Postprandial gut microbiota-driven choline metabolism links dietary cues to adipose tissue dysfunction

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ABSTRACT
The human body is an integrated circuit between microbial symbionts and our Homo sapien genome, which communicate bi-directionally to maintain homeostasis within the human metaorganism. There is now strong evidence that microbes resident in the human intestine can directly contribute to the pathogenesis of obesity and associated cardiometabolic disorders. In fact, gut microbes represent a filter of our greatest environmental exposure – the foods we consume. It is now clear that we each experience a given meal differently, based on our unique gut microbial communities. Biologically active gut microbe-derived metabolites, such as short chain fatty acids, secondary bile acids, and trimethylamine-N-oxide (TMAO), are now uniquely recognized as contributors to obesity and related cardiometabolic disorders. However, mechanistic insights into how microbe-derived metabolites promote obesity are largely unknown. Recent work has demonstrated that the meta-organismal production of the bacterial co-metabolite TMAO is linked to suppression of beiging of white adipose tissue in mice and humans. Furthermore, the TMAO pathway is becoming an increasingly attractive therapeutic target in obesity-associated diseases such as type 2 diabetes, kidney failure, and cardiovascular disease. In this commentary we discuss recent findings linking the TMAO pathway to obesity-associated disorders, and provide additional insights into potential mechanisms driving this microbe-host interaction.

KEYWORDS
microbiota; microbiome; nutrition; obesity; diabetes; adipose; trimethylamine N-oxide

Introduction
Throughout history it has been appreciated that diet is one of the most important environmental factors contributing to human disease. As early as 400 B.C. Hippocrates was quoted “Let thy food be thy medicine and thy medicine be thy food.” Given the strong association between dietary habits and human disease, a disproportionate number of Nobel Prize-winning discoveries are in the field of intermediary metabolism.1 In fact, our understanding of mammalian nutrient metabolism is quite advanced with most of the major biochemical pathways well understood. However, lagging behind substantially is our understanding of how the microorganisms that live within us metabolize common nutrients in our diets to generate unique bioactive metabolites. Although often overlooked, gut microbes represent a filter of our greatest environmental exposure – the foods we consume. In fact, it is now clear that we each experience a given meal differently, based on the enzymatic capacity of our unique gut microbial communities.2,3 Biologically active metabolites such as short chain fatty acids (SCFA),4,5 secondary bile acids (2’BA),6,7 polysaccharide A (PSA),4 4-ethylphenylsulfate (4EPS),5 indoxyl-sulfate (Indoxyl-SO4),10,11 p-cresyl-sulfate (pCresyl-SO4),10,11 N-acyl amides,12 and trimethylamine-N-oxide (TMAO)13–20 originate solely from bacteria nutrient metabolism. Importantly, circulating levels of several of these gut microbial metabolites have been recently associated with obesity and closely associated disorders such as insulin resistance, chronic kidney disease, cardiovascular disease (CVD), neurological disorders and cancer.2–20 Unfortunately, mechanistic insights into how these microbe-derived metabolites promote obesity-related cardiometabolic disorders are largely unknown. The focus of this commentary is to discuss recent studies that have shown that the gut microbial co-metabolite TMAO is linked to obesity-associated disorders in humans.20 To expand on these recent findings, we
provide additional data here supporting a role for TMA and its co-metabolite TMAO as hormone-like signals being produced after a meal that can subsequently have distant endocrine signaling effects in white adipose tissue. Collectively, the TMAO pathway represents an attractive gut microbiome-centered therapeutic target in obesity and related chronic diseases, and studies here provide some of the first clues into the rapid signaling responses elicited by TMA and TMAO in the host.

TMAO is generated by a meta-organismal pathway where nutrients present in high fat foods (phosphatidylcholine, choline, L-carnitine, and γ-butyrobetaine) are first metabolized by gut microbial enzymes such as CutC/D21,22 CntA/B,23 and YeaW/X15 to generate the primary bacterial metabolite trimethylamine (TMA). TMA is then further metabolized by the host enzyme flavin-containing monooxygenase 3 (FMO3) in the liver to produce trimethylamine-N-oxide (TMAO)26,27 (Fig. 1). Within the last six years the TMAO pathway has been identified in several independent human and animal studies as a potential contributory factor in the pathogenesis of obesity, type 2 diabetes, chronic kidney disease, and CVD.2,3,13–20 The connection between the TMAO pathway and cardiometabolic disease has been recently reviewed in detail in several excellent reviews,2,3,26,27 so here we only provide a brief overview of the key discoveries. Broad interest in the TMAO pathway began when large-scale human metabolomics-based discovery studies originally demonstrated a strong association between circulating TMAO levels and cardiovascular disease (CVD) risk.13 Follow up animal studies have found that feeding TMAO in the diet can worsen atherosclerosis progression in hyperlipidemic mouse models.2,3,13–17 Fulfilling Koch’s postulate of disease susceptibility, delivery of microbial communities with a high capacity to generate TMA, and its co-metabolite TMAO, results in enhancement of atherosclerosis development.28 Likewise, transplantation of gut microbial communities that have high TMAO production capacity results in transmission of dietary choline-induced platelet hyperresponsiveness and heightened thrombotic events in mouse models.29 In agreement, recent human studies have shown a connection between TMAO levels and thrombosis risks. Large-scale clinical studies reveal that higher TMAO levels are associated with increased incident risk for thrombotic events like heart attack and stroke.29 Moreover, TMAO levels have recently been shown to predict thrombotic event risk, and adverse outcomes, both in patients presenting with chest pain, and in acute coronary syndrome subjects.30 In further support of the TMAO-thrombosis risk link, a recent study showed that both omnivore and vegetarians or vegans alike, when placed on a dietary choline supplementation (i.e. 10–15 fold elevation in fasting plasma TMAO levels) had an accompanying enhanced platelet aggregation responses.31 In fact, the well-known anti-platelet effect of low dose aspirin therapy was blunted by elevating TMAO levels with dietary choline supplementation.31

In addition to the strong links between the TMAO pathway and atherosclerosis and thrombotic disease, several recent studies have uncovered a role for the same pathway in other closely related cardiometabolic phenotypes. Using unbiased screening approaches our group

Figure 1. Working Model by Which the Metaorganismal TMAO Pathway Promotes Obesity and Associated Cardiometabolic Diseases. Metabolism of dietary phosphatidylcholine (PC) and choline by the gut microbial enzymes CutC/D produces the microbial metabolite TMA, which is further metabolized by the host enzyme flavin-containing monooxygenase 3 (FMO3) to TMAO which can promote obesity-driven insulin resistance, atherosclerosis, heart failure, and kidney failure. Recent work by Schugar and colleagues20 demonstrated that inhibiting TMAO production with antisense oligonucleotides targeting the knockdown of FMO3 (FMO3 ASO) can prevent high fat diet-induced obesity in mice and induce the beiging of white adipose tissue.
recently discovered that the TMAO pathway is a key regulator of host cholesterol and bile acid homeostasis.\textsuperscript{18} Other recent studies performed metabolomic screening studies in mouse models of hepatic insulin resistance (liver insulin receptor knockout mice; LIRKO mice) to identify a link between the TMAO pathway and type 2 diabetes.\textsuperscript{19} Collectively, a wealth of human and animal model data supports the conclusion that the gut microbe-derived metabolite TMAO has both strong clinical prognostic value, and is a potential causative pathway in a wide variety of cardiometabolic disorders. These recent findings have provided substantial support for the concept that therapeutically inhibiting TMA or TMAO production may be a viable strategy for a number of chronic diseases.

A regulatory role for TMAO pathway in obesity and the beiging of white adipose tissue

In addition to clear links to CVD pathogenesis, we recently discovered that the TMAO pathway is also associated with obesity and energy metabolism in mice and humans.\textsuperscript{20} First, we examined how circulating TMAO levels associated with various obesity-related traits in the Hybrid Mouse Diversity Panel (HMDP) fed an obesogenic high-fat and high-sucrose diet,\textsuperscript{32} and found that circulating TMAO was positively correlated with adiposity across the numerous mouse strains represented in the HMDP.\textsuperscript{20} Given the association between TMAO and obesity in mice, we next set out to determine whether expression of the TMAO-producing enzyme \textit{FMO3} was differentially expressed in the adipose tissue of overweight or obese humans.\textsuperscript{20} In human adipose tissue \textit{FMO3} expression was positively correlated with body mass index and waist-to-hip ratio, and negatively correlated with insulin sensitivity.\textsuperscript{20} We also found that \textit{FMO3} mRNA expression levels in human white adipose tissue (WAT) were negatively correlated with several genes that represent selective markers of beige or brown adipocytes.\textsuperscript{20} Importantly, these studies were validated in three independent ethnically diverse human cohorts.\textsuperscript{20} These studies prompted us to hypothesize that the TMAO-producing enzyme \textit{FMO3} may be a negative regulator of WAT beiging, thereby impacting energy expenditure and adiposity.

To test this we generated antisense oligonucleotides (ASO) to inhibit the expression of \textit{Fmo3} in mice challenged with a high fat diet. \textit{Fmo3} ASO-treated mice were strikingly protected against high fat diet-driven obesity, which was characterized by large reductions in major WAT depots and increases in lean mass.\textsuperscript{20} Given the fact that \textit{Fmo3} knockdown resulting in a lean phenotype, and \textit{FMO3} expression was negatively correlated with brown and beige adipocyte gene markers in human adipose tissue, we next examined the effect of \textit{Fmo3} knockdown on cold-induced thermogenic reprogramming in adipose tissue. In the gonadal WAT depots, \textit{Fmo3} knockdown was associated with increased expression of brown and beige marker gene expression in the basal state.\textsuperscript{20} Likewise, the well described cold-induced upregulation of the thermogenic transcriptional regulator peroxisome proliferator-activated receptor gamma coactivator 1 alpha (\textit{Ppargc1a})\textsuperscript{33} was much higher in mice treated with \textit{Fmo3} ASO in multiple adipose depots.\textsuperscript{20} In parallel, cold-induced increases in energy expenditure were significantly enhanced in the \textit{Fmo3} ASO-treated mice housed at 4°C.\textsuperscript{20} Interestingly, dietary provision of TMAO was able to reverse \textit{Fmo3} ASO-driven increases in \textit{Ppargc1a} in WAT, indicating that TMAO may be a novel negative regulator of \textit{Ppargc1a}.\textsuperscript{20} To confirm the effects of \textit{Fmo3} ASO on WAT beiging were specific, we generated a global \textit{Fmo3} knockout (\textit{Fmo3}\textsuperscript{−/−}) mouse model using CRISPR-Cas9-mediated gene editing. In agreement with ASO-mediated knockdown, \textit{Fmo3}\textsuperscript{−/−} mice were protected against western diet-induced obesity, and also had elevated brown and beige marker gene expression in WAT depots.\textsuperscript{20} Collectively, these data provide pharmacologic and genetic evidence that the TMAO-producing enzyme \textit{Fmo3} is a negative regulator of beiging programs in white adipose tissue.

TMA and TMAO are generated postprandially and can reorganize host signal transduction in white adipose tissue

As a logical progression from our recent published work,\textsuperscript{20} we next hypothesized that TMA and TMAO may be mechanistically linked to the pathogenesis of obesity by acting as gut microbe-derived hormones that are sensed in WAT.\textsuperscript{2} To test this, we first examined the kinetics of TMA and TMAO production following a high fat test meal in mice. In fasted mice, a single gavage of a choline-containing high fat diet resulted in a postprandial spike in the appearance of both TMA and TMAO, which peaked around four hours post gavage (Fig. 2). These data suggested that TMA and TMAO are made postprandially, exhibiting hormone-like oscillations linked with TMA source nutrient ingestion. To test whether TMA or TMAO could elicit signaling responses similar to other host hormones, we next used an unbiased approach to identify the early signaling events elicited by either TMA or TMAO \textit{in vivo} (Fig. 3). For this we directly injected physiological levels (after injection portal blood reached levels ranging from 125–320 \(\mu\)M)
of each metabolite directly into the portal circulation draining the gut (i.e. portal vein) of fasted mice, and examined global phosphorylation events stimulated in WAT ten minutes later. We found a total of 15 WAT proteins that exhibited site-specific hypo- or hyper-phosphorylation 10 minutes after administration of TMA or TMAO treatment relative to vehicle (Fig. 3). Several of the TMA- and TMAO-driven phosphorylation events represented proteins enriched in key hormonal signaling pathways that are known to impact host metabolism. For example, TMA and TMAO treatment both resulted in altered phosphorylation of proteins involved in protein kinase A (PKA) signaling, including A kinase anchor protein 12 (Akap12) and the regulatory subunit of PKA (Prkar1a) (Fig. 3). Interestingly, TMA and TMAO reciprocally regulated the phosphorylation of insulin-like growth factor 2 (Igfr2), also known as cation-independent mannose-6-phosphate receptor precursor, which has been previously implicated in cellular insulin responses in adipocytes.34,35 Also, TMAO induced hyperphosphorylation of synaptosome-associated protein 23 (Snap23) which is a well known regulator of insulin-stimulated glucose transporter trafficking to the cell surface in adipocytes.36,37 Collectively, these data have identified TMA- and TMAO-driven signaling events in WAT in vivo, which potentially link these gut microbial metabolites to PKA- and insulin-signaling cascades. These data also provide the first evidence that these gut microbial co-metabolites can have hormone-like signaling actions in adipose tissue. For these microbial co-metabolites to act like hormones, there are likely dedicated host receptor systems that allow for regulated sensing of their levels.3 However, at this point there is very little known about the identity of host TMA and TMAO receptors. Recently, a G protein-coupled receptor called trace amine-associated receptor 5 (TAAR5) has been recently described as a selective cell surface receptor for TMA, but not its co-metabolite TMAO.38,39 Unfortunately, a similar receptor system for TMAO has yet to be identified.

Conclusions and perspectives

Although drug discovery has historically targeted human enzymes, a new frontier in biomedical research lies ahead where instead we target the microorganisms that live within us to improve human health. The gut microbial TMAO pathway has quickly gained traction as a potential therapeutic target in a wide variety of cardiometabolic diseases, and drug discovery efforts remain active to identify some of the first drugs that selectively target microbial of TMA production. Preclinical proof of concept was recently established given that a non-lethal small molecule inhibitor of gut microbial TMA lyase activity protects mice from thrombotic events.17 Although recent studies suggest that targeting the mammalian TMAO-producing enzyme FMO3 may provide protection against cardiometabolic disease,18–20 this is not a likely tractable therapeutic strategy due to the fact that FMO3 has many xenobiotic substrates and loss of function mutations in FMO3 result in trimethylaminuria, also known as fish odor syndrome.25 Therefore, a more attractive therapeutic strategy would be to provide a non-absorbable small molecule inhibitor to the gut microbial enzymes that generate TMA. Alternatively, if the host receptors for TMA or TMAO can be identified
TMA- and TMAO-Stimulated Phosphorylation Events in Mouse White Adipose Tissue

Figure 3. TMA and TMAO Can Rapidly Reorganize Adipose Tissue Signal Transduction In Vivo. Female C57BL/6 mice were fasted overnight (12 hour fast) and then injected directly into the portal vein with vehicle (saline), or pathophysiological levels of TMA or TMAO, and only 10 minutes later gonadal white adipose tissue was harvested for phosphoproteomic analysis to identify TMA- and TMAO-responsive phosphorylation events in mouse liver (n = 4 per group). (A) List of proteins that were differentially phosphorylated upon in vivo TMA or TMAO administration. (B) TMAO injection results in diminished phosphorylation of the regulatory subunit of protein kinase A. A doubly charged ion was present in the phosphopeptide sample that was identified as the EDEIpSPPPPNPVVK derived from cAMP-dependent protein kinase type I-alpha regulatory subunit. The CID spectra of this ion is dominated by H3PO4 loss from the precursor ion consistent with the presence of a pS or pT residue. The mass difference between the y10 and y9 ions is consistent with modification at S83. The observed chromatograms for this peptide from the Saline and TMAO samples is shown and the TMAO/Saline ratio was determined to be 0.39 (p-value 0.01907). (C) TMAO induces hyperphosphorylation of synaptosomal-associated protein 23 (Snap23). A doubly charged ion was present in the phosphopeptide sample that was identified as the ATWGDGGDNpSPSNVK from Synaptosomal-associated protein 23. The CID spectra of this ion is dominated by H3PO4 loss from the precursor ion consistent with the presence of a pS or pT residue. The mass difference between the y8 and y7 ions is consistent with modification at S110. The observed chromatograms for this peptide from the Saline, TMA, and TMAO samples is shown and the TMAO/Saline ratio was determined to be 8.1 (p-value 0.201) and the TMAO/Saline ratio was determined to be 7.9 (p-value 0.03198).
and selectively targeted, there also would lie tremendous therapeutic potential. Collectively, the gut microbial co-metabolites TMA and TMAO have been linked to several common human diseases associated with the metabolic syndrome, and may represent a novel therapeutic target at the microbe-host interface.

**Material & methods**

**Meal-Related kinetic TMA and TMAO production experiment in mice**

To examine whether TMA and TMAO are generated postprandially, we examined the levels of these gut microbial metabolites kinetically after a single meal. C57BL/6 were purchased from Harlan (Indianapolis, IN, USA), and allowed to equilibrate for several weeks before experiments. The test meal was made by adding 1% choline chloride (wt/wt) to a base liquid diet (PMI Rodent Liquid Diet LD 102; Test Diets, St. Louis, MO), which contain 49%, 36%, and 15% of kilocalories from carbohydrate, protein, and fat respectively. Mice were fasted overnight (12 hour fast), and a baseline blood sample was taken. Thereafter, mice were orally gavaged with 50 µl of the test diet. Mice were then bled kinetically after 0.5, 1, 2, 4, and 8 hours post gavage, and plasma was collected. Each plasma sample was rapidly acidified by the addition of 1 µl of 1 M hydrochloric acid to 20 µl of fresh plasma ensure that the volatile amine TMA was adequately trapped in solution. Quantification of TMA and TMAO in mouse plasma was performed using stable isotope dilution HPLC with online electrospray ionization tandem mass spectrometry on an API 365 triple quadrupole mass spectrometer (Applied Biosystems, Foster, CA) with upgraded source (I onics, Bolton, ON, Canada) interfaced with a Cohesive HPLC (Franklin, MA) equipped with phenyl column (4.6 × 250 mm, 5 µm Silica (2), Morton Grove, IL), and the separation was performed as reported previously (Wang et al., 2011; Wang et al., 2014). TMAO and TMAO were monitored in positive MRMS mode using characteristic precursor-product ion transitions: m/z 76 → 58 and m/z 60 → 44, respectively. The internal standards TMAO-trimethyl-d9 (d9-TMAO) and TMAO-d9 (d9-TMA) were added to plasma samples before sample processing and were similarly monitored in MRM mode at m/z 85 → 68 and m/z 69 → 49, respectively. Various concentrations of TMAO and TMAO standards and a fixed amount of internal standards were spiked into control plasma to prepare the calibration curves for quantification of plasma TMAO and TMA. For d9-TMA and d9-TMAO quantification in FMO activity analyses, 1,1,2,2-d4 choline (Sigma) was used as an internal standard followed by a 0.5 ml 3 K cutoff centrifugal filter (Millipore) of sample prior to LC/MS/MS analysis. The characteristic precursor-product ion transition for 1,1,2,2-d4 choline is m/z 108 → 60 monitored in positive MRM MS mode.

**Direct portal vein TMA and TMAO administration and phosphoproteomic analysis**

The goal of this experiment was to unbiasedly identify TMA- and TMAO-responsive signaling events in mouse liver. To closely mimic physiological route of delivery, we delivered the metabolites directly into the portal vein in fasted mice. Briefly, C57BL/6 mice were fasted overnight (12 hour fast), and between the hours of 9:00-10:00 am (2–3 hours into light cycle), mice were anesthetized using isoflurane (4% for induction and 2% for maintenance). Once fully anesthetized, a midline laparotomy was performed, and the portal vein was visualized under a Leica M650 surgical microscope. Briefly, fresh 10 mM stocks of TMA-HCL or TMAO were made in sterile saline, and the pH of stock solutions were adjusted to 7.4. Mice then received 20 µl of either saline vehicle, TMA-HCL, or TMAO via direct syringe infusion (Becton-Dickson product # 309306). 9.75 minutes later a small aliquot (~50 µl) of portal blood was collected by pulling back on injection syringe left in place following injection. In saline vehicle injected mice, portal blood levels of TMA ranged from 0.49–2.22 µM and TMAO levels ranged from 2.53–7.14 µM. In mice injected with TMA-HCL, portal blood levels of TMA ranged from 125.36–319.55 µM and TMAO levels ranged from 9.68–17.48 µM. Finally, in TMAO injected mice, portal blood levels of TMAO ranged from 2.23–4.10 µM, and TMAO levels ranged from 220.24–251.72 µM. Exactly 10 minutes after initial injection, the liver and gonadal white adipose tissue was rapidly snap frozen by immersion in liquid nitrogen. A total of 1 mg of protein from each sample was digested with trypsin and the resulting tryptic peptides were subjected to phosphoserine and phosphothreonine enrichment using the Thermo Scientific Pierce™ TiO2 Phosphopeptide Enrichment and Clean-up Kit (Fisher # PI88301) according to manufacturer’s instructions. The enriched peptide samples were subjected to C18 clean-up prior to LC-MS analysis. The LC-MS system was a Finnigan LTQ-Obitrap Elite hybrid mass spectrometer system. The HPLC column was a Dionex 15 cm × 75 µm id Acclaim Pepmap C18, 2 µM, 100 Å reversed-phase capillary chromatography column. 5 µL volumes of the extract were injected and the peptides eluted from the column by an acetonitrile/0.1% formic acid gradient at a flow rate of 0.3 µL/min were introduced into the source of the mass spectrometer online. The microelectrospray ion source is operated at
1.9 kV. The digest was analyzed using the data dependent multitask capability of the instrument acquiring full scan mass spectra to determine peptide molecular weights and product ion spectra to determine amino acid sequence in successive instrument scans. The data were analyzed by using all CID spectra collected in the experiment to search the human reference sequence databases with the programs Mascot and Sequest considering serine, threonine, and tyrosine phosphorylation. The data from these samples was searched against the full mouse database considering S, T, and Y phosphorylation as a dynamic modification. The resulting search results were filtered based on an Sequest XCorr scores $>1.5$ (+1), 2.0 (+2), 2.25 (+3), and 2.5 (+4), and Mascot Peptide ion score $>40$. A total of 1179 peptides were identified with 997 (85%) of these containing a phosphorylated residue. The relative abundance of the phosphopeptides was determined by using the program Sieve (Thermo Scientific version 2.2).

**Disclosure of potential conflict of interest**

Z.W. is named as co-inventor on pending patents held by the Cleveland Clinic relating to cardiovascular diagnostics. Z.W. reports that he has the right to receive royalty payment for inventions or discoveries related to cardiovascular diagnostics from Proctor & Gamble and the Cleveland Heart Lab. All other authors have no potential conflicts of interest to disclose.

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**References**

1. McKnight SL. On getting there from here. Science. 2010;330:1338–1339. doi:10.1126/science.1199908. PMID:21127243.

2. Brown JM, Hazen SL. The gut microbial endocrine organ: Bacterially derived signals driving cardiometabolic diseases. Annu Rev Med. 2015;66:343–359. doi:10.1146/annurev-med-060513-093205. PMID:25587655.

3. Brown JM, Hazen SL. Targeting of microbe-derived metabolites to improve host health: The next frontier for drug discovery. J Biol Chem. 2017;292:8560–8568. doi:10.1074/jbc.R116.765388. PMID:28389555.

4. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. Cell. 2016;165:1332–1345. doi:10.1016/j.cell.2016.05.041. PMID:27259147.

5. Brown AJ, Goldsworthy SM, Barnes AA, Eilert AA, Tcheang L, Daniels D, Muir AI, Wiggins MJ, Kinghorn I, Fraser NJ, et al. The orphan G protein-coupled receptors GPR41 and GPR43 are activated by proprionate and other short chain carboxylic acids. J Biol Chem. 2003;278:13112–13119. doi:10.1074/jbc.M211609200. PMID:12496283.

6. Wahlström A, Sayin SI, Marschall HU, Bäckhed F. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. Cell Metab. 2016;24:41–50. doi:10.1016/j.cmet.2016.05.005. PMID:27320064.

7. Watanabe M, Houten SM, Matakai C, Christofidele MA, Kim BW, Sato H, Messaddeq N, Harney HW, Ezaki O, Kodama T, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. Nature. 2006;439:484–489. doi:10.1038/nature04330. PMID:16400329.

8. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directly matures the host immune system. Cell 2005;122:107–118. doi:10.1016/j.cell.2005.05.007. PMID:16009137.

9. Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, Codelli JA, Chow J, Reisman SE, Petrosino JF, et al. Microbiota modulate behavioral and physiological abnormalities associated with neuro-developmental disorders. Cell. 2013;155:1451–1463. doi:10.1016/j.cell.2013.11.024. PMID:24315484.

10. Ramezani A, Raj DS. The gut microbiome, kidney disease, and targeted interventions. J Am Soc Nephrol. 2014;25:657–670. doi:01.1681/ASN.2013080905. PMID:24231662.

11. Guo L, Lu L, Hua Y, Huang K, Wang I, Huang L, Fu Q, Chen A, Chan P, Fan H, et al. Vasculopathy in the setting of cardiorenal syndrome: Roles of protein-bound uremic toxins. Am J Physiol Heart Circ Physiol. 2017;313:H1–H13. doi:10.1152/ajpheart.00787.2016. PMID:28411233.

12. Cohen LJ, Estherazy D, Kim SH, Lement C, Aguilar RR, Gordon EA, Pickard AJ, Cross JR, Emilano AB, Han SM, et al. Commensal bacteria make GPCR ligands that mimic human signaling molecules. Nature. 2017;549:48–53. doi:10.1038/nature23874. PMID:28854168.

13. Wang Z, Klipfell E, Bennett BJ, Koeth RA, Wang Z, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011;472:57–63. doi:10.1038/nature09922. PMID:21475195.

14. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med. 2013;19:576–585. doi:10.1038/nm.3145. PMID:23563705.

15. Koeth RA, Levison BS, Culley MK, Buffa J, Wang Z, Gregory JC, Org E, Wu Y, Li L, Smith JD, et al.
γ-Butyrobetaine is a proatherogenic intermediate in gut microbiota metabolism of L-carnitine to TMAO. Cell Metab. 2014;20(5):799–812. doi:10.1016/j.cmet.2014.10.006. PMID:25440057.

16. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbiobal metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med. 2013;368:1575–1584. doi:10.1056/NEJMoai1309400. PMID:23614584.

17. Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, Gu X, Huang Y, Zamanian-Daryoush M, Culley MK, et al. Non-lethal inhibition of gut microbiobal trimethylamine production for the treatment of atherosclerosis. Cell. 2015;163:1585–1595. doi:10.1016/j.cell.2015.11.055. PMID:26687352.

18. Warrier M, Shih DM, Burrows AC, Ferguson D, Gromovsky AD, Brown AL, Marshall S, McDaniel A, Schugar RC, Wang Z, et al. The TMAO-generating enzyme flavin monooxygenase 3 is a central regulator of cholesterol balance. Cell Rep. 2015;10:1–13. doi:10.1016/j.celrep.2014.12.036. PMID:25600868.

19. Miao J, Ling AV, Manthena PV, Gearing ME, Graham MJ, Crooke RM, Croce KJ, Esquejo R, McDaniel A, Schugar RC, Wang Z, et al. The TMAO-generating enzyme flavin monooxygenase 3 is a potential player in diabetes-associated atherosclerosis. Nat Commun. 2015;6:6498. doi:10.1038/ncomms7498. PMID:25849138.

20. Schugar RC, Shih DM, Warrier M, Helsley RN, Burrows A, Ferguson D, Brown AL, Gromovsky AD, Heine M, Chatterjee A. The TMAO-producing enzyme flavin-containing monooxygenase 3 regulates obesity and the beiging of white adipose tissue. Cell Rep. 2017;20:279. doi:10.1016/j.celrep.2017.06.053. PMID:28683320.

21. Craciun S, Balskus EP. Microbial conversion of choline to trimethylamine requires a glycyel radical enzyme. Proc Natl Acad Sci USA. 2012;109:21307–21312.; doi:10.1073/pnas.1215689109. PMID:23151509.

22. Kalnins G, Kuka J, Grinberga S, Makrecka-Kuka M, Liepinsh E, Dambrova M, Tars K. Structure and function of CutC choline lyase from human microbiota bacterium klebsiella pneumonae. J Biol Chem. 2015;290:21732–21740. doi:10.1074/jbc.M115.670471. PMID:26187464.

23. Zhu Y, Jameson E, Crosatti M, Schäfer H, Rajakumar K, Bugg TD, Chen Y. Carnitine metabolism to trimethylamine by an usual Rieske-type oxygenase from human microbiota. Proc Natl Acad Sci USA. 2014;111:4268–4273. doi:10.1073/pnas.1316595111. PMID:24591617.

24. Bennett BJ, de Aquiar Vallim TQ, Wang Z, Shih DM, Meng Y, Gregory J, Allayee H, Lee R, Graham M, Crooke R, et al. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. Cell Metab. 2013;17:49–60. doi:10.1016/j.cmet.2012.12.011. PMID:23312283.

25. Yamazaki H, Shimizu M. Survey of variants of human flavin-containing monooxygenase 3 (FM03) and their drug oxidation activities. Biochim Pharmacol. 2013;85:1588–1593. doi:10.1016/j.bcp.2013.03.020. PMID:23567996.

26. Tang WH, Hazen SL. The contributory role of gut microbiota in cardiovascular disease. J Clin Invest. 2014;124:4204–4211. doi:10.1172/JCI72331. PMID:25271725.

27. Aron-Wisnewsky J, Clément K. The gut microbiome, diet, and links to cardiometabolic and chronic disorders. Nat Rev Nephrol. 2016;12:169–181. doi:10.1038/nrneph.2015.191. PMID:26616538.

28. Gregory JC, Buffa JA, Org E, Wang Z, Levison BS, Zhu W, Wagner MA, Bennett BJ, Li L, DiDonato JA, Lusis AJ, Hazen SL. Transmission of atherosclerosis susceptibility with gut microbiota transplantation. J Biol Chem. 2015;290:5547–5560. doi:10.1074/jbc.M114.618249. PMID:25550161.

29. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, Li L, Fu X, Wu Y, Mehrabian M, et al. Gut microbial metabolism of TMAO enhances platelet hyperreactivity and thrombosis risk. Cell. 2016;165:111–124. doi:10.1016/j.cell.2016.02.011. PMID:26972052.

30. Li X, Obied S, Klingenberg R, Gencer B, Mach F, Räber L, Winnacker S, Rodondi N, Nanchen D, Muller O, et al. Gut microbiota-dependent trimethylamine N-oxide in acute coronary syndromes: A prognostic marker for incident cardiovascular events beyond traditional risk factors. Eur Heart J. 2017;38:814–824. doi:10.1038/euheartj.2016.280. PMID:28077467.

31. Zhu W, Wang Z, Tang WH, Hazen SL. Gut microbiobal generated trimethylamine N-oxide from dietary choline is prothrombotic in subjects. Circulation. 2017;135:1671–1673. doi:10.1161/circulationaha.116.025338. PMID:28438808.

32. Parks BW, Nam E, Org E, Kostem E, Norheim F, Hui ST, Pan C, Civelek M, Rau CD, Bennett BJ, et al. Genetic control of obesity and gut microbiota composition in response to high-fat, high-sucrose diet in mice. Cell. 2013;17:141–152. doi:10.1016/j.cell.2012.12.007. PMID:23312289.

33. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell. 1998;92:829–839. PMID:9529258.

34. Yu ZW, Wickman A, Eriksson JW. Cryptic receptors for insulin-like growth factor II in the plasma membrane of rat adipocytes — a possible link to cellular insulin resistance. Biochim Biophys Acta. 1996;1282:57–62. PMID:8679660.

35. Corvera S, Folander K, Clairmont KB, Czech MP. A highly phosphorylated subpopulation of insulin-like growth factor II/manosse-6-phosphate receptors is concentrated in a clathrin-enriched plasma membrane fraction. Proc Natl Acad Sci USA. 1988;85:7567–7571. PMID:2971973.

36. Grusovin J, Macaulay SL. Snares for GLUT4-mechanisms directing vesicular trafficking of GLUT4. Front Biosci. 2003;8:d620–641. PMID:12700065.

37. Foster LJ, Yaworsky K, Trimble WS, Klip A. SNAP23 promotes insulin-dependent glucose uptake in 3T3-L1 adipocytes: Possible interaction with cytoskeleton. Am J Physiol. 1999;275:C1108–C1114. PMID:10439659.

38. Li Q, Korzan WJ, Ferrero DM, Buchi M, Lemon JK, Kaur AW, Stowers L, Fendt M, Liberles SD. Synchronous evolution of an odor biosynthesis pathway in T37 and links to cariometabolic and chronic disorders. Nat Rev Nephrol. 2016;12:169–181. doi:10.1038/nrneph.2015.191. PMID:26616538.