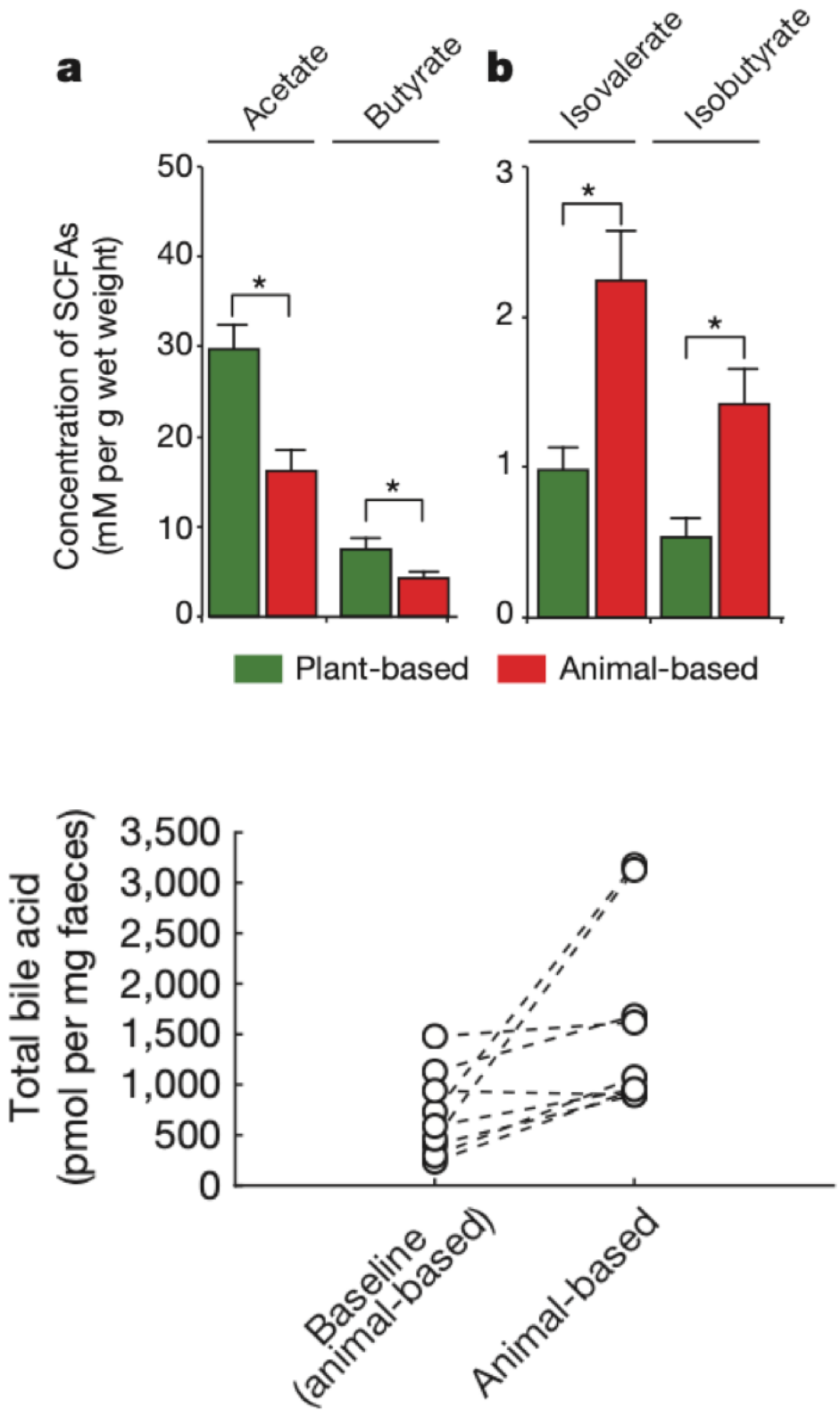

Figure *from the paper illustrating [**top**] the change in the SCFA which are byproducts of carbohydrate fermentation [**left graph**] and those which are byproducts of protein fermentation [**right graph**]. The graph on **bottom** indicates the increased concentration of bile acids from the animal-based diet.*

The Critical Breakdown

Pros: The study tested a novel intervention, and it is rare to see such extreme contrasts in diet in a study. Participants were young and otherwise healthy, thus the effects observed cannot be attributed to reverse causality given that we know that obesity and metabolic disease are associated with shifts in the microbiota. The analytical methods employed were all highly sophisticated and provided a wealth of insight into the changes observed, from bacterial composition to RNA.

Cons: The study was very small, and very short-term [although to be fair, that was the point]. For obvious reasons, neither investigators nor participants were blind to the treatment, which is typical in short experiments of this nature in nutrition research. Although the analysis clearly separated the single vegetarian participant, the diet of the vegetarian and the omnivores during the PBD phase were significantly different. For example, the vegetarian consumed 90g fibre per day, while the omnivores achieved 40g per day. The lower level achieved by omnivores could explain why there was less variation observed in the shift to a PBD, compared to the more extreme shift in the ABD. Greater control over the achieved targets, particularly for total energy intake and fibre, could have strengthened the study.

Key Characteristic

Sapiens. As in, the study was conducted in them. This study provided evidence of effects in humans which previously had been confined to animal models. In particular, the study found significant increases in levels of *B.wadsworthia* during the ABD. Previous research in mice has implicated the potential role for *B.wadsorthia* in inflammatory intestinal conditions, as feeding mice a high saturated fat diet increased expression of this pro-inflammatory bacteria [(6)]. The present study thus added to this evidence by demonstrating the significant increase in abundance of *B.wadsorthia* in humans, evident in as little as two days after shifting to an exclusively ABD rich in saturated fat.

Interesting Finding

The study group included a single male participant who happened to be a lifelong vegetarian. This provided some scope for interesting comparisons with this individuals' microbiota, and the response to the dietary interventions, which sheds some light on the difference between short-term abrupt dietary change vs. sustained long-term dietary practices.

At baseline, this participant had an average fibre intake of ~70g per day. The bacterial composition of this participant at baseline was dominated by the *Prevotella* genus of bacteria, which is associated with fibre-degradation and is observed in populations consuming lower fat, lower animal protein and high carbohydrate and fibre diets [3-5]. This participant also lacked any appreciable levels of *Bacteroides*, which you' ll recall increased significantly during the ABD. Nevertheless, by Day 4 on the ABD, this participants previous composition was flipped on its head; *Prevotella* decreased significantly, and *Bacteroides* increased significantly.

Although this is only one participant in a small study, it is instructive of certain characteristics of the microbiota that have emerged in the research:

1. That the composition of an individuals gut bacteria is primarily stable over adulthood and reflects long-term dietary intake, and;
2. That the composition of gut bacteria can shift rapidly in response to changing diet, reflecting a short-term adaptive capacity of the microbiota.

The fact that the microbiota of participants had returned to their pre-intervention structure reflects the stability of the longer term influence over short-term changes.

Relevance

When this paper was published, I don't think the "Carnivore Diet" had even been concieved. To have some fun in setting the context for this Deepdive, I did a quick Google search of the following terms: "carnivore diet gut health". I found this, from 'The Carnivore Diet Coach':

"Those on the carnivore diet, like predatory animals, by contrast, don't require the same range of gut bacteria to break down food residues. They no longer need all the specialists to attack starches and indigestible sugars in plant matter that regular people eat. Specialist bacteria that breakdown plant cells might have positive effects, but they could also be entirely negative. We just don't know at this point. What we do know, however, is that many historical populations lived on something like the carnivore diet in the eons before the neolithic revolution."

Please also note the comparison between carnivore dieters and predators, highlighting the crisis of masculinity which appears to go hand-in-hand with this diet. Gentlemen, your rulers please.

Bacteria are specialised in the fermentation of different dietary substrates, therefore food composition provides the raw materials for the selective growth of specific species. From the perspective of the ABD in the present study, this is not good. The ABD was associated with increased levels of pro-inflammatory bacteria, increased secondary bile acids, increased pathogenic bile-tolerant bacteria, all of which are potentially carcinogenic and implicated in Inflammatory Bowel Disease [7]. In a previous Deepdive, we looked at a diet-swap study in which African-American subjects consuming a Western diet showed increased conversion of bile acids into pro-carcinogenic secondary bile acid metabolites, while age-matched native Africans consuming a high-fibre diet showed an abundance of SCFA-producing bacteria and high butyrate levels [5]. Switching African-American and native African subject's diets resulted in a reversal of these effects [5]. Cumulatively, the evidence demonstrates a strong association between diet and colon cancer [4,5].

Is there potential harm to the PBD? In the study, there was evidence that a plant pathogen found in spinach, which was a key component of the PBD, was detectable in the gut. However, this is likely to reflect the specific food, and does not appear to be any reflection of fibre-degrading bacteria. Importantly, whether it is the increase in SCFA production, the protective effects against pathogenic adherence to the intestinal lining, the lower [or non-existent] levels of inflammation, carcinogenesis, or tumorigenesis, it is difficult to argue that the bacterial species which specialise in degrading fibrous carbohydrates may have harmful effects [1-8]. It's not a case of "we just don't know"; we have evidence.

Bear in mind that after the ABD, the composition of the participant's gut bacteria returned to its baseline structure in only 2-days. Hope for the Carnivores yet, but jesting aside, the point remains that long-term compositional shifts in the microbiota may only be sustained as long as the particular diet is sustained. This is not particularly good news for Carnivore dieters who may continue on this diet for years, because the constellation of changes observed in the present study say "inflammatory bowel disease" and "colorectal cancer" [4-8]. This is also not a case of "we just don't know"; we have the evidence.

And what about the pathetic ancestral justifications? If only our 'Carnivore Diet Coach' had applied his "we just don't know" to evolutionary speculation, and sought out evidence; ancestral diets may have had up to ~100g/d dietary fibre in the Paleolithic period [9].

Application to Practice

Studies like this are often difficult to take much direct application from, given their small size and very experimental design. Nevertheless, where part of the study becomes congruent with wider evidence, we can place into that context.

Diversity of bacteria in the gut is a good thing, when the gut bugs are themselves 'good'. A recent study found that eating 30 plant foods per week on average was associated with the greatest bacterial diversity [10]. I personally don't like emphasising studies like these because their application can seem privileged and far removed from the realities facing achieving that type of dietary pattern for the average person. But simply encouraging as wide a variety of plant-sourced foods, fibrous vegetables, fruits, and legumes in particular, is as broad a conclusion as we can take from the current research on gut health.

There is practically nothing which would justify a zero fibre, all animal meat and produce diet, for gut health. Or any other health outcome. But you know this.

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