

TMAO FROM DIETARY CHOLINE

Abstract

Diet plays very significant role in maintaining overall health of well being but certain alterations in significant amounts of nutrients can cause various health discomforts among which one is an oxidative compound TMAO (Trimethylamine N Oxide) a metabolite from dietary choline present in many foods and taken as vital nutrient to maintain body vital functions and its deficiency can cause fatality . However its increase in blood serums ie the increase of its metabolite can lead toxic damage thus involve various life threatening diseases like ckd, cardiovascular disease, obesity, metabolic errors and severity to death. The chapter details about choline as a dietary compound and hazards of its metabolites If not consumed in significant way. This emerging micro metabolite lead new trends of studying health complications both in medical and nutritional way.To promote optimal health as well as to approach nutrition goals in holistic way TMAO is a choice of concern. Optimal health at cellular level is the key factor of every diet incorporation. Thus chapter deals with food development enhancing food therapy in considering TMAO based on various investigations and paper researches and its effect on people with or without disease.

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Choline is a type of nutrient which is present in certain foods naturally. The compound gains its essentiality as it helps to synthesize phospholipids which helps in the formation of plasma membranes. It is a water-soluble organic compound, and has similarities with **vitamin B complex**. Infact, it affects a number of vital body functions. It impacts liver function, development in brain, muscular movement, nervous system and body metabolism. Choline is needed to make fats which is essential to maintain structure of cell membranes. It does the formation of cell messengers. It helps in removing cholesterol from liver. Choline with other vitamins, such as B12 and folate, helps in synthesis of DNA. From choline an important neurotransmitter acetylcholine is formed which helps in muscle movement, regulating heart beat, memory and other functions. Deficiency of choline can lead to mostly liver disease and the groups who are at the higher risks are pregnant women, endurance athletes, postmenopausal women and those who consume alcohol.

I. TMAO AND CHOLINE

Gut bacteria can convert nutrient choline into Trimethylamine N –oxide(TMAO).TMAO is a compound which is linked to cardiovascular disease though its significance depends on the amount of choline present in the body and gut microbiota of an individual. Studies suggest choline which is found in beef liver, eggs, chicken liver, salmon, fresh cod can raise TMAO levels in individuals and can cause platelet aggregation while as plant source choline from cauliflower, broccoli, soyabean oil have no affect on individuals with chronic disease.

II. DETAILED CONFIGURATION OF CHOLINE METABOLITES

As we know dietary choline is important though its vitality to perform vast functions in an individual despite excess intake lead to several repercussions. According to National library of Medicine the metabolism of choline shows relation to the pathogenesis of atherosclerosis (thickening or hardening of the arteries caused by a buildup of plaque in the inner lining of an artery) by inflammation. Phosphatidylcholine gives the component choline which follows oxidation and forms precursor to betaine. Choline and other trimethylamine (TMA) containing species like carnitine are metabolized to TMA by the gut microbiota. TMA is subsequently oxidized by at least one member of the flavin-containing mono-oxygenases, FMO3, forming trimethylamine-N-oxide (TMAO), which is then released into circulation. Several studies have shown that an elevated level of TMAO increases the risk of atherosclerosis and subsequently of cardiovascular disease. Also, a recent study revealed a link between the TMAO-producing enzyme FMO3 and obesity. Further it has been revealed that the relationship between circulating levels of choline, betaine, carnitine, TMA and TMAO cause inflammatory disease affecting the joints and connective tissue and is associated with psoriasis of the skin and nails. A recent study showed that expression of FMO3 in adipose tissue in overweight patients positively correlated with BMI and waist-to-hip ratio, and negatively correlated with insulin sensitivity suggesting a link between TMAO-producing enzyme FMO3 and obesity.

III. PROPERTIES OF TMAO

TMAO an amine compound with formula $(\text{CH}_3)_3\text{NO}$ with molecular weight 75.1 Da. It's obtained chemically from TMA as formation is mentioned above by oxidation reaction and is usually in the form of dihydrate. Its physical appearance is colorless and is odorless with water solubility. It has lot of physiological and biochemical functions in the stability of protein structure, in osmotic regulation, resistance to ionic stability and water pressure. Its synthesis is influenced by three factors;

1. On composition of food precursors. Researches show that higher dietary phosphatidylcholine leads to enhanced plasma TMAO concentrations in humans.
2. Intestinal microbial activity and
3. Third factor is oxidation of TMA.

IV. GUT MICROBIOTA AND TMAO

Studies have suggested that TMAO is a co-metabolite of the gut microbiota. Dietary choline or L-carnitine is metabolised by gut microbiota into TMA in the intestine. Researches also end up in germfree gut cannot produce TMA thus cause increase in choline further antibiotic treatment decrease TMA levels. Thus it indicates irreplaceable role of gut microbiota in synthesis of TMAO. The distinct bacteria participating in TMA formation have been identified, including

Anaerococcus hydrogenalis(bacteria present in vaginal discharges and ovarian abscesses), *Clostridium asparagiforme*(anaerobic gramnegative bacterium causing agent of tyzzer's disease) , *Clostridium hathewayi*(bacterium cause hepatic abscess and is isolated from feces of healthy human), *Clostridium sporogenes*, *Edwardsiella tarda*(pathogen causing intestinal and extra intestinal infections), *Escherichia fergusonii*(it cause urinary tract infections), *Proteus penneri*(bacterium causing gastroenteritis UTI and wound infections), and *Providencia rettgeri*(bacterium found in both water and land environments). Under anaerobic conditions,

TMAO is an electron acceptor for some bacteria species (e.g., **Escherichia coli**) that colonize the human intestine. (source net).

V. TMAO AND WESTERN DIET

According to Journal of thrombosis and haemostasis, Trimethylamine N-oxide (TMAO) enhances platelet hyper-reactivity and increases thrombosis risk after eating a Western diet. Western diet, predispose to chronic disease such as, CVD, colorectal cancer and type-2 diabetes (T2D). The Western diet contains excessive saturated and trans type saturated fatty acids, animal protein and simple sugars, and are low in fiber, vegetables and other plant-based foods. However, studies have shown evidence that high-protein and high-fat diets are associated with gut microbiota that are associated with *Bacterial* enterotype; whereas diets rich in carbohydrates and simple sugars are associated with a *Prevotella* enterotype. High-fat diets have shown increase in plasma TMAO levels. Advanced TMAO levels induce cardiovascular and kidney

damage and full fat dietary products plays significant role in enhancing these levels. Processed meat and ham, pork, cream cheese and butter are good sources to contribute higher levels of TMAO. TMAO significantly increased Ces1 protein (*liver carboxylesterase 1 is an enzyme that in human is encoded by the CES1 gene and it plays role in metabolism of several drugs*) expression activity and clopidogrel (*an anti platelet medicine which prevents platelets to stick together so as to prevent formation of blood clot*) hydrolysis in the liver as well as intracellular ROS (*stress induced reactive oxygen species*) and CES1 levels and Nrf2 gene nucleus translocation in HepG2 (*it's a human liver cancer cell line*) cells decreased the formation of clopidogrel active metabolite. Plasma TMAO, protein levels of clopidogrel-metabolizing enzymes in the liver and adenosine diphosphate-induced platelet aggregation thus cause thrombosis.

VI. TMAO AND CKD

Chronic kidney disease (CKD) is a global problem which leads to end stage renal disease (ESRD). It's basically the chronic inflammation caused by uremic toxins, oxidative stress and microbial infections. These inflammations may vary with renal circulations and regulations which aggravate renal injury. In CKD IL-1, IL-6 and TNF which are several cytokines are increasing significantly and these are related with poor prognosis of CKD such as albuminuria and loss of renal function.

In CKD imbalance in intestinal flora leads to immune disorders. Studies suggested that intestinal flora of CKD patients decreased significantly in probiotics while as it increases in toxigenic flora. Indoxyl sulphate and p-cresyl sulphate have been to promote pro inflammatory cytokines.

By metabolising foods that contain choline, lecithin, betaine, carnitine TMAO is produced by gut microflora. It's dominantly excreted by kidneys so its level in ESRD patients is increased. It has been demonstrated that choline TMA-lyase inhibitor can suppress renal tubulointerstitial fibrosis caused by high choline diet. In 2006, Bain et al. showed that the concentrations of TMA and TMAO in pre-dialysis plasma were significantly higher than the corresponding levels in healthy ones. In addition Stubbs et al. described a strong inverse association of serum TMAO concentration with estimated glomerular filtration rate (eGFR). It has been suggested that this was due to the decreased GFR rather than tubular dysfunction. GFR measuring rate determine the association between plasma TMAO and CKD stages 3 to 5. Moreover, some studies have reported that the elevation of TMAO in CKD is associated with an increased risk of mortality. Although a 2.5 to 40-fold increase in TMAO levels has been reported in HD patients when compared to normal kidney function.

VII. DIET AND TMAO: FOOD DEVELOPMENT ENHANCING FOOD THERAPY

According to institute of food science and research (V.Garcia and C.Simo) their study shows link between TMAO and pathogenic molecular processes which are associated with atherosclerosis and thrombosis. Choline metabolite is on the trend as it has become a new therapeutic target for the prevention and treatment of Cardiovascular disease. In different

biochemical and physiological pathways various strategies have been put forward in order to reduce TMAO levels. Limiting the consumption of food enriched in dietary trimethylamines or choline and some animal source foods could help in reducing various diseases.

The inhibition of hepatic FMO3 activity is another approach but it has been seen the impaired FMO3 activity gives rise to the inherited disorder trimethylaminuria (TMAU), a condition called *Fish odor syndrome*. Individuals with such effect cannot produce TMAO and excrete large amounts of odorous TMA in their urine, sweat, and breath. On another part studies and experimentation done on mice suggest hepatic FMO3 is increased in insulin-resistance and recent research by Chen *et al.* (2019) suggests that FMO3 inhibition could be a suitable target to control TMAO production. The data shows that FMO3 inhibition by dietary 3,3'-diindolylmethane or indole-3-carbinol (phytochemicals in cruciferous vegetables such as cauliflower and Brussel sprouts) supplementation reduced hepatic TMAO in insulin-resistant humans leading to a decrease in activated PERK and FoxO1 (gene that regulates cellular metabolism in the liver, adipose tissue and pancreas) and improved glucose tolerance. It was reasoned that partial inhibition of FMO3 (gene that provides instructions for making enzyme which is the part of larger enzyme family flavin containing dimethylaniline) activity could be a tradeoff for having the beneficial TMAO-lowering effects without causing odor.

Oral administration of probiotics alter gut microflora. These have beneficial effect on health as it reduces the extent of atherosclerosis being non-invasive and to have little or no side effects. Din *et al.* (2019) reviewed the various possible mechanisms by which probiotics could modulate the microbial metabolism and antagonize other strains to attenuate CVD.

Dietary fibers are known to provide direct benefits, that is increase in fecal bulking and laxation. Dietary fibers have the ability to modify microbiota structure and function, and decrease CVD risk, it made them suitable for functional food development. Water-soluble dietary fiber has been recognized to exert beneficial effects on health by different ways. The effects of supplementation of soluble fibers on TMA/TMAO metabolism and the related gut microbiota have recently gained great attention. For instance, Cheng *et al.* (2017) observed that galacto-oligosaccharides plus inulin and ordinary dietary fiber, supplemented individually or mixed for three weeks, had different effects on the gut microecology. Results revealed that functional oligosaccharides (galacto-oligosaccharides and inulin) and ordinary dietary fiber supplementations elevated TMAO levels, while as the combination of both types of fibers favored reduction of TMAO levels in blood serum.

TMAO is considered a uremic toxin, and it reaches maximum circulating levels in individuals with CKD. A report by Hill *et al.* (2019) explored the feasibility of consuming a high β -glucan oat supplement and its effect on uremic toxins and mineral metabolism in CKD patients. Consuming such supplement cause serum TMAO level decreased by 17% and after discontinuation of the β -glucan oat supplement, TMAO levels were raised. It could be indicative that β -glucan exerts its greatest effects in CKD patients with high baseline TMAO concentrations.

The study report by Obeid *et al.* (2016) showed the effects of dietary supplements other than TMA precursors on plasma TMAO levels in humans. The study was conducted on volunteers without prevalent obesity and diabetes which were supplemented with either vitamin D and calcium, folic acid, and vitamins B5 and B12 for 12 months, a 67 percent reduction of plasma TMAO was observed with these supplementation therapy.

VIII. MEDITERRANEAN DIET (MD)

Mediterranean diet (MD) is a diet that revolutionized the diet and is based on abundant plant-based foods like (fruit, nuts, vegetables, legumes, cereals), where olive oil is prioritizing the source of fat, intakes of fish, consumption of eggs, poultry and dairy products (i.e cheese and yogurt). It is characterised by low or avoidance of consumption of processed meats, red meat and sweets. It also accounts for low wine consumption. It lowers the risk of CardioVascular Diseases and certain types of cancer. MD has been attributed to eating and lifestyle behaviors. Dietary interventions with MD have shown an inverse association between adherence to the TMAO and cardiovascular risk.

IX. SUMMARY

Dietary Choline plays vital role in maintaining body's vital function. It promotes gut health, muscle health, neural health and metabolic statistics. Deficiency can lead to fatality so its important to maintain it in our blood serum but formation of its oxidative compound TMAO (trimethyl-N-acetyl oxide) more than its normal level i.e 0 - 6.2 uM in blood serum can lead to severe lifestyle disease like Ckd, cardiovascular disease, obesity and metabolic errors which have severe repercussions and can even lead to mortality. Review of studies and experimentations have even suggested the effect on genomic enzymes by TMAO. National institute of health and nutritional society suggested many approaches that could be incorporated even at the cellular level to counter the effect of TMAO to promote optimum health. Role of gut microflora is the key part which promotes oxidation of choline metabolites and so does the formation of TMAO. Carnitine, betaine, lecithin oxidation by gut microflora lead formation of precursors which in turn forms such oxide. Various studies related advanced levels of this compound lead to toxicity and insulin resistance. This emerging micro metabolite lead to new trend of studying health complications both in medicinal way and nutritional way.

Diet plays significant role as eventually TMAO is the nutritional precursor. Consumption of TMAO rich foods and development of interest in TMAO reduced food ingredients is the subject to study. To reduce the damage, novel food approach is optimised as it provide a great functionary to circulate lower levels of TMAO in blood serum, thus various risk of diseases can be lowered.

- Foods rich in TMAO levels are
- Beef liver
- Chicken liver
- Eggs
- Salmon

- Cauliflower
- Broccoli
- Soybean Oil
- Foods low in TMAO levels:
- Whole food plant based diet merely free from animal products and cold pressed olive oil, balsamic vinegar and red wine.