Does Our Gut Microbiome Predict Cardiovascular Risk?
A Review of the Evidence From Metabolomics

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Abstract—Millions of microbes are found in the human gut, and are collectively referred as the gut microbiota. Recent studies have estimated that the microbiota genome contains 100-fold more genes than the host genome. These microbiota contribute to digestion by processing energy substrates unutilized by the host, with about half of the total genome of the gut microbiota being related to central carbon and amino acid metabolism as well as the biosynthesis of secondary metabolites. Therefore, the gut microbiome and its interaction with the host influences many aspects of health and disease, including the composition of biofluids such as urine and blood plasma. Metabolomics is uniquely suited to capture these functional host-microbe interactions. This review aims at providing an overview of recent metabolomics evidence of gut microbiota-host metabolic interactions with a specific focus on cardiovascular disease and related aspects of the metabolic syndrome. Furthermore, the emphasis is given on the complexities of translating these metabolite signatures as potential clinical biomarkers, as the composition and activity of gut microbiome change with many factors, particularly with diet, with special reference to trimethylamine-oxide.

Metabolomics, Metabonomics, and the Gut Microbiome

The word metabolomics,[1–3] and the related term metabonomics,[4,5] is used to describe the use of analytical chemistry techniques to measure the metabolite compliment of a cell, tissue, or organism, often to derive a better understanding of a disease process or a genetic modification. The field was in part developed by the use of nuclear magnetic resonance spectroscopy to follow changes in metabolites found in urine to monitor potential drug toxicity as a part of drug safety assessment. Using such an approach Phipps et al[6] made the intriguing observation that changes in diet in rats could alter the urinary excretion of phenolic compounds, in particular hippuric acid and meta-(hydroxyphenyl)-propionic acid. Through a series of different experiments involving different rat strains and diets, the authors were able to determine that this was a diet-dependent effect, although the diets used did not have a major difference in the content of aromatic amino acids. The authors suggested that in fact the major difference in the excretion of hippuric acid or meta-(hydroxyphenyl)-propionic acid arose from diet-induced alterations in gut microflora. Since this first report of the gut microbiome influencing the urinary metabolomics profile, several studies have observed how the metabolome is influenced by different gut microbes, and there is an increasing evidence that some of these interactions between the gut microflora and the host may result or contribute to disease pathology.[7]

Developing the observations of Phipps et al[6] further, Nicholls et al[8] housed germ-free mice for 21 days in metabolism cages and observed their urinary profiles as the mice established a gut microflora. These changes were fairly dramatic with increases in the concentrations of hippurate, trimethylamine-N-oxide (TMAO), phenylacetylglycine, and 3-hydroxypropionic acid. It is now well established for many types of metabolic profiling studies that 2 metabolomes must be considered for mammals, particularly when examining urinary profile changes.[9–11] Since these reports of gut microflora affecting the metabolism of amines and aromatic metabolites, a wide range of studies have suggested a role for the gut microbiotica in a diverse range of diseases and modifications,[12] including obesity, type 2 diabetes mellitus and aspects of the metabolic syndrome,[13–15] drug metabolism,[9] autism spectral disorder,[16–18] colitis,[19] and Chron disease.[20] Just how large the influence of the gut microbiome has on host metabolism was examined by Wikoff et al,[21] who showed that hundreds of metabolites were perturbed in concentration between germ-free mice and control conventionally raised mice. Indeed certain classes of metabolites were entirely absent from the metabolome of blood plasma from germ-free mice. Many of the identified modifications associated with gut microflora resembled phase 2 drug metabolites.

However, because of the complex interactions between the huge quantities and diverse range of microbes found in the gut and the human body, although we know that gut microbiotica do interact with a diverse range of disease, we still do not understand the underlying mechanisms. This brief review examines the potential role of the gut microbiome in cardiovascular disease (CVD). We first assess how the gut microbiome interacts with diet in healthy individuals, and then its potential role in altering risk of CVD as well as the related comorbidity type 2 diabetes mellitus and aspects of the metabolic syndrome. These studies have centered on a set of metabolites associated with choline metabolism and, in particular the formation of

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TMAO, a metabolite that has been linked to a variety of interventions and diseases, including CVD.

TMAO, the Gut Microbiome, and Metabolomics

One of the most important pathways linked to gut microbial metabolism involves the turnover of choline to produce amines, such as trimethylamine and TMAO. Given this central importance, we felt that it is important to consider how these metabolites are produced in the mammalian body and how physiological changes affect flux through this pathway. TMAO is a ubiquitous metabolite found in a variety of biological fluids and tissues and features as a major source of variation in many metabolomics studies for a range of physiological and pathophysiological states.[22–24] One of the main roles of TMAO in the body is as a renal osmolyte[25,26] and elevation of TMAO concentrations in urine and plasma has previously been associated with renal medullary damage and chronic renal failure.[26,27] The increased concentration of blood TMAO could reflect renal medullary damage as a result of effects on the renin–angiotensin system and hypertension associated with CVD.[28]

TMAO is the result of the N-oxidation of its trimethylamine precursor by liver flavin-containing monooxygenase (FMO) enzymes in the liver, in particular associated with the FMO3 isoform.[29] Trimethylamine itself can be formed by endogenous N-dealkylation of choline by liver cytochrome P450 enzymes, but recent interest has concentrated on the bacterial degradation of choline by the gut microbiome.[30–33] Dietary sources of choline include red meat, eggs, fish, and brassica vegetables and choline can be found in its free form or in an esterified form (phosphocholine, glycerophosphocholine, phosphatidylcholine, and sphingomyelin).[34] However, these forms of dietary choline have different bioavailabilities,[35] with the lipid-soluble forms bypassing the liver, whereas the water-soluble forms enter the enterohepatic circulation.[34] Choline can also undergo metabolism to betaine via a 2-step process involving choline dehydrogenase (EC 1.1.99.1; Figure). Betaine acts as a methyl donor in the conversion of homocysteine to methionine in the presence of betaine-homocysteine methyltransferase (EC 2.1.1.5) and it has been reported that higher concentrations of plasma homocysteine correlate with increased CVD risk.[36]

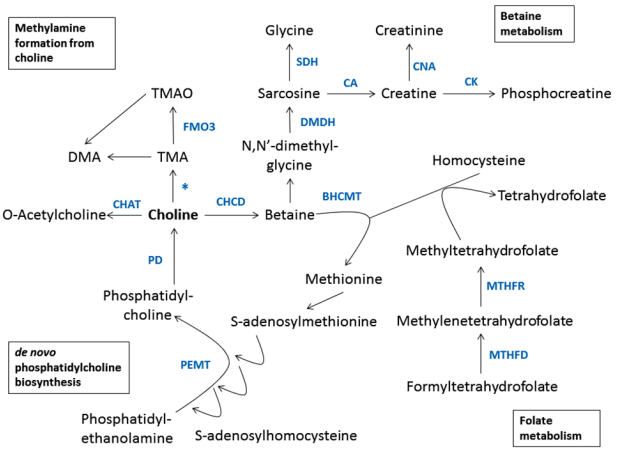

Figure. Choline metabolism: The figure shows key metabolic pathways involved in the degradation of choline and production of urinary amines, in part via the gut microbiome. *Bacterial degradation of choline by the gut microbiome. BHCMT indicates betaine-homocysteine methyl transferase (EC 2.1.1.5); CA, creatinase (EC 3.5.3.3); CHAT, choline O-acetyltransferase (EC 2.3.1.6); CHCD, choline dehydrogenase (EC 1.1.99.1); CK, creatine kinase (EC 2.7.3.2); can, creatininase (EC 3.5.2.10); DMA, dimethylamine; DMDH, dimethylglycine dehydrogenase (EC 1.5.99.2); FMO3, flavin-containing monoxygenase; MTHFD, methylene-tetrahydrofolate dehydrogenase (EC 1.5.1.5); MTHFR, methylene-tetrahydrofolate reductase (EC 1.5.1.20); PD, phospholipase D (EC 3.1.4.4); PEMT, phosphatidylethanolamine methyltransferase (EC 2.1.1.17, 2.1.1.71); SDH, sarcosine dehydrogenase (EC 1.5.99.1); TMA, trimethylamine; and TMAO, trimethylamine-N-oxide.

Sex-related differences in urinary TMAO concentrations have been identified in murine models with female rats[22] and mice[23] showing higher concentrations of TMAO. Urinary TMAO has also been shown to fluctuate at different stages of the rat estrus cycle, which has in turn been linked to changing estrogen concentrations.[24] In humans, the FMO3 enzymes responsible for the N-oxidation of trimethylamine have been shown to be induced by estrogen[37] but suppressed by testosterone,[38] suggesting that sex-based variation in the rate of choline catabolism to form methylamine derivatives is the result of hormonal imprinting of liver enzymes, such as FMO3. Further evidence that estrogens have an important role in the control of TMAO metabolism is the metabolic condition, trimethylaminuria or fish odor syndrome, which is caused by an inherited defect in the FMO3 enzyme.[39] This condition can be exacerbated around puberty and in females the symptoms worsen before and during menstruation and with the use of oral contraceptives as a result of the hormonal inhibition of the FMO3 enzyme.[40] It is postulated that premenopausal female subjects also have a different choline requirement to males to support fetal development[34] particularly at the point where choline turnover links to folate metabolism and with the conversion of homocysteine to methionine (Figure). Estrogens are also thought to be a mediator of increased de novo biosynthesis of phosphatidylcholine, catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT, EC 2.1.1.17) which has been shown to be greater in female mice.[41]

The Gut Microbiome, CVD, and the Metabolic Syndrome

One of the most compelling studies to link gut microbiome changes to increased obesity and the risk of developing type 2 diabetes mellitus was conducted by Turnbaugh et al[42] using obese and lean mice. They demonstrated that the microbiome associated with obese mice was capable of extracting more calorific value from food compared with lean animals, and this ability was transferable by the transplantation of feces into germ-free mice. Furthermore, when the innate immune system is compromised as in Toll-like receptor 5 knock-out mice, it has been demonstrated that the gut microbiome can contribute to the development of type 2 diabetes mellitus and obesity.[43] In humans, although the gut microbiome is shared to a degree between family members, it can also be influenced by obesity, which is associated with a decrease in diversity of the microbial community in weight discordant and concordant twins.[44] Oral bacteria have also been associated with increased CVD, and bacteria associated with oral cavities have been found in atherosclerotic plaques.[45]

From these studies, there is clearly an important contribution that the gut microbiome plays in a variety of metabolic disorders, including obesity, insulin resistance, and CVD.

These relationships are also supported in the metabolomics literature. Dumas et al[46] used 1H NMR spectroscopy to examine the plasma and urine metabolic profiles of mice (129S6) prone to developing nonalcoholic fatty liver disease, determining that liver pathology was associated with changes in choline metabolism, in particular with increased excretion of amines, such as dimethylamine, trimethylamine, and TMAO derived in part by gut microbial metabolism. The authors suggested that the degradation of choline by the gut microbiome reduces the availability of choline and mimics a choline-deficient diet, which in turn is known to induce nonalcoholic fatty liver disease. Dumas et al[47] further went on to define the interaction between host and microbial metabolism by conducting chromosomal mapping in control and diabetic rats, and then cross correlating this information with metabolic profiles measured in plasma using 1H NMR spectroscopy. Using this approach, they identified a correlation between benzoate and uridine diphosphate glucuronosyltransferase, identifying a metabotypic quantitative trait loci that relied on a strong gut microbe/host interaction.

Velagapudi et al[48] examined the blood serum from germ-free mice and conventionally raised mice and correlated changes in blood metabolites with alterations in the lipidome of the liver and adipose tissue. As with similar studies, they revealed profound changes in blood serum metabolism with conventionally raised animals having increased pyruvic acid, citric acid, fumaric acid, and malic acid, and reduced concentrations of cholesterol and fatty acids. More remarkably, triglyceride and phosphocholine metabolism was also altered in the liver and adipose tissue, with conventionally raised mice having improved clearance rate of triglycerides. Given that bile acids have a key role in terms of lipid uptake from the gut, Sayin et al[49] identified a novel key regulatory role for gut microflora in terms of bile acid synthesis. They demonstrated a signaling pathway between bile acid synthesis and activation of the nuclear receptor farnesoid X receptor (FXR), which relied on gut microbiotica regulated by the production of β- and α-muricholic acid in the gut. Activation of FXR in turn reduced the production of bile acids in the liver. Li et al[50] further defined the role of bile acid metabolism by gut microflora by showing that tempol, an antioxidant which reduces obesity in mice, alters the gut microbiome to increase the production of tauro-β-muricholic acid, which is an FXR antagonist. When they compared FXR-null mice with wild-type controls on a high-fat diet, the mutant mice showed less obesity, suggesting that intestinal FXR mediates the effects of tempol.

Wang et al[30] applied metabolomics to identify potential metabolites of CVD by looking at 50 individuals who went into hospital for elective cardiac evaluation and then subsequently experienced a myocardial infarction, stroke, or death during the next 3 years, along with 50 age-matched controls. The analysis was performed using a nontargeted liquid chromatography–mass spectrometry approach, and to validate the approach, a further group of 25 subjects and 25 controls were analyzed in a validation group. This analysis identified 18 metabolites in both studies that robustly discriminated the disease group from the controls in both the biomarker discovery and validation tests. Of these, 3 metabolites of phosphatidylcholine metabolism, choline, TMAO, and betaine were particularly strong at predicting subsequent CVD disease. To test causality, these metabolites were then supplemented into the diet of apolipoprotein E–null mice, which increased the inflammatory state of the CVD system by upregulating multiple macrophage receptors linked to atherosclerosis. Given, as we have seen above, that the production of TMAO is regulated in part by gut microflora, the authors then examined what would happen in germ-free animals, where TMAO production was prevented, and so too was the stimulation of the inflammatory state in apolipoprotein E mice after choline feeding, as well as producing a reduction in aortic plaque size in the mice.

Although the study as a whole makes an impressive argument for the role of gut microflora in CVD, mediated through the break down of choline to produce TMAO, it did raise several questions. As we have seen, there is an important influence of diet on the urinary and blood plasma concentrations of TMAO, betaine, and choline. First, red meat consumption will increase TMAO excretion, and increased red meat consumption is associated with CVD, so could TMAO actually be a biomarker for red meat consumption in the human cohorts examined? Furthermore, metabolomics investigations have found that plasma and urine concentrations of TMAO increase dramatically with increasing consumption of fish, but the evidence is that increased fish consumption is protective from CVD.[51–55]

Furthermore, metabolites produced by the oxidation of choline have been shown to be poor markers of plaque formation in the mouse. We have previously shown that the oxidation of choline is increased by feeding C57BL mice, a diet containing a high-fat and cholate, but decreased in the low-density lipoprotein receptor knock-out mouse, a transgenic mouse model with a known predisposition to CVD.[56] Furthermore, betaine supplementation is reported to attenuate atherosclerotic lesion in apolipoprotein $E^{-/-}$ mice.[57] In animal models, it also reduces plasma homocysteine concentration,[58] a known atherosclerotic risk factor that is associated with enhanced vascular inflammation and oxidative stress.[59] These studies raise the question as to whether the correlation observed by Wang et al[30] is causative, as claimed in the study, or a surrogate marker of the effect. Furthermore, the dietary variation we describe above will affect the use of TMAO as a potential biomarker because the sources cannot be delineated from a measure of the plasma. Hence, the background effect for the use of TMAO as a biomarker is limited to situations where exclusion criteria are applied before sampling and such criteria will be strict. However, the relationship between CVD and TMAO concentrations has been borne out in a larger study of >4000 individuals, where TMAO was again found to be correlated with subsequent CVD events,[31] even after correcting for major risk factors, including age, sex, smoking, systolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and type 2 diabetes mellitus status. Thus, if TMAO is a surrogate marker for another pathological process, it must be separate to these classic risk factors.

To characterize the role of TMAO in CVD further, Bennett et al[60] examined the regulation of TMAO concentrations in mice, identifying that FMO 1 and 3 are responsible for the conversion of trimethylamine to TMAO, with FMO3 having the highest activity. Intriguingly, the expression of FMO3 is induced by dietary bile acids involving the FXR, linking lipid uptake to TMAO concentrations. In mice, the induction of FMO1 and 3 was in part negatively regulated by the production of testosterone, which the authors use to explain the greater susceptibility of female mice to atherosclerosis, in marked contrast to human disease.

High urine concentrations of TMAO directly correlate to the consumption of a high-meat content diet[61] and this can be attributed to high concentrations of not only choline but also carnitine (found in high concentrations in red meat, fish, and dairy products as well as some energy drinks and diet supplements), which has also been shown to be associated with bacterial degradation to the trimethylamine precursor.[62] It has also been observed that vegetarian and vegan subjects who consume a single meal of meat had lower blood levels of TMAO than those eating a regular omnivorous diet which was attributed to the lower levels of the intestinal bacteria responsible for the catabolism of carnitine to trimethylamine in vegetarian and vegan subjects.[62] However, these studies were rather underpowered and further clinical studies are required to fully understand the relationship between the dietary sources of trimethylamine (whether from dietary choline or carnitine), the role of the liver FMO3 enzymes responsible for the N-oxidation of trimethylamine to TMAO and the potential imprinting of the gut microbiome from different baseline diets (ie, omnivorous, pescetarian, vegetarian, and vegan). Furthermore, Ussher et al[63] have reviewed both the positive and negative effects of carnitine supplementation, suggesting that potential negative effects associated with raised production of TMAO contributing to CVD may be balanced by increased glucose metabolism in muscle and heart.

In addition to interactions where the gut microbiome influences the health of the host, there are also examples where surgical intervention on the host, in turn, affects the microbiome. Ashrafian et al[64] examined Roux-en-Y gastric bypass surgery in the Wistar rat using a combination of ^{1}H NMR spectroscopy and liquid chromatography–mass spectrometry–based metabolomics to study metabolic changes in blood plasma and cardiac tissue. In humans, the Roux-en-Y gastric bypass procedure has a profound effect on patient health, often dramatically improving insulin resistance and reducing cardiovascular risk. This approach identified perturbations in a variety of metabolic pathways, including bile acids, phosphocholines, amino acids, nucleosides and amines in blood plasma, and glycogen and amino acids in the heart. The authors suggest that these changes were in part mediated by the alterations in gut microflora after the gastric bypass, demonstrating the complexities between the host–gut microbiome interactions.

Conclusions

There is an increasing evidence that the gut microbiome has a profound effect on the systemic health of the host for a range of diseases. Obesity, type 2 diabetes mellitus, and the metabolic syndrome are some of the best characterized pathologies that have been identified as examples of these host–gut microbiome interactions, and metabolomics is increasingly being used to understand how the gut microbiome may aggravate disease processes. This has identified many metabolites that are readily observed in human blood plasma and urine that could be used to follow these interactions. There is also an evidence that some of these metabolites could be used to identify patients at risk of disease, although given the dynamic nature of the gut microbiome one must take care with these correlations, particularly in terms of whether these changes are causative or a consequence of the pathology. Furthermore, diet has an even more profound effect on the gut microbiome and we must be vigilant that suggested biomarkers of obesity and cardiovascular risk are not proxies for dietary changes.

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Disclosures

None.

References

1. Paton NW, Khan SA, Hayes A, Moussouni F, Brass A, Eilbeck K, et al. Conceptual modelling of genomic information. *Bioinformatics*. 2000;16:548–557.
2. Raamsdonk LM, Teusink B, Broadhurst D, Zhang N, Hayes A, Walsh MC, et al. A functional genomics strategy that uses metabolome data to reveal the phenotype of silent mutations. *Nat Biotechnol*. 2001;19:45–50. doi: 10.1038/83496.
3. Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chem Soc Rev*. 2011;40:387–426. doi: 10.1039/b906712b.
4. Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica*. 1999;29:1181–1189. doi: 10.1080/004982599238047.
5. Nicholson JK, Connelly J, Lindon JC, Holmes E. Metabonomics: a platform for studying drug toxicity and gene function. *Nat Rev Drug Discov*. 2002;1:153–161. doi: 10.1038/nrd728.
6. Phipps AN, Stewart J, Wright B, Wilson ID. Effect of diet on the urinary excretion of hippuric acid and other dietary-derived aromatics in rat. A complex interaction between diet, gut microflora and substrate specificity. *Xenobiotica*. 1998;28:527–537. doi: 10.1080/004982598239443.
7. Goodacre R. Metabolomics of a superorganism. *J Nutr*. 2007;137(suppl 1):259S–266S.
8. Nicholls AW, Mortishire-Smith RJ, Nicholson JK. NMR spectroscopic-based metabonomic studies of urinary metabolite variation in acclimatizing germ-free rats. *Chem Res Toxicol*. 2003;16:1395–1404. doi: 10.1021/tx0340293.
9. Wilson ID, Nicholson JK. The role of gut microbiota in drug response. *Curr Pharm Des*. 2009;15:1519–1523.
10. Nicholson JK, Holmes E, Lindon JC, Wilson ID. The challenges of modeling mammalian biocomplexity. *Nat Biotechnol*. 2004;22:1268–1274. doi: 10.1038/nbt1015.
11. Nicholson JK, Holmes E, Wilson ID. Gut microorganisms, mammalian metabolism and personalized health care. *Nat Rev Microbiol*. 2005;3:431–438. doi: 10.1038/nrmicro1152.
12. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota metabolic interactions. *Science*. 2012;336:1262–1267. doi: 10.1126/science.1223813.
13. Cani PD, Delzenne NM. Gut microflora as a target for energy and metabolic homeostasis. *Curr Opin Clin Nutr Metab Care*. 2007;10:729–734. doi: 10.1097/MCO.0b013e3282efdebb.
14. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*. 2008;57:1470–1481. doi: 10.2337/db07-1403.
15. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One*. 2010;5:e9085. doi: 10.1371/journal.pone.0009085.
16. Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*. 2013;155:1451–1463. doi: 10.1016/j.cell.2013.11.024.
17. Clayton TA. Metabolic differences underlying two distinct rat urinary phenotypes, a suggested role for gut microbial metabolism of phenylalanine and a possible connection to autism. *FEBS Lett*. 2012;586:956–961. doi: 10.1016/j.febslet.2012.01.049.
18. Yap IK, Angley M, Veselkov KA, Holmes E, Lindon JC, Nicholson JK. Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls. *J Proteome Res*. 2010;9:2996–3004. doi: 10.1021/pr901188e.

19. Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature*. 2012;487:477–481. doi: 10.1038/nature11228.
20. Sinagra E, Tomasello G, Cappello F, Leone A, Cottone M, Bellavia M, et al. Probiotics, prebiotics and symbiotics in inflammatory bowel diseases: state-of-the-art and new insights. *J Biol Regul Homeost Agents*. 2013;27:919–933.
21. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci U S A*. 2009;106:3698–3703. doi: 10.1073/pnas.0812874106.
22. Stanley EG, Bailey NJ, Bollard ME, Haselden JN, Waterfield CJ, Holmes E, et al. Sexual dimorphism in urinary metabolite profiles of Han Wistar rats revealed by nuclear-magnetic-resonance-based metabonomics. *Anal Biochem*. 2005;343:195–202. doi: 10.1016/j.ab.2005.01.024.
23. Gavaghan McKee CL, Wilson ID, Nicholson JK. Metabolic phenotyping of nude and normal (Alpk:ApfCD, C57BL10J) mice. *J Proteome Res*. 2006;5:378–384. doi: 10.1021/pr050255h.
24. Bollard ME, Holmes E, Lindon JC, Mitchell SC, Branstetter D, Zhang W, et al. Investigations into biochemical changes due to diurnal variation and estrus cycle in female rats using high-resolution (1)H NMR spectroscopy of urine and pattern recognition. *Anal Biochem*. 2001;295:194–202. doi: 10.1006/abio.2001.5211.
25. Balaban RS, Knepper MA. Nitrogen-14 nuclear magnetic resonance spectroscopy of mammalian tissues. *Am J Physiol*. 1983;245(5 pt 1):C439–C444.
26. Bell JD, Lee JA, Lee HA, Sadler PJ, Wilkie DR, Woodham RH. Nuclear magnetic resonance studies of blood plasma and urine from subjects with chronic renal failure: identification of trimethylamine-N-oxide. *Biochim Biophys Acta*. 1991;1096:101–107.
27. Hauet T, Baumert H, Gibelin H, Hameury F, Goujon JM, Carretier M, et al. Noninvasive monitoring of citrate, acetate, lactate, and renal medullary osmolyte excretion in urine as biomarkers of exposure to ischemic reperfusion injury. *Cryobiology*. 2000;41:280–291. doi: 10.1006/cryo.2000.2291.
28. Madsen K, Tinning AR, Marcussen N, Jensen BL. Postnatal development of the renal medulla; role of the renin-angiotensin system. *Acta Physiol (Oxf)*. 2013;208:41–49. doi: 10.1111/apha.12088.
29. Lang DH, Yeung CK, Peter RM, Ibarra C, Gasser R, Itagaki K, et al. Isoform specificity of trimethylamine N-oxygenation by human flavin-containing monooxygenase (FMO) and P450 enzymes: selective catalysis by FMO3. *Biochem Pharmacol*. 1998;56:1005–1012.
30. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011;472:57–63. doi: 10.1038/nature09922.
31. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med*. 2013;368:1575–1584. doi: 10.1056/NEJMoa1109400.
32. Loscalzo J. Gut microbiota, the genome, and diet in atherogenesis. *N Engl J Med*. 2013;368:1647–1649. doi: 10.1056/NEJMe1302154.
33. Rak K, Rader DJ. Cardiovascular disease: the diet-microbe morbid union. *Nature*. 2011;472:40–41. doi: 10.1038/472040a.
34. Zeisel SH. Choline: critical role during fetal development and dietary requirements in adults. *Annu Rev Nutr*. 2006;26:229–250. doi: 10.1146/annurev.nutr.26.061505.111156.
35. Cheng W-L, Holmes-McNary MQ, Mar M-H, Lien EL, Zeisel SH. Bioavailability of choline and choline esters from milk in rat pups. *J Nutr Biochem*. 1996;7:457–464.
36. Guba SC, Fink LM, Fonseca V. Hyperhomocysteinemia. An emerging and important risk factor for thromboembolic and cardiovascular disease. *Am J Clin Pathol*. 1996;106:709–722.
37. Lattard V, Lachuer J, Buronfosse T, Garnier F, Benoit E. Physiological factors affecting the expression of FMO1 and FMO3 in the rat liver and kidney. *Biochem Pharmacol*. 2002;63:1453–1464.
38. Ayesh R, Mitchell SC, Smith RL. Dysfunctional N-oxidation of trimethylamine and the influence of testosterone treatment in man. *Pharmacogenetics*. 1995;5:244–246.
39. Mitchell SC, Smith RL. Trimethylaminuria: the fish malodor syndrome. *Drug Metab Dispos*. 2001;29(4 pt 2):517–521.
40. Li M, Al-Sarraf A, Sinclair G, Frohlich J. Fish odour syndrome. *CMAJ*. 2011;183:929–931. doi: 10.1503/cmaj.100642.
41. Noga AA, Vance DE. A gender-specific role for phosphatidylethanolamine N-methyltransferase-derived phosphatidylcholine in the regulation of plasma high density and very low density lipoproteins in mice. *J Biol Chem*. 2003;278:21851–21859. doi: 10.1074/jbc.M301982200.
42. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006;444:1027–1031. doi: 10.1038/nature05414.
43. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science*. 2010;328:228–231. doi: 10.1126/science.1179721.
44. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. *Nature*. 2009;457:480–484. doi: 10.1038/nature07540.
45. Koren O, Spor A, Felin J, Fåk F, Stombaugh J, Tremaroli V, et al. Human oral, gut, and plaque microbiota in patients with atherosclerosis. *Proc Natl Acad Sci U S A*. 2011;108 Suppl 1:4592–4598. doi: 10.1073/pnas.1011383107.
46. Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc Natl Acad Sci U S A*. 2006;103:12511–12516. doi: 10.1073/pnas.0601056103.
47. Dumas ME, Wilder SP, Bihoreau MT, Barton RH, Fearnside JF, Argoud K, et al. Direct quantitative trait locus mapping of mammalian metabolic phenotypes in diabetic and normoglycemic rat models. *Nat Genet*. 2007;39:666–672. doi: 10.1038/ng2026.
48. Velagapudi VR, Hezaveh R, Reigstad CS, Gopalacharyulu P, Yetukuri L, Islam S, et al. The gut microbiota modulates host energy and lipid metabolism in mice. *J Lipid Res*. 2010;51:1101–1112. doi: 10.1194/jlr.M002774.
49. Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall HU, Bamberg K, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab*. 2013;17:225–235. doi: 10.1016/j.cmet.2013.01.003.
50. Li F, Jiang C, Krausz KW, Li Y, Albert I, Hao H, et al. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. *Nat Commun*. 2013;4:2384. doi: 10.1038/ncomms3384.
51. Zuppi C, Messana I, Forni F, Rossi C, Pennacchietti L, Ferrari F, et al. 1H NMR spectra of normal urines: reference ranges of the major metabolites. *Clin Chim Acta*. 1997;265:85–97.
52. Svensson BG, Akesson B, Nilsson A, Paulsson K. Urinary excretion of methylamines in men with varying intake of fish from the Baltic Sea. *J Toxicol Environ Health*. 1994;41:411–420. doi: 10.1080/15287399409531853.
53. Zhang AQ, Mitchell SC, Smith RL. Dietary precursors of trimethylamine in man: a pilot study. *Food Chem Toxicol*. 1999;37:515–520.
54. Lenz EM, Bright J, Wilson ID, Hughes A, Morrisson J, Lindberg H, et al. Metabonomics, dietary influences and cultural differences: a 1H NMR-based study of urine samples obtained from healthy British and Swedish subjects. *J Pharm Biomed Anal*. 2004;36:841–849. doi: 10.1016/j.jpba.2004.08.002.
55. Stella C, Beckwith-Hall B, Cloarec O, Holmes E, Lindon JC, Powell J, et al. Susceptibility of human metabolic phenotypes to dietary modulation. *J Proteome Res*. 2006;5:2780–2788. doi: 10.1021/pr060265y.
56. Cheng KK, Benson GM, Grimsditch DC, Reid DG, Connor SC, Griffin JL. Metabolomic study of the LDL receptor null mouse fed a high-fat diet reveals profound perturbations in choline metabolism that are shared with ApoE null mice. *Physiol Genomics*. 2010;41:224–231. doi: 10.1152/physiolgenomics.00188.2009.
57. Lv S, Fan R, Du Y, Hou M, Tang Z, Ling W, et al. Betaine supplementation attenuates atherosclerotic lesion in apolipoprotein E-deficient mice. *Eur J Nutr*. 2009;48:205–212. doi: 10.1007/s00394-009-0003-4.
58. Schwahn BC, Wang XL, Mikael LG, Wu Q, Cohn J, Jiang H, et al. Betaine supplementation improves the atherogenic risk factor profile in a transgenic mouse model of hyperhomocysteinemia. *Atherosclerosis*. 2007;195:e100–e107. doi: 10.1016/j.atherosclerosis.2007.06.030.
59. Hofmann MA, Lalla E, Lu Y, Gleason MR, Wolf BM, Tanji N, et al. Hyperhomocysteinemia enhances vascular inflammation and accelerates atherosclerosis in a murine model. *J Clin Invest*. 2001;107:675–683. doi: 10.1172/JCI10588.
60. Bennett BJ, de Aguiar Vallim TQ, Wang Z, Shih DM, Meng Y, Gregory J, 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.
61. Stella C, Beckwith-Hall B, Cloarec O, Holmes E, Lindon JC, Powell J, et al. Susceptibility of human metabolic phenotypes to dietary modulation. *J Proteome Res*. 2006;5:2780–2788. doi: 10.1021/pr060265y.
62. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, 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.
63. Ussher JR, Lopaschuk GD, Arduini A. Gut microbiota metabolism of L-carnitine and cardiovascular risk. *Atherosclerosis*. 2013;231:456–461. doi: 10.1016/j.atherosclerosis.2013.10.013.
64. Ashrafian H, Li JV, Spagou K, Harling L, Masson P, Darzi A, et al. Bariatric surgery modulates circulating and cardiac metabolites. *J Proteome Res*. 2014;13:570–580. doi: 10.1021/pr400748f.

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