New Insight into the Dietary Cause of Atherosclerosis:

Implications for Pharmacology

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d) Abbreviations (alphabetical order) 1→14

   1. ASCVD, atherosclerotic cardiovascular disease
   2. BMI, body mass index
   3. CAD, coronary artery disease
   4. CHD, coronary heart disease
5. CV, cardiovascular

6. DMB, 3, 3-dimethyl-1-butanol

7. FMO3, Flavin mono-oxygenase 3

8. HDL, high density lipoprotein

9. HRT, hormone replacement therapy

10. Lyase, cut C/D choline TMA lyase; Yea W/X TMA lyase

11. MI, myocardial infarction

12. RCT, reverse cholesterol transport

13. TMA, trimethylamine

14. TMAO, trimethylamine N-oxide

e) Cardiovascular Pharmacology
Abstract

At present, the guideline approach to the medical treatment and prevention of atherosclerotic cardiovascular disease (ASCVD) is to classify patients by risk, and treat the known risk factors (contributory causes), e.g., hypertension, diabetes, obesity, smoking, and poor diet as appropriate. All high risk patients should receive statins. This approach has had substantial success but ASCVD still remains the number one cause of death in the United States. Until recently, the underlying cause of ASCVD remained unknown although a potential dietary cause was suggested by the fact that vegetarians, especially vegans, have a much lower incidence of ASCVD than animal flesh eaters. Recently, consistent with the vegetarian data, substantial evidence for a cause of ASCVD in animals and humans has been discovered. Trimethylamine (TMA)-containing dietary compounds in meat, milk, and other animal foods (e.g., lecithin, choline, and carnitine) are converted by closely related gut bacterial TMA lyases to TMA which is absorbed and converted predominantly by flavin mono-oxgenase 3 to the toxic trimethylamine N-oxide (TMAO). TMAO causes atherosclerosis in animals and is elevated in patients with coronary heart disease. Inhibition of bacterial lyases prevents TMA and secondarily TMAO formation and atherosclerosis in mice and provides strong evidence for the TMAO hypothesis. At present, the challenge for the pharmaceutical industry is to discover and develop a potent “broad spectrum” bacterial lyase inhibitor which along with diet and exercise could, if the TMAO hypothesis is correct, revolutionize the preventive treatment of ASCVD.
Introduction

In the United States, atherosclerosis in the coronary and cerebral arteries is generally a causal factor in over 600,000 cardiac and a 130,000 stroke deaths per year (Mozaffarian et al., 2016). In most cases, the critical event (the “coup de grace”) is a clot which forms over a rough or cracked, non-obstructive atheromatous lesion, acutely obstructing blood flow and causing distal tissue necrosis, although less commonly emboli can also obstruct blood flow (Blumenthal and Kapur, 2006). Depending on where this occurs, the result may be a fatal or non-fatal myocardial infarction (MI), ischemic stroke, bowel infarction or other less common syndromes. Based on careful clinical research, seven prominent contributory causes (risk factors) for atherosclerosis have been identified: increased serum cholesterol and blood pressure, diabetes, obesity, a positive family history, smoking and an atherogenic diet (Mozaffarian et al., 2016). However, the pathophysiology in each case is complex; for example, increased blood pressure not only accelerates atherosclerosis in the large arteries but also causes arteriolosclerosis with arteriolar hyaline change or even medial necrosis in malignant hypertension (Mozaffarian et al., 2016).

However, none of these “contributory causes” fully explains the underlying cause and pathophysiology of the atherosclerotic process in most cases. Yet, a somewhat successful approach has been to control these risk factors when present: lowering blood pressure with safe and effective drugs and blood cholesterol with statins; controlling type II diabetes and obesity with drugs, diet and weight loss; stopping smoking and improving diets e.g., with a vegetarian-type diet (Mozaffarian et al., 2016). All these measures are useful and have lowered age adjusted mortality due to atherosclerotic cardiovascular disease (ASCVD) by ~ 30% from 2003 to 2013 (Mozaffarian et. al., 2016). As a consequence, for the first time, in 2016, cancer (with greater
than 600,000 projected deaths) may overtake heart disease as the leading cause of death in the
US. Stroke, which used to be the second leading cause of death, has now descended to fifth place
after cancer, heart disease, respiratory ailments and accidents, thus attesting to the success of
pharmacologic control of risk factors, primarily hypertension in this case. (Mozaffarian et al.,
2016).

Notwithstanding these successes, myocardial and cerebral infarctions (ASCVD) together
remain the leading cause of death in the United States and developed world (Mozaffarian et al.,
2016). Based on convincing epidemiology work, there has been a strong suspicion that a dietary
factor(s) might be an underlying cause, i.e., a necessary and sufficient cause exacerbated by the
risk factors (contributory causes) enumerated above. This hypothesis, described in detail below,
has recently been supported by substantial evidence in animals and humans (Wang, et al., 2011;
Koeth et al., 2013; Tang et al., 2013; Troseid et al., 2015; Shih et al., 2015; Warrier et al.,
2015; Wang et al., 2015; Johnson and Backhed, 2015). Briefly, trimethylamine (TMA)
containing dietary compounds found mainly in meat, milk, some fish and other animal foods
(e.g., lecithin, choline, betaine, carnitine) are converted by TMA lyases (lyases) in gut bacteria to
TMA which is absorbed and then oxidized by hepatic flavin mono-oxygenase 3 (FMO3) to the
toxic trimethylamine N-oxide (TMAO). TMAO causes atherosclerosis in animals and probably
humans, and is elevated in patients with atherosclerotic coronary heart disease (CAD). Before
reviewing the TMAO evidence, I will review the cholesterol hypothesis which for decades has
been widely accepted as the principal cause of atherosclerosis. I will also briefly comment on
the management of diabetes and hypertension since this is relevant to prevention of
atherosclerosis and finally the epidemiology work on dietary causes of atherosclerosis.
The Cholesterol Hypothesis

Initially the cholesterol hypothesis as a cause of atherosclerosis had three components: elevated high density lipoprotein (HDL) was protective and elevated low density lipoprotein (LDL) and triglycerides in blood were harmful. The HDL and triglyceride components of the hypothesis are not well supported and will not be discussed further (Spector, 2013; Medical Letter, 2013). In favor of the LDL component of the cholesterol hypothesis are: 1) the higher the LDL cholesterol, the greater the risk of CAD; 2) certain patients with rare genetic disorders and very high serum cholesterol have accelerated atherosclerotic cardiovascular disease (ASCVD); 3) feeding large quantities of cholesterol to rabbits and other animals causes atherosclerosis and 4) statins which lower cholesterol are unequivocally beneficial (Scandinavian Simvastatin Survival Study Group, 1994; Spector 2013; Mozaffarian et al., 2016).

There is, however, persuasive evidence that the LDL cholesterol is not the underlying causal factor in most patients with ASCVD; 1) more than one-third of MI patients have normal or even low serum cholesterol and many patients with elevated LDL never manifest ASCVD thus showing that increased serum LDL is not a necessary or sufficient cause of ASCVD (Spector 2013); 2) in fact, statins although they lower serum cholesterol and LDL cholesterol, do not work primarily by slowing or regressing the atherosclerotic process (Blumenthal and Kapur, 2006). In a large two year placebo-controlled randomized study in patients with CAD treated with high dose lovastatin (80mg) or placebo, there was no difference in changes in coronary anatomy as measured by quantitative coronary angiography between drug and placebo groups both at baseline and after two years of treatment (Blankenhorn et. al., 1993). With maximal dose rosuvastatin (40 mg) and atorvastatin (80 mg.), in two studies employing ultrasound, there was an ~1% decrease in coronary atheroma volume compared with baseline over two years (Nissen et
al., 2006; Nicholls et al., 2011); however, there were no controls in these atheroma studies and no luminal measurements, so these data are difficult to interpret unlike the Blankenhorn et al. (1993) study referred to above with lovastatin (Blumenthal and Kapur, 2006). But what can be said from these studies is that statins do not alter the atheroma process much if at all. Yet, in a carefully done double-blind, randomized clinical trial of secondary prevention, there was ~ a 30% decrease in all-cause mortality as well as similar decreases in MI’s and strokes in patients randomized to moderate dose simvastatin compared to placebo patients (Scandinavian Simvastatin Survival Study Group, 1994; Spector 2013, Mozaffarian et. al., 2016). In this trial there was 100% follow up for mortality, so there is absolutely no ambiguity about the results. The mechanism of this effect we now know is not due to decreasing atherosclerosis per se but to stabilizing non-obstructing atheromatous plaques and preventing clotting on them when these plaques are rough or rupture (Blumenthal and Kapur, 2006). Anti-platelet agents (e.g., aspirin) also help prevent clots on such lesions (Mora, 2013); 3) statin efficacy is independent of baseline serum cholesterol. This has been shown in studies of both simvastatin (Heart Protection Study Collaborative Group, 2002) and pravastatin (Sacks et. al., 2000); 4) feeding huge amounts of cholesterol to rabbits is not a good model of human ASCVD unlike the TMAO data discussed below; 5) many drugs that lower serum LDL cholesterol and raise HDL (e.g., hormone replacement therapy [HRT] and niacin) either have no effect on ASCVD or are actually harmful (Hulley et. al., 1998; AIM-HIGH Investigators, 2011; Spector, 2013). For example, in the first large placebo controlled trial of HRT in women who had CAD, there was no CV benefit but significantly more gall bladder and thromboembolic disease in the HRT arm (Hulley et. al., 1998). Subsequent controlled trials confirmed no CV benefit of HRT or actual harm (Spector and Vesell, 2002); and 6) on Feb. 15, 2016, the FDA made its decision about the utility of Vytorin, a
drug which combines 40 mg of simvastatin with 10 mg of ezetimibe, a drug which blocks cholesterol absorption. As expected, Vytorin lowers serum total and LDL cholesterol more than simvastatin (40 mg.) alone. However, after a careful review of a 7-year ~18,000 patient randomized controlled trial of Vytorin versus simvastatin (40 mg) alone (Cannon, et al, 2015) and other data, the FDA and its advisory committee concluded that Vytorin (40/10) was no better than simvastatin (40 mg.) in preventing cardiovascular death, strokes and MIs. In short, Vytorin which lowers total and LDL serum cholesterol more than simvastatin alone is not more effective clinically. This result is consistent with a recent analysis of the statin data from the extant randomized clinical trials (Takagi and Umemoto, 2013). This analysis does not make unjustified linear assumptions and suggests the clinical benefit of statins is achieved with doses of 40 mg. of atorvastatin or simvastatin and further lowering of blood cholesterol achieves very little (Takagi and Umemoto, 2013; Spector and Snapinn, 2011). For all the above reasons, serum cholesterol must be considered a contributory cause of ASCVD in some cases only.

It is worth noting that the new national guidelines (by the Veterans Administration/ US Department of Defense/American Heart Association/ American College of Cardiology) for the treatment and prevention of ASCVD have finally recognized these facts about statins (Downs and O’Malley, 2015). Based on the current treatments available, the guidelines now correctly focus on risk: BP and weight control; cessation of smoking; exercise; and statins and aspirin (Mora, 2012) for high risk patients employing moderate dose inexpensive generic statins (e.g., 20 mg or preferably 40 mg of atorvastatin or simvastatin) for almost all high risk patients irrespective of serum cholesterol. The guidelines have eschewed hypothetical unproven schemes like treatment to arbitrary LDL goals (Downs and O’Malley, 2015). In other words, the guidelines implicitly recognize that statins do not appreciably alter atherosclerosis but do prevent
MIs, strokes and death by the mechanism noted above, and thereby reduce risk (Downs and O’Malley, 2015; Krumholz and Hayward, 2010; Spector 2013).

Hypertension and Diabetes

Hypertension and diabetes both have in common the ability to accelerate the development of atherosclerosis in large arteries and, in their more severe forms, to cause arteriolosclerosis, a separate problem. Arteriolosclerosis can manifest itself as lacunar infarcts in brain, kidney damage, retinal destruction, and, in diabetes, neuropathy due to decreased blood flow to peripheral nerves. Current treatment of hypertension with inexpensive safe drugs (ace inhibitors, angiotensin receptor blockers, β-blockers, calcium channel blockers and diuretics) is effective in lowering blood pressure; in many patients these drugs drastically reduce the risks of arteriolosclerosis and stroke (Mozaffarian, et. al., 2016). Treatment of hypertension also slows the atheromatous process in large arteries. However, good control of diabetes slows the arteriolar damage but for unclear reasons does not have much effect on large artery atherosclerosis. In diabetes statins and other drugs are needed to combat the risks of atherosclerosis (Mozaffarian, et. al., 2016; Downs and O’Malley, 2015).

The Role of Diet

A tremendous number of epidemiology/observation studies and a few controlled trials have been published on the relationship between diet and ASCVD. The vast majority of these epidemiological/observation studies suffer from multiple methodologic deficiencies and the results are often ambiguous, misleading or actually quantitatively incorrect as pointed out by ourselves, and many others (Spector and Vesell, 2000; 2002; 2006a; Campbell and Campbell...
2006). These methodologic deficiencies include poor validation of food questionnaires, no or infrequent assessment of change in diet, inattention to the Hill criteria for proving causality, improper use of statistics in non-randomized studies, futile attempts to focus on one variable, “data dredging” (also termed data mining, i.e., looking for correlations in large data sets without a predetermined hypothesis, a procedure that leads to many false positive correlations) and many other errors (Spector and Vesell, 2000; 2002; 2006a; Campbell and Campbell 2006). Two important examples of false positives studies include those that concluded that HRT and mega-vitamin E prevented ASCVD (Spector and Vesell, 2002). However, subsequent controlled trials show they were both either ineffective or harmful (Spector and Vesell, 2002). Another example is the advice some decades ago to switch from butter (saturated fat) to margarine based on incorrect epidemiology/observation studies suggesting that saturated fat was harmful (Siri-Tarino et. al., 2010); this was terrible advice since most margarine contains trans-fats, now known to be atherogenic. In recent years it has been recognized that eating saturated fat is in fact not harmful per se and the original studies were incorrect. Stampfer, the head of epidemiology at the Harvard School of Public Health which carried out many of these earlier epidemiology/observation studies admitted, “This has been our greatest failure and disappointment - that we have not learned what people can do to lower their risk” (Quoted in Campbell and Campbell, 2006). Please see Campbell and Campbell (2006) for a detailed critique of such studies.

Because of the tremendous variability in such studies several authors have published meta-analyses of non-randomized epidemiology/observation studies in an attempt to answer certain questions. For example, one question was: does red meat increase the risk of ASCVD? A recent meta-analysis of 20 epidemiology/observation studies with over one million individuals suggests it does not (Micha et. al., 2010). Is this correct? See below.
However, there have been several, I believe, more revealing epidemiology studies. During and after the Second World War, there was over a fifty percent decline in England in the incidence of diabetes and deaths from heart disease (Trowell, 1974). This was ascribed to the meager diets during and after the war. Consistent with the dietary hypothesis were studies of CAD and diabetes in Japanese in Japan, Hawaii, and California (Kagan et al., 1974; Marmot, 1975; Worth et., al., 1975; Tsunehara et. al., 1990). At the time of these studies in Japanese men, there was a gradient with CAD and diabetes being lowest in Japan, next Hawaii and highest in California. The investigators in these studies suggested that the diet in Japan at the time when these studies were performed was mainly a vegetarian-type diet and not the “richer” animal protein/fat diet of California. However, from these studies of Japanese in Japan, Hawaii and California, only qualitative conclusions can be drawn. Third the “China Study” showed that a mainly plant based diet in China was associated with much less ASCVD than a non-vegetarian animal based Western diet (Campbell and Campbell, 2006). Moreover, there have been several small controlled trials that have also suggested a vegetarian-type diet is better on both morbidity and mortality than a standard Western diet in patients with CAD (Campbell and Campbell, 2006).

A recent analysis of other studies in homogenous populations provides further powerful evidence for the efficacy of vegetarian diets in minimizing ASCVD mortality and morbidity. Unlike many of the negative or ambiguous studies that compared subjects who ate lessor or greater quantities of animal products noted above, e.g., the Nurses Study, these vegetarian studies compared vegetarians versus animal product eaters (Fraser 2009). The results are clear and consistent: vegetarians enjoy lower overall mortality and 32% less CHD mortality than non-vegetarians. Moreover, there is a clear gradient from vegans (no animal products permitted), to
lactovo- vegetarians (eggs and milk permitted), to pesco-vegetarians (eggs, milk and fish permitted), to semi-vegetarians to non-vegetarians in morbidity (Fraser, 2009).

However, causal interpretations are complex because there is also a gradient between vegans and non-vegetarians favoring vegans in body mass index (BMI), diabetes and hypertension. Vegans in one study had an 18% lower BMI, a prevalence of diabetes and hypertension of 22% and 25%, respectively, of non-vegetarians. Therefore, these results only allow one to conclude that a vegetarian (especially a vegan diet) is helpful in preventing ASCVD but not exactly why. Is it the diet, the decreased BMI, blood pressure, diabetes, or a combination?

New Information

Thus, in 2010, by lowering risk factors we could effectively treat some patients with established ASCVD or prevent clinical ASCVD in those with risk factors. However, the underlying cause of atherosclerosis in the larger arteries remained unknown, although as noted above, there was convincing evidence that a vegetarian diet (especially a vegan diet) was healthier than the Western (animal-based) diet. At this point (2010), the best advice was Aristotle’s: “a sound mind in a sound body” and “moderation in all things.” This was translated into: keep one’s weight between BMI of 20 – 25, be sure the diet contains the known essential nutrients (ions, vitamins, essential fatty and amino acids), enjoy a balanced diet even including an occasional egg or two (Virtanen, et. al., 2016), and tend toward a vegetarian diet; finally do not forget to exercise.
However, beginning in 2011, investigators in the Cleveland Clinic and other venues published a series of groundbreaking papers suggesting TMAO formed from dietary TMA containing nutrients, especially animal products, might, in fact, be the “toxic” metabolite, a prime cause of atherosclerosis (Wang et al., 2011; Koeth et al., 2013; Tang et al., 2013; Troseid et al., 2015; Shih et al., 2015; Warrier et al., 2015; Wang et al., 2015; Johnson and Backhed, 2015). Basically, what these investigators have shown is that the TMA covalently incorporated in lecithin, choline, betaine or carnitine can be metabolized in gut by bacterial lyases to release TMA, a gas. TMA is readily absorbed by mice and humans and converted to TMAO by liver FMO 3; TMAO can cause atherosclerosis in mice (Wang et al., 2011). This process however is variable and complex. First, different gut bacteria have different abilities to form TMA from TMA-containing nutrients. This is in part because there are two distinct bacterial lyases; one consists of the cut C and cut D gene products and is termed cut C/D choline TMA lyase. The other consists of the Yea W/X gene products (Yea W/X TMA lyase). This promiscuous lyase can convert lecithin, choline, betaine, carnitine, and butyrobetaine to TMA (Falony et al, 2015; Wang et al., 2015). Both of these lyases are inhibitable to a greater or lesser extent by 3, 3 dimethyl-1-butanol (DMB) (Wang et al., 2015). There is also a third enzyme in human gut bacteria consisting of the gene products Cnt A and Cnt B termed carnitine Cnt A/B oxygenase/reductase that convert carnitine to TMA and malic semi-aldehyde (Wang et al., 2015; Falony et al., 2015). The quantitative importance of this enzyme remains uncertain. It is not inhibited by DNB (Wang et al, 2015). Some gut bacteria contain no TMA-forming enzymes; others contain 1, 2 or all three of these enzymes (Falony et al., 2015). Further complicating matters, different individuals contain different levels of gut bacteria that can form TMA in vivo.
When TMA formation in mice is blocked with either DMB or antibiotics, TMAO is not formed and, in mice, atherosclerosis is greatly diminished especially on a high choline diet (Wang et al., 2015). Moreover, especially interesting is the finding that DNB blockade of lyase activity leads to gut bacteria with less lyase activity (Wang et al., 2015). It is worth noting the non-toxic DMB is found in balsamic vinegars, red wines and extra-virgin olive and grapeseed oils (Wang et al., 2015).

After absorption, TMA is N-oxidized mainly by FMO3 (Wang et al., 2015; Warrier et. al., 2015). Congenital absence of FMO3 leads to “fish odor syndrome” due to inability to metabolize TMA; TMA smells like rotting fish (Wang, et. al., 2015). To avoid the odor, such patients avoid TMA-containing foods. In normal humans, TMA is almost completely N-oxidized to TMAO after a 600 mg TMA base “load” (Zhang et al., 1995). However, obligate heterozygotes (parents of fish odor affected children) although on a normal diet oxidize greater than 90% of the TMA to TMAO, after a 600 mg TMA base load N-oxidize only 76 ±3% to TMAD, clearly different than normal (96±2%). (Zhang et al., 1995). Furthermore in a study of one hundred volunteers challenged with 600 mg base TMA, only one was within the range found among obligate heterozygotes (Zhang et al., 1995). Interestingly, in three normal Japanese women during menstruation, the ratio of TMA to TMAO in the urine increased to levels seen in obligate heterozygotes suggesting a hormonal effect (Shimizu et al., 2007). If this observation can be confirmed and extended, it might explain why menstruating women are protected from ASCVD i.e., less production of the toxic TMAO.

In mice, there are large effects on FMO3 activity with cholic acid causing up-regulation and testosterone down-regulation; inhibition of FMO3 has effects on reverse cholesterol transport (RCT), hepatic inflammation and bile formation (Bennet et al., 2013; Warrier et. al.,
2015). Thus, inhibition of FMO3 to block formation of TMAO would not only cause fish odor syndrome but also might have effects on RCT, exacerbate hepatic inflammation and have other complex effects. However, humans lacking FMO3, beside fish odor, are said to be healthy. Moreover, as noted above, normal humans (with the possible exception of menstruating women) efficiently convert TMA to TMAO and the relevance of the mouse studies dealing with regulation of FMO3 (Bennet et al., 2013; Warrier et al., 2015) remains to be determined. In any case, it may be more acceptable to reduce dietary precursors of TMA than to reduce FMO3 activity.

In human studies, consistent with animal studies, TMAO was elevated in patients with ASCVD, and could be suppressed with antibiotics presumably by killing gut bacteria (Wang et al., 2015). Especially revealing was a 3-year prospective cohort study of 4007 patients with CAD in which an elevated TMAO concentration was associated with an increased risk of major ASCVD events (the risk in the highest quintile of TMAO was 2.5 times the lowest quintile; 95% confidence interval, 2 to 3.3; p < .001) (Tang et al., 2013). Similarly, TMAO was increased in heart failure patients due to ischemic heart disease (Troseid et al., 2015).

This work has important implications for understanding much previous data. Assuming TMAO is the principal “culprit agent” (cause) of atherosclerosis, then it is clear why patients with normal cholesterol, weight, blood pressure, and blood sugar (i.e., without diabetes) and who don’t smoke could have severe ASCVD. They may produce excess TMAO because of diet and lyase-containing intestinal bacteria. In a steady weight individual, it is also clear that saturated fats and sugar which are metabolized to CO₂ and H₂O for energy (ATP formation) should not be harmful per se (Siri-Tarino et al., 2010). Moreover, there is no evidence that dietary cholesterol is harmful (Virtanen et al., 2016). Thus, if the TMAO hypothesis is correct, the recent focus of
dietary therapy (beyond a balanced diet containing essential nutrients) should not be on dietary sugar, cholesterol and saturated fat if preventing atherosclerosis is the objective, but on TMA-containing nutrients like lecithin, carnitine, choline and betaine; in other words, a diet more like a vegetarian-type diet should be followed. The potential benefit of a vegetarian-type diet which minimizes TMAO formation thus becomes clear. However, a word of caution is necessary; there might be other unknown dietary factors and metabolites not present or formed in vegetarian-type diets that, along with TMAO, are toxic.

The TMAO hypothesis also is consistent with the fact that high doses of statins which lower LDL cholesterol by 40-60% have no or minimal effect on coronary atherosclerosis (by arteriography or ultrasound) (Blankenhorn et al., 1993; Blumenthal and Kapur, 2006). As noted above, statins stabilize plaques thereby preventing clotting, obstruction and distal ischemia/necrosis. Aspirin and other anti-platelets agents also prevent platelet clotting (Mora, 2012).

Implications for Pharmacology

One of the most important decisions in drug discovery and development is the selection of the correct target (Spector and Vesell, 2006b). When this is done, the pharmaceutical industry has made remarkably safe and effective drugs and vaccines. In the current case, we have very strong pre-clinical and clinical data that TMA lyases in gut bacteria are a worthy target although there are different forms of this lyase in different bacterial species as noted above (Wang et al., 2015). DMB, which has an IC$_{50}$ of ~10 μM in lysates of bacterial cells, is a weak competitive inhibitor but has been able to provide proof of principle (Wang et al., 2015; Johnson and
Backhed, 2015). The IC$_{50}$ is likely much higher in intact bacterial cells. Extrapolating from the mouse work – probably 10 – 20 grams a day of DMB in divided doses would be required in humans to inhibit intestinal bacterial lyase activity. What is needed is a full-throttle approach by the pharmaceutical companies to make a potent “broad-spectrum” bacterial lyase inhibitor, an inhibitor with an IC$_{50}$ of more like 10 nM, preferably one not absorbed so it “worked” only on bacteria in the gut or, if ultimately absorbed, rapidly metabolized. If such a compound was discovered, phase I and II trials in humans could be performed to test the compound’s ability to block TMAO production in vivo. Further development in large long term phase III clinical trials versus placebo could be done to test (on top of statins and aspirin) whether such a compound could decrease MI, strokes and CV deaths. If the TMAO hypothesis is correct, there should be a major effect. What is not known is exactly how TMAO might “cause” ASCVD in humans. There is speculation that TMAO may interfere with RCT (Wang et. al., 2015; Warrier et. al., 2015). There are also convincing data that TMAO enhances platelet hyperreactivity in animals and humans, and thrombosis risk in mice (Zhu et al., 2016). Such an effect if it occurs in vivo in humans could at least potentially explain the ability of TMAO to “cause” ASCVD in humans. With a potent lyase inhibitor as a tool, these hypotheses (RCT inhibition, platelet hyperreactivity, and others) could be tested to help uncover the mechanism of TMAO toxicity if, in fact, TMAO is a causative factor in ASCVD.

In summary, in the past five years, several investigative groups have brought forward an exciting hypothesis for the actual cause of atherosclerosis. These investigators have also potentially explained the benefits of vegetarian-type diets. Moreover, they have provided strong evidence for a pharmacological target for the treatment and prevention of ASCVD (Wang et. al., 2015; Zhu et al., 2016). If an effective broad-spectrum bacterial lyase inhibitor (or blocker of
TMAO toxicity) can be discovered and developed that can prevent ASCVD, especially when coupled with a prudent vegetarian-type diet and exercise, we may at long last have effective tools to decrease substantially the morbidity and mortality currently associated with ASCVD.

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