CARDIOVASCULAR DISEASE

The diet–microbe morbid union

A common dietary component that some people even take as a supplement is converted by the gut microbiota to harmful metabolites linked to heart disease. This finding has cautionary implications. SEE ARTICLE P.57

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Everyone knows that a 'bad diet' can lead to heart disease. But which dietary components are the most harmful? Some lay the blame on saturated fatty acids, others point a finger at excess carbohydrates, which also lead to obesity and insulin resistance. On page 57 of this issue, Wang *et al.*[1] outline a remarkable chain of events that links diet, intestinal bacteria and liver metabolism to the generation of a chemical that promotes the build-up of arterial plaque and cardiovascular disease.

Intestinal bacteria currently hold centre stage for their role in maintaining digestive health[2]. Although the main focus has been on detailed molecular characterization of the gut microbiome[3], there is increasing interest in the impact of these seemingly innocuous gut microorganisms on metabolic disease in humans. Indeed, recent evidence[4,5] has implicated gut microbiota in insulin resistance and non-alcoholic fatty-liver disease.

A burgeoning area of research is metabolomics — an unbiased approach to identifying and measuring the small-molecule metabolites in a system — and determining the relationship of the metabolome to disease. Nonetheless, the scope of the blood metabolome that arises from the gut microbiome has not been fully defined; this knowledge could lead to insights connecting diet, the gut microbiota and disease.

Wang *et al.*[1] tell a compelling story — which starts with a metabolomics approach — of their search for circulating small molecules associated with coronary heart disease. They screened blood from patients who had experienced a heart attack or stroke and compared the results with those from blood of people who had not.

The authors found major differences in choline, betaine and trimethylamine *N*-oxide (TMAO) — three metabolites of the ubiquitous dietary lipid phosphatidylcholine (also called lecithin). Choline is an essential nutrient[6], and lack of dietary choline can lead to non-alcoholic fatty-liver disease and muscle damage. This knowledge has sparked the use of choline as a dietary supplement to prevent liver damage and to increase muscle performance[7]. Furthermore, lecithin is marketed as a dietary supplement to reduce the risk of heart disease, despite the absence of data to support this claim.

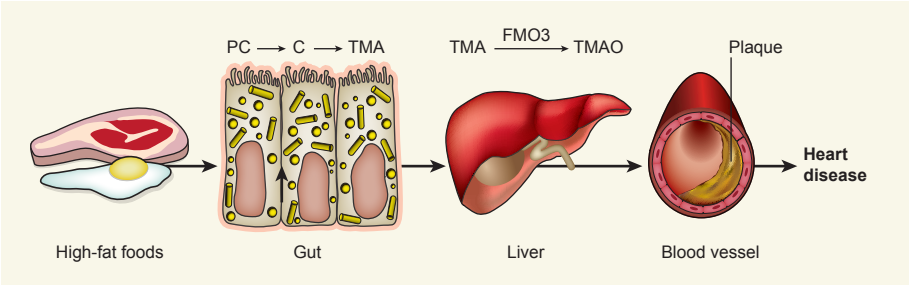

Figure 1 | From diet to disease. High-fat foods are rich in the lipid phosphatidylcholine (PC) and its metabolite choline (C). Intestinal bacteria convert C to TMA. In the liver, the enzyme FMO3 processes TMA to TMAO — a metabolite that makes its way into the blood. Wang *et al.*[1] show that circulating TMAO may contribute to greater plaque development in the arteries, and so to heart disease.

After choline is released from phosphatidylcholine by phospholipase enzymes, gut microbiota metabolize much of it into trimethylamine (TMA) — a gas that smells like rotten fish. When TMA reaches the liver, oxidizing flavin monooxygenase enzymes convert it into TMAO (Fig. 1). Wang and colleagues gave mice isotopically labelled phosphatidylcholine orally and later found the label in TMAO in the animals' plasma, confirming the metabolic link between dietary intake of phosphatidylcholine and the production of TMAO. They also report that, in mice prone to atherosclerosis, increased dietary choline leads not only to increased plasma levels of TMAO, but also to greater plaque development in the animals' arteries.

To demonstrate the role of gut microbiota in this process, the researchers treated mice with broad-spectrum antibiotics, effectively abolishing the animals' intestinal flora. In this setting, phosphatidylcholine administration did not result in TMAO in the blood and, more strikingly, a high-choline diet did not increase the severity of atherosclerosis. A lingering question is whether increased TMAO production contributes to cardiovascular disease or is simply a marker of disease risk.

This paper[1] raises the possibility of several new approaches to prevent or treat atherosclerosis. The most obvious is to limit dietary choline intake. Although phosphatidylcholine is found in a wide range of foods, it tends to be particularly high in foods with greater fat content[6]. Indeed, people with trimethyluria, who cannot convert TMA to TMAO, are prescribed a low-fat, low-choline diet to reduce TMA production[8]. What's more, our diet comparisons show that a very low carbohydrate (Atkins) diet contains roughly 2.5 times more choline than a typical very low fat (Ornish) diet. It is thus tempting to speculate that a very low fat diet may reduce the risk of heart disease in part because of its low choline content (Fig. 1). These results also call into question the safety of using choline and lecithin as dietary supplements.

Another approach is to reduce the load of gut bacteria that generate TMA from dietary choline. Intriguingly, low-dose antibiotics have been used[8] to reduce TMA production in people with trimethyluria. It is of note that antibiotic trials in humans[9] set up to test the hypothesis that certain microorganisms, such as chlamydia, may directly infect the arterial wall have not shown cardiovascular benefit. If bacterial species responsible for metabolizing choline to TMA are identified, their selective elimination would be ideal because it would be therapeutically sufficient, and less disruptive to the intestinal microbiota than the broad-spectrum antibiotics Wang *et al.* used in mice.

A third approach is to use probiotics — live microorganisms that both inhibit and promote various species in the gut microbiome. In a mouse model carrying a 'humanized' microbiome[10], administration of a certain probiotic reduced TMAO production, whereas another probiotic increased it. Clinical studies

of the effect of probiotics on plasma TMAO levels and on cardiovascular disease in humans would be of interest.

Because TMAO is produced in the liver by the action of the flavin monooxygenase FMO3, inhibition of this enzyme in the liver might be another strategy by which to reduce TMAO production and cut the risk of heart disease. Although complete absence of FMO3 — for instance, in the disease trimethylaminuria — is undesirable, its reduced activity might be beneficial. Whether variations in the gene encoding FMO3 that reduce its activity are associated with reduced plasma TMAO levels and, more importantly, with reduced incidence of cardiovascular disease, should be tested.

Although Wang and colleagues' work[1] suggests that excess dietary choline might lead to cardiovascular disease, choline is an essential nutrient for several cellular metabolic pathways. So any attempt to reduce the levels of choline or its metabolites for therapeutic purposes requires caution. Nonetheless, this study has added phosphatidylcholine and other sources of dietary choline — such as the widely used food supplements — to the list of dietary culprits with the potential to increase the risk of heart disease. What's more, it implicates the gut microbiome in promoting heart disease in the setting of a high-choline diet. The implications for prevention of cardiovascular disease are tangible, and the subsequent chapters in this story will make fascinating reading. ■

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ELECTRONICS

Industry-compatible graphene transistors

An innovative technique has been developed to manufacture graphene transistors that operate at radio frequencies and low temperatures. The process brings the devices closer to applications. SEE LETTER P.74

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To an increasing extent, modern society relies on advances in wireless communications. The backbone of wireless systems is radiofrequency (RF) transistors that are able to amplify signals and provide electronic gain at high frequencies. Unfortunately, these abilities degrade with increasing frequency, but emerging applications require ever higher operating frequencies. On page 74 of this issue, Wu *et al.*[1] describe transistors made from graphene — a carbon sheet just one atom thick — that hold promise for RF applications.

Two parameters are used to assess the frequency performance of an RF transistor: the cut-off frequency, f_T, at which the device's current gain drops to unity; and the maximum frequency of oscillation, f_{max}, at which the power gain becomes unity. One way to enhance the frequency performance of transistors is to use new materials that have high charge (carrier) mobility. The ultra-high mobilities observed[2,3] in graphene attracted the attention of device engineers immediately after their discovery, and intensive research[4,5] on RF graphene transistors is now under way.

Significant progress has been made since the demonstration[6] of the first gigahertz graphene transistors in 2008. Most notably, in February 2010, a group reported[7] a field-effect transistor (FET, the type of transistor most frequently used in electronics) made from graphene that broke the 100-GHz-f_T mark. And only a few months later, researchers demonstrated[8] a graphene FET that has an f_T of 300 GHz. Wu *et al.*[1] now report graphene FETs with gate electrodes of remarkably short length (40 nanometres) and f_T as high as 155 GHz. This result certainly does not represent a new record in frequency performance for RF transistors, and one might say that this is just another report on the good performance of graphene transistors. In fact, it is more than that in several respects.

Most groups make graphene by mechanical exfoliation, a method described[2] by Nobel prizewinners Konstantin Novoselov and Andre Geim. Mechanical exfoliation consists of peeling graphene flakes off a graphite crystal, and is a neat and practical method for university labs; the 300-GHz-f_T transistor mentioned above is made from exfoliated graphene. To make graphene attractive for the electronic-chip industry, however, reliable large-scale preparation schemes are needed. One such scheme, pioneered by Berger and de Heer[9], is the growth of graphene, by a method known as epitaxy, on silicon carbide wafers. A second option is to use a process known as chemical vapour deposition (CVD) to grow graphene on a metal, and then to transfer the graphene from the metal onto an insulating substrate, which most commonly consists of silicon with a top layer of silicon oxide (SiO_2)[10]. Wu and colleagues[1] now present a promising modification of the latter approach, which is to use a diamond-like carbon film as the top layer. Using this instead of SiO_2 is thought to result in better carrier transport in graphene FETs.

The authors[1] fabricated graphene transistors with gate lengths in the 40–550-nm range. They demonstrate reproducible measured characteristics for 30 devices and cut-off frequencies up to 155 GHz. Although this f_T value does not exceed that obtained previously[8], it is the highest f_T reported for CVD graphene transistors. Wu *et al.* also provide the first RF data for CVD graphene FETs on diamond-like carbon. With this work, another industry-compatible technology option for RF graphene FETs is now available.

What's more, Wu and colleagues are the first to investigate graphene FETs at very low temperatures. They show that the f_T of their transistors remains essentially constant between 300 kelvin and liquid-helium temperatures (4.2 kelvin), proving that graphene transistors could represent an alternative to conventional silicon- and III-V-semiconductor-based FETs for use at cryogenic temperatures, for example in space-based applications. It should be noted that proper operation of devices at 4.2 K is not a matter of course. There were serious concerns that carrier freeze-out might degrade the performance of silicon transistors of the MOSFET type at low temperatures. Fortunately, experiments[11] showed that this was not the case.

Finally, Wu and co-workers discuss not only the merits but also, and quite thoroughly, the problems of graphene transistors. The f_T performance of graphene FETs is known to be very competitive. For most applications, a high f_T is certainly desirable, but more important than a high f_T would be high power gain and f_{max}. Unfortunately, graphene FETs still suffer from low f_{max}. The 550-nm-gate graphene FETs of Wu *et al.* display an f_{max} of only 20 GHz. Although this is the highest f_{max} reported so far for graphene RF FETs, it is much lower than that of competing RF FETs (Fig. 1). Moreover, in