الجمهورية الجزائرية الديمقراطية الشعبية

وزارة التعليم العالي والبحث العلمي

Democratic and Popular Republic of Algeria

Ministry of Higher Education and Scientific Research

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قسم البيولوجيا الجزئية و الخلوية

Faculty of Nature and Life Siences

Department of Molecular and Cell Biology

Master's thesis

For obtaining: the Acadimic Master degree in Biology

Specialization: Basic and Applied Toxicology

Theme

Sardine trimethylamine (*Sardina pilchardus*): a probable antagonist of the cerebral effect of alcohol

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Acadimic year: 2019-2020

Order number (library):……

Acknowledgment

First of all, I thank ALLAH for giving me strength, patience and willingness to accomplish this modest work.

I would like to express my sincere appreciation and gratitude to my supervisor Dr. BALLI Nassima, whose guidance and supervision provided me the opportunity and conducive environment to complete this study, she gave me support, hope and optimism throughout the course of my studies.

A special thanks goes to Pr. LAHOUEL Mesbah and Pr. BOUALEM Mayache for their precious advice and guidance. I would also like to thank the reading committee, Dr. LAHOUEL Asma and Dr. HABILA Safia, for agreeing to evaluate my work and for their honorable presence.

My sincere thanks to my father Mr.Boullouf Mohammad and mother Ms. Alioua Noura who supported me from childhood until graduation.

Last but not least I would like to thank my loving and supportive friends and families.

Thanks for everyone.

Boullouf Chahinez

List of abbreviations

Symbol	Nomenclator
ADH	Alcohol dehydrogenase.
ALDH	Aldehyde dehydrogenase.
ALSPAC	The Avon Longitudinal Study of Parents and Children.
ATP	Adenosine triphosphate.
AUD	Alcohol use disorder.
BBB	Blood bain barriere
CH_3CHO	Acetaldehyde.
CH_3COO^-	Acetate.
CH_3COOH	Acetic acid.
$C_6H_{12}O_6$	Sugar.
C_2H_5OH or CH_3CH_2OH	Ethanol.
CNS	Central nervous system.
CO_2	Carbon dioxide.
COA	Coenzyme A.
COVID-19	Corona Virus Disease 2019.
CSF	Cerebrospinal fluid.
Cyclic AMP	Cyclic adenosine monophosphate.
Cyclic GMP	Cyclic guanosine monophosphate.
DNA	Deoxyribonucleic acid.
EDP	Edition Diffusion Presse Sciences.
EUMOFA	European Market Observatory for fisheries and aquaculture.
FAO	The Food and Agriculture Organization of the United Nations.

FASEB	The Federation of American Societies for Experimental Biology.
FMO3	Flavin-containing monooxygenase 3.
FMOs	Flavin-dependent monooxygenases.
GABA	Gamma-Aminobutyric acid.
GBB	γ-butyrobetaine.
GGT	γ-glutamyl transferase.
HDL-cholesterol	High-density lipoprotein cholesterol.
Hg	Mercury.
H_2O	Dihydrogen monoxide.
IARC	International Agency for Research on Cancer.
mAChRs	Muscarinic acetylcholine receptors.
MeHg	Methylmercury.
MPRH	Ministry of Fisheries and Fishery Resources.
NADH	Nicotinamide adenine dinucleotide.
NMDA	N-methyl-D-aspartate.
O_2	Oxygen.
OECD	Organization for Economic Co-operation and Development.
S. pilchardus	***Sardina pilchardus***.
SAH	S-adenosylhomocysteine.
SAM	S-adenosylmethionine.
TMA	Trimethylamine.
TMAO	Trimethylamine oxide.
TMAU	Trimethylaminuria.
USA	United States of America.

USTHB	University Of Science and Technology Houari Boumediene.
VOCs	Volatile Organic Compounds.
WHO	World Health Organization.

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Introduction

Introduction

Globally, alcohol consumption represents a major public health challenge, where it is the main factor in premature death and disability for people aged from 15 to 49 and the seventh cause in the general population (**Collaborators, 2018**).

Alcohol is a psychoactive substance with toxic and dependence-producing properties. It is widely consumed throughout the world; the prevalence of current drinking differs widely by location and has attracted human concernment for thousands of years.

More than 2 billion people, or about three of ten individuals, are current drinkers globally. According to a World Health Organization (WHO) report, in 2012, almost 3.3 million deaths, or 5.9 % of the total worldwide, were attributable to alcohol consumption (**OMS, 2018**). They are more frequent in summer according to WHO statistics published in 2015 and the road accidents are the 9th leading cause of death (**Scheen, 2019**).

Alcohol consumption is a sensitive subject in the world; researchers are always looking for permanent solutions to fight alcohol with chemical or organic substances, such as plants, substances of animal origin, currently there is no treatment for alcohol from animal source, but in future it is possible; could it be fish!

Universally, fish is considered as an essential amino acid and protein source for the global population (**Ghaly et al., 2013**). An average Algerian consumes 3.2kg of fish per year; nevertheless, 70.000 to 100.000 tons of fish are being catched on the Algerian coast per year (**Ibp Inc, 2013**), witch pelagic fishery is dominated by landed ***Sardina pilchardus*** represents 58 % of total captured (**MPRH, 2008**).

Seafood spoilage leads to the formation of low-molecular-weight volatile amines. The main compound responsible for the typical smell of spoiled fish is trimethylamine (TMA), the latter is a good chemical marker of freshness (**Popelka et al., 2014**): its concentration increases with spoilage by bacterial degradation of trimethylamine N-oxide (TMAO), an important osmoregulatory organic molecule that is commonly found in the muscle of marine fish (**Treberg and Driedzic, 2002**).

Fish consumption has long been known for its important role in nutrition and chronic diseases prevention (**Sioen et al., 2007**), but it may be also a solution to fight the effect of alcohol.

TMA (the responsible for the smell of fish) may have a probable effect on alcohol drinkers due to the similarity of its structure with choline. The latter is a complex essential nutrient widely distributed in foods especially milk, eggs and fish and involved in several diverse body functions (**cho et al., 2006; Penry and Manore, 2008; Wiedeman et al., 2018**). Recent data have shown that choline supplementation of rat pups reduces the effects of alcohol on neurobehavior (**Tang et al., 2014**).

In this way we are submitting a new hypothesis starting from similarity between choline and TMA, to the best for knowledge TMA has never been studied for alcohol.

So, the first goal of this study was to try to answer via a bibliographic synthesis on some basic theoretical questions such as: what is the general definition of TMA, what is its source, and how it is produced, what is the definition of alcohol and it propriety, how it effects the human body and what is relation between alcohol, TMA and choline?

Chapter I
Trimethylamine of *Sardina pilchardus*

I.1 Generalities about *Sardina pilchardus*

I.1.1 Definition

S. pilchardus is the most abundant small pelagic fish species migratory, widely distributed throughout the Mediterranean basin and its adjacent seas, as well as in the North-Eastern Atlantic (**Whitehead et al., 1988**).

S. pilchardus is a short-lived and fast-growing fish that migrate towards coastal waters for spawning, where they release their spawning products multiple times (**Zorica et al., 2016**).

I.1.2 Classification

Sardines belong to a complex taxonomic group (Figure 01) which includes marine pelagic fish. In the genus ***Sardina***, there is only one species, ***S. pilchardus***. The classification of this species is as follows (**Whitehead, 1985**):

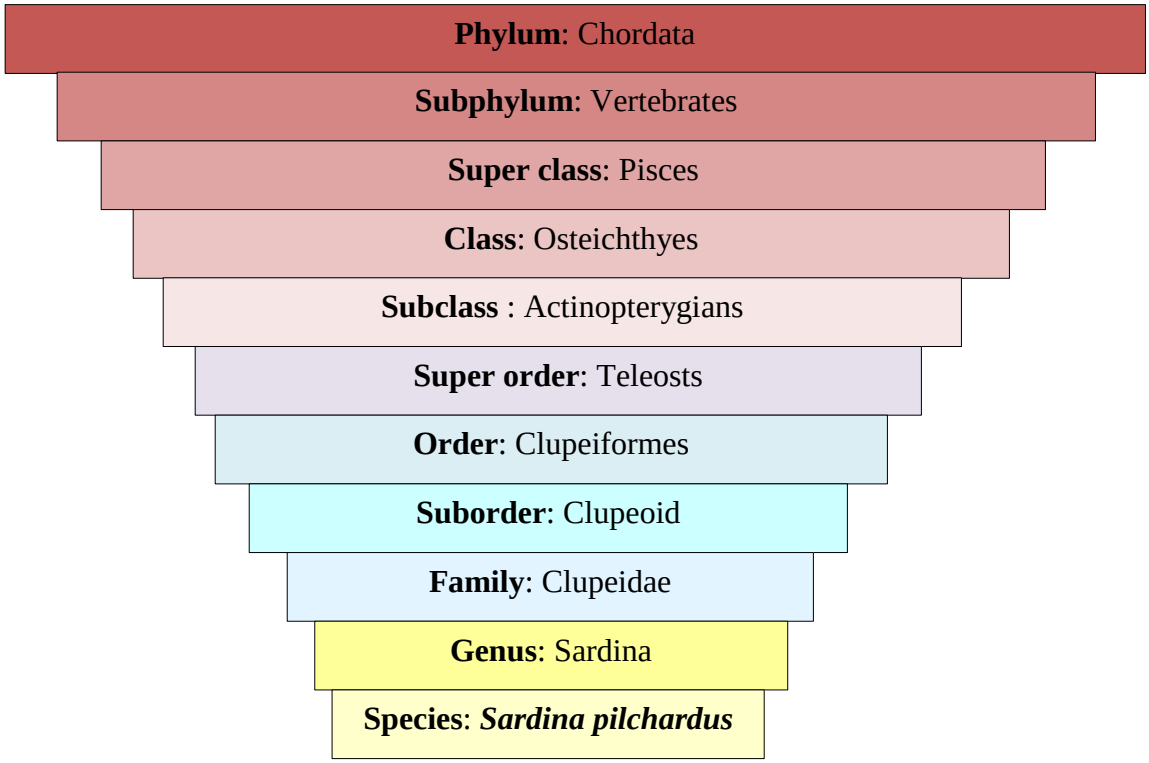

Figure 01: Taxonomy of ***S. pilchardus*** (**Whitehead, 1985**) (**modified**).

I.1.3 Morphology

S. pilchardus is a small fish has a fusiform body which slightly compressed on the flanks and covered with large scales. It also wears a short dorsal fin without spines (**Belkhodja et al., 2016**), it has a shiny silver belly, a bluish back and series of dark spots along the upper flanks (Figure 02) (**Whitehead, 1985**).

Figure 02: Morphology of ***S. pilchardus***.

In the region of North Africa, the size of sardine increasingly varies from North to South and from East to West. Table 01 represents differences in volume of ***S. pilchardus***.

Table 01: Differences in volume of ***S. pilchardus*** in North Africa according to several authors.

	Females	**Males**	**References**
Tunisian coast	12.3 cm	11.8 cm	(**Bedairia et al., 2016**)
Algiers' coast	12.9 cm	11.9 cm	(**Mouhoub, 1986**)
Laayoune region (Morocco)	17.5 cm	16.3 cm	(**Amenzoui et al., 2004**)

The size of ***S. pilchardus*** can reach to 27cm; 90 % of which is reached during the first year of its life cycle (**Whitehead, 1985**). The same note was observed in the Algerian ***S. pilchardus*** from the port of Jijel, which was caught in the month of July 2020, lengths of which vary from 11 cm to 26 cm (Figure 03).

Figure 03: Differences in volume of ***S. pilchardus*** caught in Jijel.

I.1.4 Repartition

S. pilchardus is an epipelagic and neritic marine species widely distributed in the northeastern Atlantic (**Sinovčić et al., 1991**) extends from the Southern Celtic Sea and North Sea to Mauritania and Senegal, with residual populations also off the Azores, Madeira, and the Canary Islands (**Parrish et al., 1989**) as well as in the Mediterranean, including its adjacent seas (Figure 04).

In the Adriatic Sea, sardine is more densely distributed along the Eastern coast (**Sinovčić et al., 1991**).

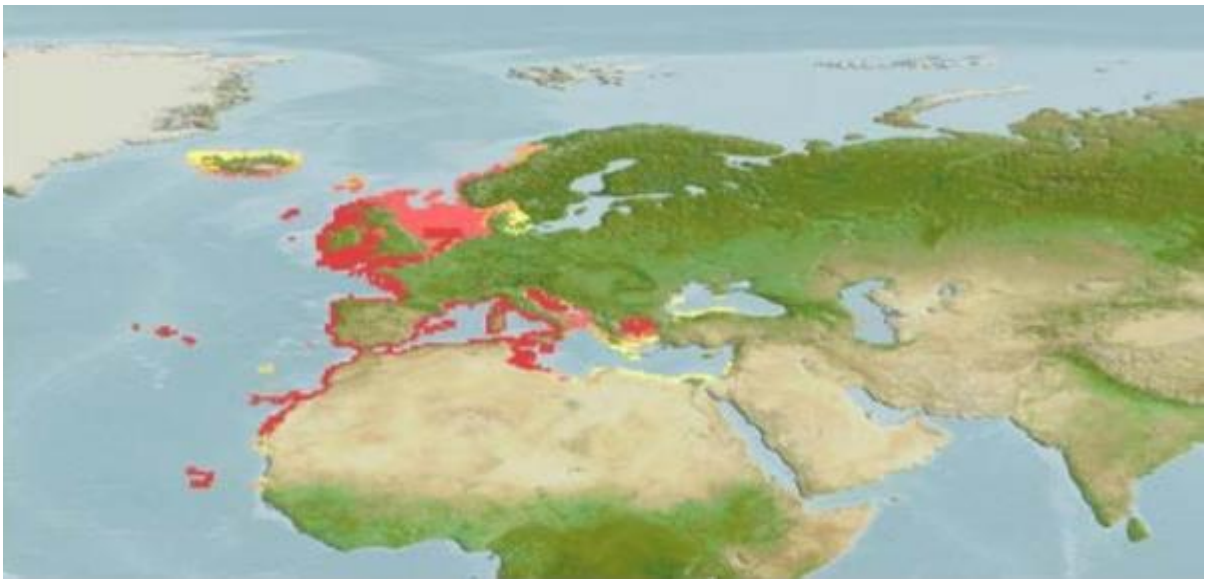

Figure 04: Distribution of ***S. pilchardus*** (**Kaschner et al., 2013**).

I.1.5 Alimentation

S. pilchardus generally has highly diverse diets which vary geographically and seasonally (**Ganias, 2014**) feeds mainly on plankton and derive the majority of their dietary carbon from zooplankton, and not phytoplankton as previously described, in particular small crustaceans such as the copepods, mollusc larvae, fish eggs and fry (**Massuti and Oliver, 1948**).

In the Mediterranean Sea sardine diet was dominated by zooplankton such as planktonic copepods and the various larvae present in zooplankton (**Massuti and Oliver, 1948**), however in England in Celtic Sea the European sardine diet was numerically dominated by decapods, amphipods, euphausiids, diatoms, peridinians and molluscans (**Hickling, 1945**).

I.1.6 Reproduction

Sardine has a life cycle which is essentially characterized by rapid growth, a short lifespan and rapid maturation associated with high fertility; each female can release up to 35,000 pelagic eggs (**Rochet, 2000; Rose et al., 2001; Whitehead, 1985**).

The larval phase lasts 60 days and the latter lives between 10 and 40 m deep and disperses more widely at night (**Olivar et al., 2001; Ramirez et al., 2001**). The favorite ecology of reproduction of sardines is represent in (Table 02).

Table 02: The favorite ecology of reproduction of sardines (**Berraho, 2007**) (**modified**).

Parametre		**Favorite**
Sardine period		September- June
Egg-laying peaks		January- February
Conditions for laying	Salinity	37.6 and 38‰
	Temperature	15.5 and 17.5°C

In Northwest Africa, ***S. pilchardus*** spawning occurs throughout the year, although seasonal peaks of spawning depend on time and location (**Ganias, 2014**), between September and June it lays eggs mainly on the European Atlantic coasts and from October to June on the African coasts (**Bernal et al., 2007; Ettahiri et al., 2003**).

I.1.7 The benefit of sardine

Fish is one of the main food constituents in the human diet and prime source of protein, vitamins, minerals, lipid and essential fatty acids, which have been proven to have positive effects on human health (**Borgstrom, 1961**).

Commonly, fish consumption can lead :

- To a reduction in the level of cholesterol.

- To a reduction in the occurrence of stroke.

- To protect against cardiovascular disease (**Aminah et al., 2013**).

- Development of the neural system in children and enhancing cognitive development (**Cardoso et al., 2018; Daniels et al., 2004**).

- Brain development and prevention of mild cognitive decline in the elderly (**Cardoso et al., 2016; Coletta et al., 2010**).

I.1.8 Risk factor of *Sardina pilchardus*

S. pilchardus is one of the most important fishing resources in the Mediterranean (**Balcells et al., 2018**). However recent landings of ***S. pilchardus*** became even lower than during the investigated period before the 1960s. This decrease might be linked to over-exploitation and to impact of environmental and climatic conditions (**Van Beveren et al., 2016**) like:

- Temperature (**Garrido et al., 2016**).

- Predation and food unavailability (**Houde, 2008**).

- Extreme wind speed (**Solari et al., 2010**).

- Impact of industries and human activities; emission of various environmental pollutants such as heavy metals such as mercury (**Yabanli, 2013**).

Many study in Algeria confirmed the presence of mercury (Hg) and methylmercury (MeHg) concentrations in ***S. pilchardus*** fished in three Algerian coasts (Béjaia, Algiers, and Oran) but lower than the national and European regulatory thresholds (**Mehouel et al., 2019**), two years ago other study showed that mercury concentrations in different sardine samples were found to be higher than the legal limits in Beni saf, Mostaganem, Ghazaouet, Algiers and Jijet coast (**Benguendouz, 2017**).

I.2 *Sardina pilchardus* as a source of trimethylamine

I.2.1 Generalities about trimethylamine

I.2.1.1 Trimethylamine's sources

TMA is generated in the gut from betaine, L-carnitine and its metabolite γ-butyrobetaine (GBB), choline and other choline-containing compounds, which are present in the diet like fish, by host intestinal bacteria producing its precursor TMA (**Zeisel and Warrier, 2017**).

I.2.1.2 Definition and chemical properties of trimethylamine

TMA (Figure 05) is a basic compound, miscible and readily absorbed by water and soluble in solvents with lower dielectric constants (**Mitchell, 2016; Smith, 2010**). It is a volatile low-molecular-weight tertiary aliphatic amine, as mention before TMA can be produced from dietary carnitine and choline but also from TMAO as a result of a bacterial enzyme activity in the decomposition process of fish (**Qiu et al., 2017; Wang et al., 2011**).

TMA is the main component responsible for an unpleasant ‘‘fishy’’ odour and the indicator of the conservation status of seafood; increases during spoilage (**Huss, 1995; Nevigato, 2018**).

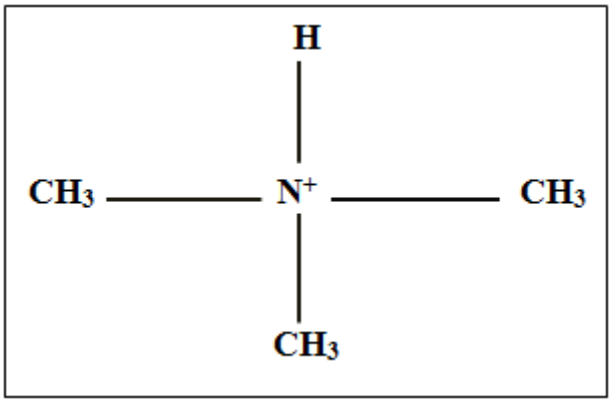

Figure 05: Chemical structure of TMA (**He and Chen, 2018**).

I.2.1.3 Role of trimethylamine

TMA characteristic is as a volatile gas during storage (**Lv et al., 2019**). It plays a very important role in many fields, as it is mentioned in the table below:

Table 03: Different roles of TMA.

Field	Role	References
Fish	Provides the best indication of bacterial deterioration in sea fish of any of the various spoilage effects.	**(Dyer and Mounsey, 1945)**
Economic	Producing formaldehyde and ammonium from variety of aerobic and anaerobic bacteria, which can utilize TMA as their energy source like ***Paracoccusand Hyphomicrobium.***	(**Kim et al., 2003**; **Meiberg and Harder, 1978**; **Qiu et al., 2017**)
	TMAO is commonly used in various industrial processes such as in pharmaceutical production and food processing.	(**Knölker, 1996**; **Rappert and Müller, 2005**)
Human body	TMA associated with humans and their constituents, it present throughout the body.	(**Mitchell and smith, 2016**)
	In the human body, biogenic amines as TMA play important physiological roles, such as in the formation and maintenance of synapses and in the generation of endogenous amino acids.	(**Okado et al., 2001**)

On the other hand, TMA has some shortcomings in the human body, including trimethylaminuria (TMAU); fish malodor syndrome in human body headaches, stimulating the eyes and the respiratory system, and atherosclerosis through enhanced accumulation of cholesterol (**Chen et al., 2014; Mitchell and Smith, 2001; Wang et al., 2011**).

I.2.1.4 Production of trimethylamine

Fish is an important food commodity worldwide and is popular with consumers because of its delicacy and nutrition. However, fish is highly perishable as a consequence of biochemical and microbial breakdown mechanisms (**Lv et al., 2019**).

Fish muscle is known to be a susceptible material to both chemical and bacterial deteriorations (**Kerimoğlu et al., 2020**). During storage of fish, nutrient components such as protein, carbohydrates, and fats decompose to produce many Volatile Organic Compounds (VOCs) including TMA (**Lv et al., 2019**) through three phases:

- ✓ **Phase 1**: The fish is very fresh and has a sweet and delicate taste.
- ✓ **Phase 2**: There is a loss of the characteristic odor and taste but the texture is still pleasant.
- ✓ **Phase 3**: There is sign of spoilage and a range of volatile, unpleasant-smelling substances is produced, one of the volatile compounds is TMA derived from the bacterial reduction of TMAO, by spoilage bacteria such as ***Vibrionaceae***, ***Photobacterium phosphoreum*** and ***Shewanella putrifaciens***, their Microbial enzymes which are present in fish can break down TMAO to TMA. The latter has a very characteristic "fishy" smell.

After the later stage, the texture becomes either soft and watery or tough and dry, and any other volatile compounds can be formed such as ammoniacal by microbial enzymatic degradation of other substrates, acetate, carbon dioxide and water (**Huss, 1995**).

I.2.2 Trimethylamine in the human body

It is well known that TMA is normally formed in the human body, mainly from dietary choline, where eggs, liver and soybeans are the main sources.

- ➢ **In digestive system:**

Gut flora produces TMA either from foods containing TMA or TMAO, which is present in seafood, or from diet precursors that are identified as choline, betaine and carnitine.

TMA is then actively absorbed through the intestinal barrier into the bloodstream, and re-oxidized in the liver to TMAO, by the hepatic enzyme flavin-containing monooxygenase isoform 3 (FMO3) in humans, which, together with small amounts of non-oxidized TMA, is excreted through the urine (Figure 06) **(Pelletier et al., 2019; Wolrath et al., 2005)**

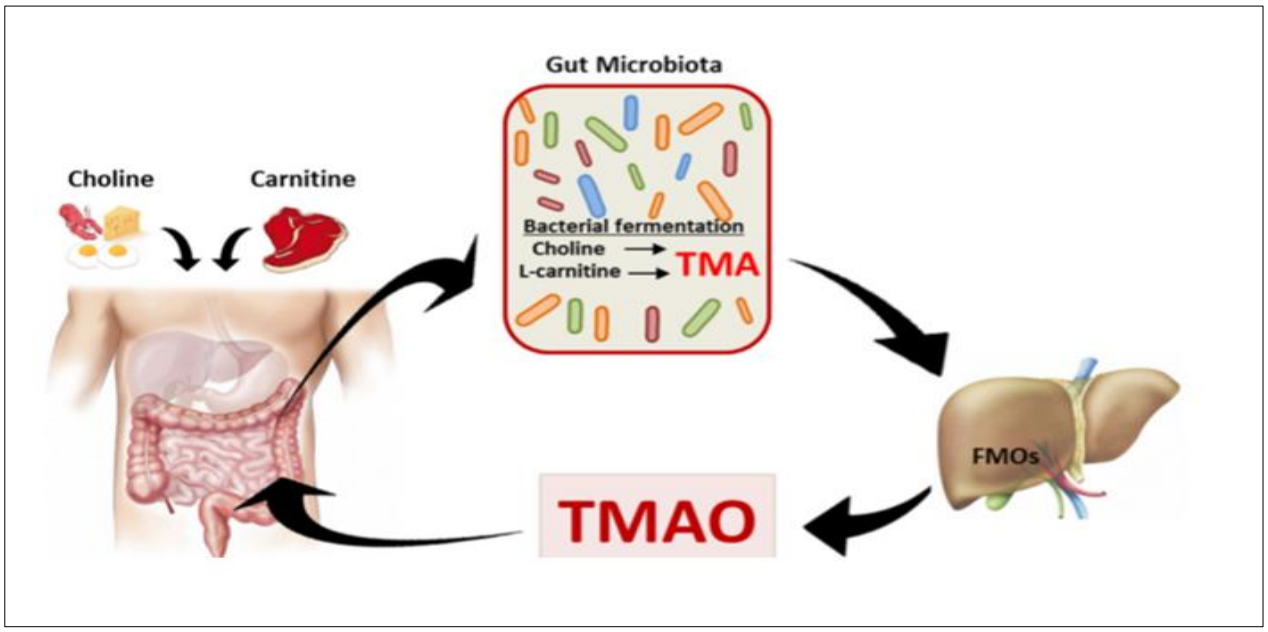

Figure 06: TMA in the human body (**Velasquez et al., 2016**).

- **In receptors:**

Protonated TMA may combine with the same receptor of acetylcholine; desensitize the end-plate receptor to further action of acetylcholine and competitively inhibits cholinesterase in the presence of acetylcholine chloride (**Furukawa and furukawa, 1959; Hobbiger, 1956; MAKC, 2014**)

TMA blocks endplate ionic currents and thus nerve signal transmission, and it can blocks N-methyl-D-aspartate (NMDA) receptor but its mechanism of action is not clear (**Dwyer et al., 1980; Zarei and Dani, 1995**).

Tetramethylammonium, the smallest open-channel blocker, which has similarity structure with TMA, block the NMDA receptor which comprises a subtype of glutamate receptors that are ligand-gated cation-selective channels, highly permeable to calcium, and blocked by magnesium in a voltage-dependent manner. These ion permeability properties underlie the initiation of long-term potentiation by NMDA receptors at some excitatory pathways. Although the calcium permeability and blockade by magnesium have been well characterized and site-directed mutagenesis has shown the importance of particular amino acids, the structural basis and molecular mechanisms for these

and related properties are controversial. Ion permeation and blockade of the channel depend most strongly on ion binding within the narrow region of the pore.

The NMDA receptor channel is impermeable to the symmetrical cation tetramethylammonium, but is permeable to larger asymmetrical cations (**Zarei and Dani, 1995**).

Tetramethylammonium causes also the release of cholinergic transmitter from excitatory nerve endings-releasing acetylcholine from nerve terminals and hemicholine inhibits this action; both acetylcholine and tetramethylammonium combine with the same receptor ,the most likely interpretation concerning the site of action of acetylcholine and tetramethylammonium is that they act upon the 'muscarinic' receptor, so that tetramethylammonium has been suggested like carbamylcholine, acts on preganglionic nerve endings to release transmitter (**Mitchelson, 1971**).

- **In blood–brain barrier**

TMA and TMAO affect blood–brain barrier (BBB**)** function in a metabolite-specific manner, likely through modulation of the actin cytoskeleton and tight junctions. Variation in circulating levels of these metabolites with aging may play a role in the BBB changes seen in Alzheimer's disease (**Mcarthur et al., 2018**)

Small fraction of liver-derived TMAO can cross the BBB, but cannot rule out that a fraction of the TMAO detected in cerebrospinal fluid (CSF) may derive from de novo synthesis, as expression of FMO3 has been detected in the adult brain. So that in a small tested group of subjects, TMAO levels in CSF are apparently unrelated to the diagnosed neurological disorders (**Del Rio et al., 2017**).

I.2.3 Trimethylamine N-oxide in the human body

TMAO is a small colorless amine oxide generated from choline, betaine and carnitine by gut microbial metabolism. It accumulates in the tissue of marine animals in high concentrations and protects against the protein-destabilizing effects of urea. Plasma level of TMAO is determined by a number of factors including diet, gut microbial flora and liver flavin monooxygenase activity (**Velasquez et al., 2016**).

In humans, a positive correlation between elevated plasma levels of TMAO and an increased risk for major adverse cardiovascular events and death is reported, so that several large clinical studies have shown that plasma TMAO levels associate with cardiovascular risk (**Liu and Dai, 2020; Velasquez et al., 2016**)

The atherogenic effect of TMAO is attributed to alterations in cholesterol and bile acid metabolism, activation of inflammatory pathways and promotion foam cell formation.

The mechanism by which TMAO promotes atherosclerosis also remains speculative. The proposed mechanisms include changes in cholesterol and sterol metabolism, promotion of foam cell formation by increasing expression of scavenger receptors on macrophages, and causing alternations in bile acid metabolism and sterol transporters in the liver and intestine.

Surprisingly, TMAO levels were inversely correlated with atherosclerotic lesion size in aorta. A study suggests that TMAO may confer a protective and not a causative effect on atherosclerosis development. Thus, further studies are needed to conclusively prove the role of TMAO in the pathogenesis of atherosclerosis (**Velasquez et al., 2016**).

Chapter II
Alcohol

II.1 Generalities about alcohol

II.1.1 Definition and proprieties

Alcohol is the only substance that is both a nutrient and a drug affecting brain function (**Mann and Truswell, 2017**).

It is synonymous with ethyl alcohol or ethanol of formula C_2H_5OH and of molar mass 46, indicates a family of organic molecules comprising a hydroxyl group (-OH), it is completely miscible with water, but sparingly soluble in fats. It is a volatile product which boils at 78.5°C (**Goullé, 1999; Mann and Truswell, 2017; Paquot, 2019**).

Pharmacologists classify alcohol as a central nervous system depressant in the same group as general anaesthetics (**Mann and Truswell, 2017**).

II.1.2 Production of alcoholic beverages and their containers

Alcohol is produced by alcoholic fermentation of glucose. The specific enzymes are provided by certain yeasts, saccharomyces, which are unicellular fungi. Yeast contains the enzyme, pyruvate decarboxylase, not present in animals. This converts pyruvate to acetaldehyde, and then alcohol dehydrogenase (working in the opposite direction from its role in the human body) converts acetaldehyde to ethanol. The overall reaction is:

$$\mathbf{C_6H_{12}O_6 + CO\text{-}Factors + ATP \rightarrow 2C_2H_5OH + 2CO_2}$$

The co-factors include NADH, thiamin pyrophosphate and magnesium. Grapes among fruits contain a lot of sugar, nearly all glucose (around 16%), so providing an excellent substrate for alcoholic fermentation (Figure 07).

So alcoholic beverages contain variable amounts of unfermented sugar and dextrin (in beers), small amounts of alcohols other than ethyl, moderate amounts of potassium, small amounts of riboflavin and niacin, but no thiamin, and sometimes vitamin C. They also contain a complex array of flavour compounds, colours, a preservative and sometimes additives (**Mann and Truswell, 2017**).

Figure 07: Processes production of alcoholic beverages; wine (**Haenel, 1989; Mann and Truswell, 2017**) (**Modified**).

II.1.3 Classification of alcoholic beverages and consumers

II.1.3.1 Classification of alcoholic beverages

There is a distinction between alcoholic beverages:

- Alcoholic beverages obtained by fermentation such as wine, cider or beer. They are light alcohols; these are containing less than 15% pure alcohol.

- Alcoholic beverages obtained by distillation for calvados, brandies or whiskey. They are strong alcohols; these are containing more than 50% alcohol.

- Alcoholic beverages obtained by maceration such as liqueurs (**Bouafia, 2006; Goullé and Guerbet, 2015; Morel and Anger, 2012**).

The following figure shows the percentage of alchoholic consumption by type alcoholic beverage in Algeria (Figure 08).

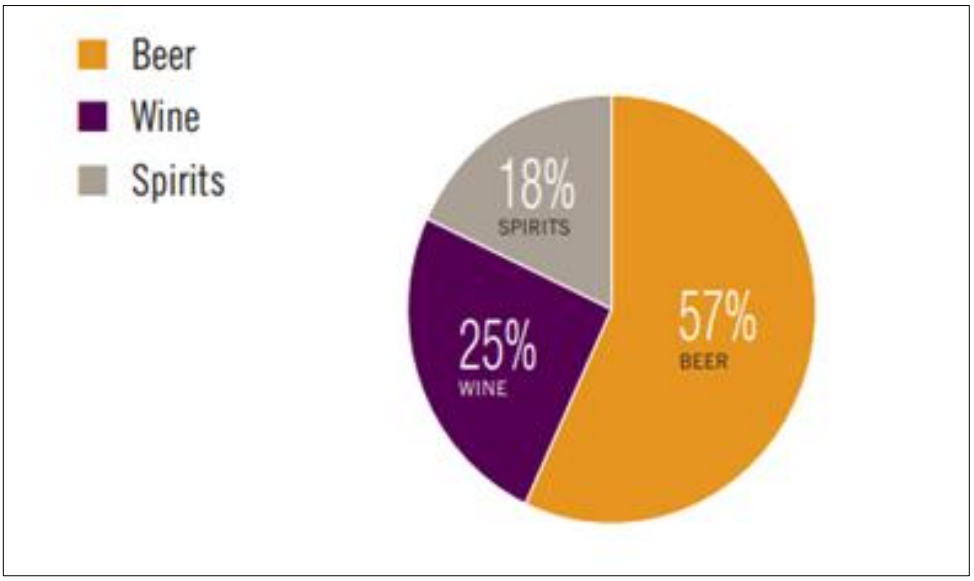

Figure 08: Recorded alcohol per capita (15+) consumption by type of alcoholic beverage, in Algeria in 2016 (**WHO$_1$, 2018**) (**Modified**).

II.1.3.2 Consumer classification

The World Organization of Health in 2007 described each group of consumers according to their daily alcohol consumption (**WHO, 2006**):

• **Low-risk consumers** have occasional or regular lower than the limit values defined by the WHO. Furthermore their consumption is moderate and adapted to the situation.

• **At-risk consumers** present a level of consumption likely to significantly increase the risk of morbidity and mortality.

• **Consumers for harmful use** are defined by the existence of at least patent (and not only latent) medical damage (clinical or biological), psychic or social induced by alcohol.

• **Alcohol addicts** are consumers with a loss of control of alcohol consumption and dependence (**APA, 2000**).

II.1.4 Sociology of alcohol consumption in the world

Alcohol is a great threat to human beings, which represents a major problem in public health, especially in this century (**Sivakumar, 2020**).

In countries like Saudi Arabia, Morocco, Iran and Algeria, alcohol intake is illegal and is considered as a sin or crime, however it make up a big rate in the Eastern Mediterranean region, according to

WHO's (**WHO$_3$, 2018**), unlike the United States law allows people to drink alcohol when they attain 21 years of age (**Sivakumar, 2020**). Alcohol consumption is strongly embedded in cultural norms and traditions leading to health and social damages that are often overlooked (**Flor and Gakidou, 2020**).

Europe ranked third in alcohol consumption, especially, France and Belgium. According to a 2017 survey by the Organization for Economic Co-operation and Development (OECD), Belgium is the world champion in alcohol consumption (**Goullé and Guerbet, 2015; OCDE, 2017**).

Alcohol consumption in Algeria seems very low, according to the latest data from the WHO. Indeed, according to the report of this organization published on the consumption of alcohol in the world, 93.5% of the Algerian population has never consumed alcohol during their life, and only 3.3% of the population consumes alcohol (**WHO$_1$, 2018**).

II.2 Alcohol and its fate in the human body

II.2.1 Alcohol absorption from the digestive tract

Alcohol is almost completely absorbed in the digestive tract by simple diffusion, mainly in the small intestine at the duodenum and proximal jejunum (80%) and for a lower proportion (-20 %) from the stomach (**Goullé, 1999; Paquot, 2019**). Certain numbers of factors (Figure 09) are likely to slow it down or speed it up:

☞ Among the parameters that will slow it:

- Some medicaments.

- Food whether it's fat, protein or most carbohydrates.

☞ Among the factors that will accelerate it:

- The emptiness of the stomach

- The acceleration of gastrointestinal motility

- Elevation of the alcoholic strength of the drink (**Goullé and Guerbet, 2015**).

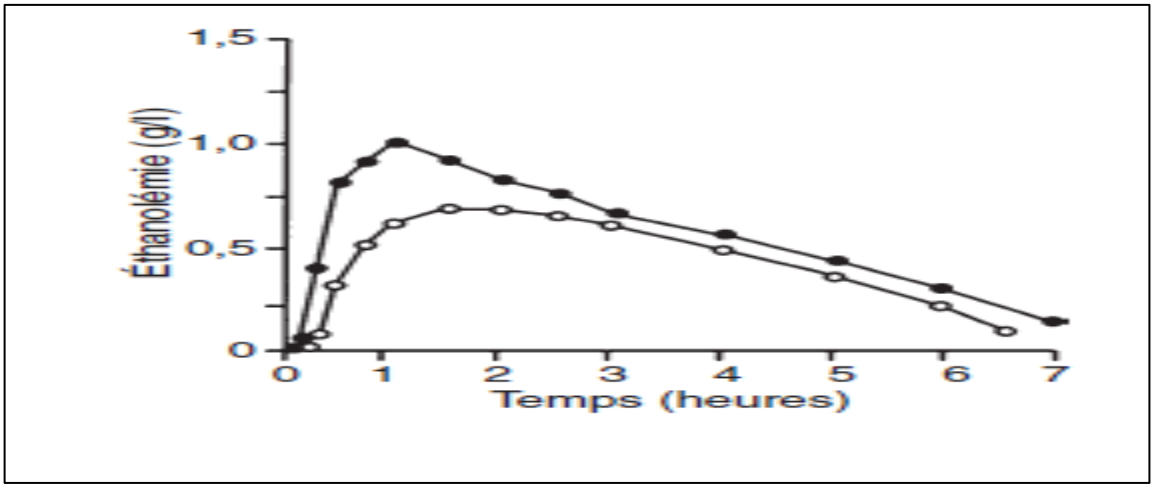

Figure 09: Toxicokinetics of alcohol absorption on an empty stomach or after a meal. Values obtained in a man having consumed 0.80 g of alcohol / kg of body weight before (●) or (○) after breakfast (**Lamiable et al., 2000**).

II.2.2 Distribution of alcohol

One of the essential characteristics of alcohol, linked to its low molecular weight and its high water solubility, is that it diffuses very easily in all body tissues because it follows the movements of water by passive diffusion in the digestive mucosa of the stomach, small intestine and colon with the exception of bones and fats in which its penetration is negligible (**Goullé and Guerbet, 2015; Paquot, 2019**).

It is distributed throughout the total body water, so that after having one drink its 10 g of alcohol is diluted in about 40 L of water in an adult, giving a peak concentration of 0.025g/dl in the blood and in the rest of body water (**Mann and Truswell, 2017**). The concentration in the brain quickly reaches that of blood (**Paquot, 2019**).

In brain (cerebral cortex, hypothalamus. medulla, pons and striatum) alcohol affects a number of neurochemical processes simultaneously (table 04) (**Grant, 1987; Mann and Truswell, 2017**). Alcohol acts in very specific ways on certain brain receptors, by increasing the main inhibitory neurotransmitter, Gamma-AminoButyric Acid (GABA) and by inhibiting the major excitatory neurotransmitter, glutamate, particularly at the NMDA glutamate receptor (**Gilpin and Koob, 2008; Tran et al., 1981**).

Table 04: Receptor involved in the action of alcohol (**Nutt, 1999**).

Experience	Receptor
Activation	Dopamine
Euphoria/ pleasure	Dopamine/ opioids/serotonin
Anxiolysis/ ataxia	Increase GABA
Sedation/ amnesia	Increase GABA and block NMDA
Nausea	Stimulation serotonin
Withdrawal	Increase calcium flux and decrease magnesium

For many years there has been conflicting opinion as to the actions of alcohol on GABA function, with enhancement being found in some models but not in others. Very elegant molecular pharmacological studies have now clarified the relationship of alcohol and GABA by showing that it is critically dependent on the molecular subtype of the GABA receptor.

GABA receptors are like hollow doughnuts that sit in the neuronal membrane; the hole in the middle is a channel that allows the passage of negatively charged ions, especially chloride. When GABA binds to the GABA receptor the hole effectively enlarges, so more chloride ions enter the cell; this reduces the cell's excitability and so decreases anxiety (**Nutt, 1999**)

Alcohol increases the ability of GABA to open the chloride channel. At higher concentrations (> 250 mg/dl) alcohol has a direct action on the receptor, causing a prolonged opening of the chloride channel that is GABA independent; excessive chloride influx result in paralysis of the neurons responsible for respiratory drive, so causing asphyxiation (**Grant, 1987; Nutt, 1999**).

As well as enhancing the actions of the brain's main inhibitory system, alcohol also acts to block some aspects of the brain's endogenous excitatory systems. This dual action of increased inhibition and decreased excitation explains why alcohol causes so much impairment of brain function.

Excitation in the brain is the key process for sensory perception, information transfer and consciousness itself. The major excitatory transmitter is the amino acid glutamate, which acts in at least three subtypes of receptor. In relation to alcohol the key subtype is the NMDA receptor, like the GABA, receptor, this is doughnut-like protein complex that sits in the cell membrane and allows the passage of calcium ions into the cell. Alcohol acts as a blocker of the NMDA channel, opposing the effects of glutamate (**Nutt, 1999**).

Alcohol also inhibition of voltage-sensitive calcium channels and increased intracellular calcium (**Mann and Truswell, 2017**). This influx of calcium activates cellular enzymes that can produce long-term alterations in neuronal, especially synaptic, function (**Nutt, 1999**).

II.2.3 Metabolism of alcohol

Alcohol is readily absorbed unchanged from the jejunum; it is one of the few substances that also absorbed from the stomach. Alcohol is nearly all metabolized in the liver with a percentage of 90 to 95 % but a small amount is already metabolized as it passes through the stomach wall; it is only 5 to 10 % (**Goullé, 1999; Mann and Truswell, 2017**).

On average, people can metabolize about 5 g of ethanol per hour, there are three stages for alcohol metabolism (Figure 10) (**Mann and Truswell, 2017**):

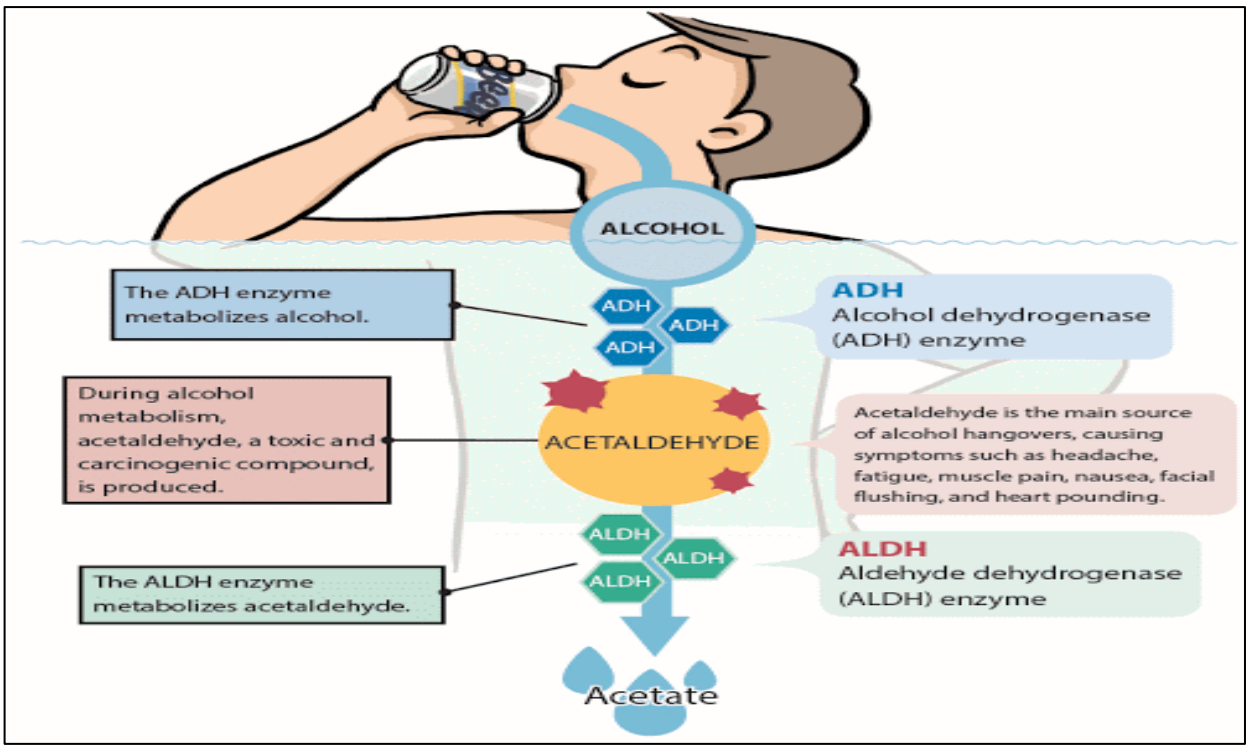

Figure 10: Alcohol metabolism in the digestive system (**Bossng, 2017**).

II.2.3.1 Oxidation of the functions alcohol to aldehyde function by ADH

The oxidation of the alcohol functions to the aldehyde which involves alcohol dehydrogenase (ADH) according to the following reaction (figure 11):

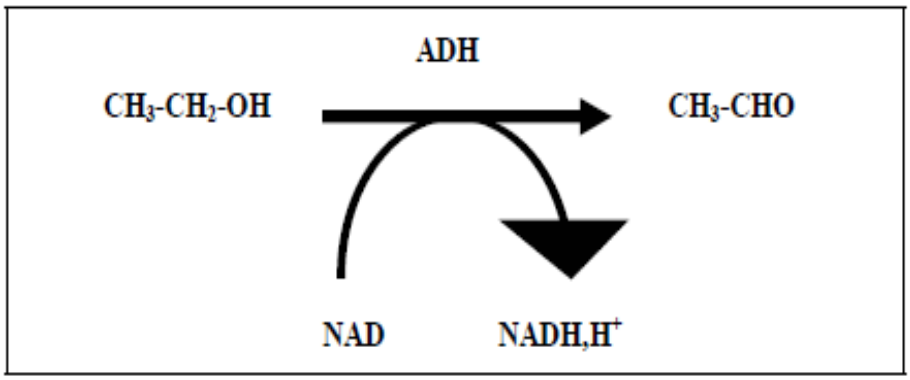

Figure 11: The reaction of oxidation alcohol by ADH (**Lhermitte and Houdret, 1999**).

ADH is a family of zinc enzymes, NAD + dependent, oxidizing ethanol to acetaldehyde. ADH is in reaction ubiquitous, catalyzing different alcohols into aldehydes (**Lhermitte and Houdret, 1999**).

This first step results in the formation of acetaldehyde, a toxic metabolite of ethanol responsible for the accumulation of symptomatology such as nausea, vomiting, headache and asthenia. (**Goullé, 1999; Goullé and Guerbet, 2015**).

II.2.3.2 Oxidation of the function aldehyde in acid function under the influence of ALDH

The oxidation of the aldehyde functions to the acid function under the influence of acetaldehyde dehydrogenase (ALDH) (figure 12):

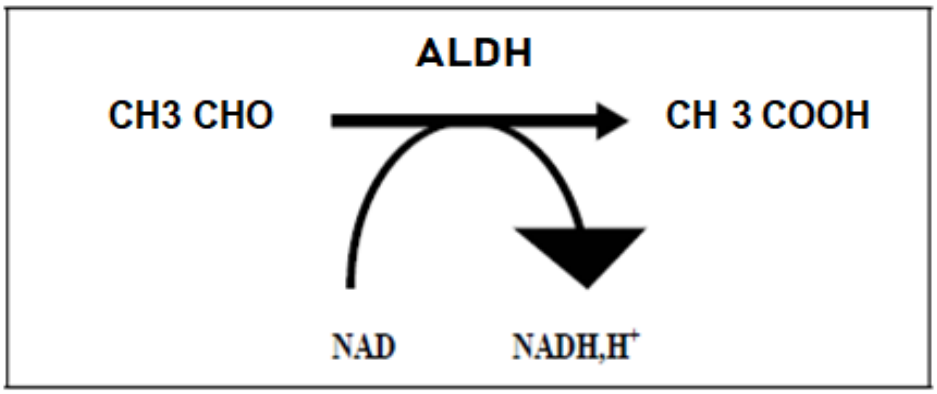

Figure 12: The reaction of oxidation alcohol by ALDH (**Lhermitte and Houdret, 1999**).

ALDH are a group enzyme of detoxification, NAD + dependent. In mitochondria acetaldehyde dehydrogenase (ALDH2) catalyzes and with a simple process transfers acetaldehyde to acetic acid according to an irreversible process (**Lhermitte and Houdret, 1999**).

II.2.3.3 Oxidation of acetate to carbon dioxide

The acetate formed is in turn oxidized to carbon dioxide and water in the peripheral tissues and some organs: muscles, heart and brain. Acetate combines with coenzyme A to give acetylcoenzyme A (figure 13), involved in the biosynthesis of cholesterol and fatty acids in peripheral tissue and the brain (**Goullé and Guerbet, 2015**).

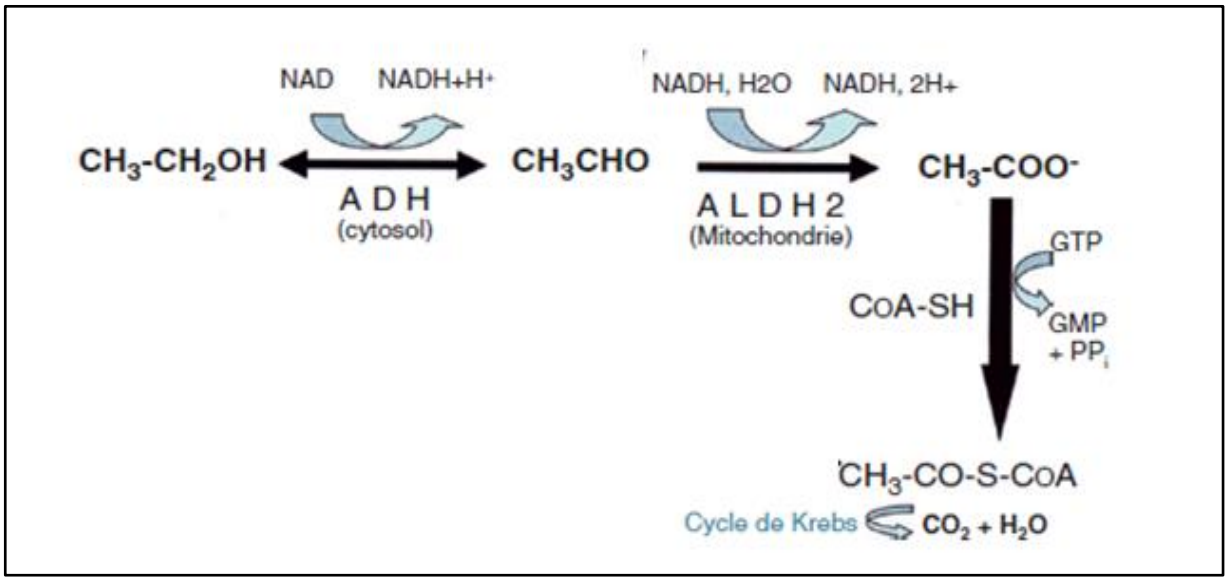

Figure 13: Metabolism of alcohol (**Morel and Anger, 2012**).

II.2.4 Elimination of alcohol

A small proportion of ethanol, of the order of 10 to 15 %, is eliminated as it is by different ways, 2 % is eliminated by the kidneys or the lungs under normal conditions, so it is found in the urine, sweat, saliva, milk, tears, air expired(**Goullé, 1999; Paquot, 2019**).

The presence of ethanol in the exhaled air constitutes an indirect analytical means for assessing the impregnation alcoholic of the person (**Goullé, 1999**).

II.3 Medical analysis

Doctors are usually trained to suspect when someone is drinking too much alcohol, the diagnostic feature, is that the eyes cannot move properly (ophthalmoplegia) and not being fully conscious (**Mann and Truswell, 2017**).

The intake of alcohol can be confirmed by the determination of ethanol in biological fluids (blood, urine, expired air, saliva) by enzymatic or chromatographic methods (**Deveaux, 2002**).

If a person has not been recently drinking, there are changes in the blood which are suggestive of long-term excessive alcohol intake (**Mann and Truswell, 2017**):

- Increased red cell volume.

- Increased γ-glutamyl transferase (GGT) activity.

- Increased plasma triglycerides.

- Increased plasma urate.

- Increased plasma transaminases.

II.4 Effects of alcohol

Alcohol causes serious physical and psychological illnesses, as that social cost of the damage caused by alcohol is very high (**Bouafia, 2006**), among them:

II.4.1 Pathologies linked to alcohol consumption

Alcohol can place the health of an individual at risk for a series of diseases (**Shield et al., 2014**). According to the WHO, heavy alcohol consumptions are associated with many chronic diseases (**WHO_2, 2018**). The effects of alcohol vary in severity, as we find the least dangerous and most dangerous for human health:

II.4.1.1 Least dangerous alcohol effect

Among these effects we can estimate (**Armor et al., 1976; Mann and Truswell, 2017**):

- Alcoholism.

- Dehydration (may be present from diuresis).

- Hypertension.

- HDL-cholesterol raises.

II.4.1.2 Most dangerous alcohol effect

The most dangerous effects of alcohol are:

- Gastrointestinal complications; chronic gastritis and acute pancreatitis.

- Cardiovascular diseases (**Mann and Truswell, 2017**).

- Alzheimer's disease (**Heymann et al., 2016**).

- Fetal alcohol syndrome; women who drink alcohol heavily during pregnancy can give birth to a baby with an unusual facial appearance (small eyes and thin upper lip), prenatal and postnatal growth impairment, central nervous system dysfunction and often other physical abnormalities.

- Liver disease, alcohol causes three types of liver damage:

Fatty liver; metabolism of large amounts of alcohol in the liver produce an increased ratio of NADH/NAD, this depresses the citric acid cycle and oxidation of fatty acids, and favours triglyceride synthesis in the liver cells.

Alcoholic hepatitis; this type is not caused by a virus but by prolonged excess of alcohol intake.

Alcoholic cirrhosis; when the liver has to metabolize large amounts of alcohol over a long time, membranes inside the cells become disordered and irregular strands of fibrous tissue criss-cross the liver.

- Cancers of the oesophagus, liver, the rectum and possibly breast cancer (**Mann and Truswell, 2017**).

- It may be the cause of human death ; 45,000 deaths are attributable to alcohol each year (11,000 cancers, 9,000 cirrhosis, 2,500 alcohol dependencies and 22,000 indirect deaths linked to mental, cardiovascular and accident disorders), making it the second cause leading to death, after tobacco (**Goullé and Guerbet, 2015; Morel and Anger, 2012**).

II.4.2 Social and psychiatric effects related to alcohol

Many consequences of alcohol consumption can be described as "social" indirectly linked to health shortcomings, to mention few:

- Accidents on the public highway or work (**Heise, 1934**).

- Hurt himself and others; acts of domestic violence (**Mann and Truswell, 2017**).

- Disruptions in relationships; according the epidemiological research, alcohol and drug abuse has been shown to be the third most cited reason why couples get divorced in the United States (**Amato and Previti, 2003**).

- Depression, in data obtained through a study of **Deykin et al., (1987)** that 424 in healthy college students which drink alcohol are always subject prone to depressive (**Deykin et al., 1987**).

- Suicide; in South Africa it was estimated that most of suicide cases are associated with alcohol, and most studies reported that females generally have a higher frequency of suicide than males (**Jamison, 2006**).

Chapter III
Choline and its relation to trimethylamine and alcohol

III.1 Generalities about choline

III.1.1 Definition and proprieties

Choline is the trivial name for 2-hydroxyethyl-trimethyl-ammonium (**Wiedeman et al., 2018**). The structure for this compound is shown in (Figure 14).

Choline was first discovered in 1862 and ever since it has been studied extensively as a nutrient, a component of cell membranes, and a precursor to the neurotransmitter acetylcholine (**Lockman and Allen, 2002**), it is usually grouped within the vitamin B complex (**Wang et al., 2011**).

All cells use choline as the precursor of certain phospholipids (phosphatidylcholine, sphingomyelin, and choline plasmalogens), which are the major constituents of all biological membranes (**Blusztajn et al., 1987**).

Choline is found in foods as free choline and as esterified forms such as phosphocholine, glycerophosphocholine, sphingomyelin, and phosphatidylcholine (**Pitkin et al., 2000**).

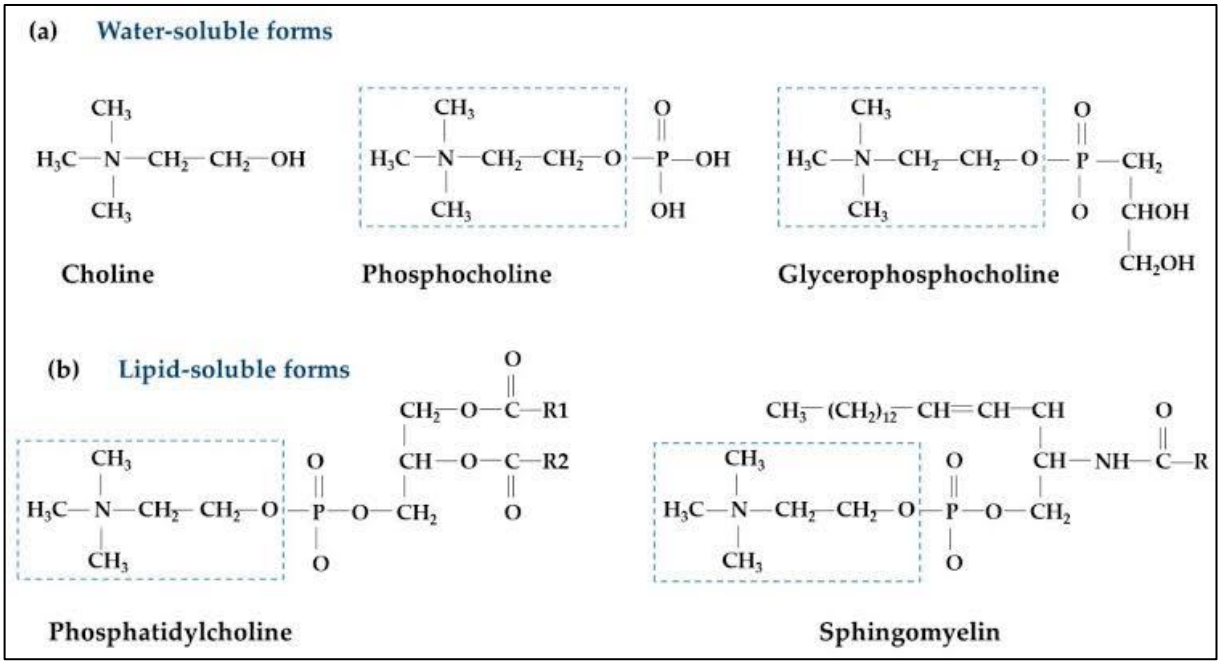

Figure 14: Structures of different choline forms: (a) Water-soluble forms; (b) lipid-soluble forms. Dashed box indicates free choline; R represents a fatty acid chain (**Wiedeman et al., 2018**).

III.1.2 Top dietary sources of choline

Choline is a complex essential nutrient involved in several diverse body functions (**Wiedeman et al., 2018**). It is obtained from the diet and endogenous synthesis but the latter is insufficient to

cover the body's needs. As such, choline needs to be obtained from the diet. (**Velazquez et al., 2020; Wiedeman et al., 2018**).

It is widely distributed in foods, with most of it in the form of phosphatidylcholine (**Penry and Manore, 2008**). Food that is especially rich in choline compounds as red meat, poultry, milk, eggs, fish, Coffee, beer, potatoes, and orange juice as represented in (Table 05) (**Cho et al., 2006; Pitkin et al., 2000; Wiedeman et al., 2018**).

Eggs have especially high choline moiety content (about 300 mg choline/egg, mostly in the form of phosphatidylcholine), milk is also a good food source for total choline, as it is usually consumed on a daily basis (**Wiedeman et al., 2018; Zeisel, 2000**).

Table 05: Food sources of choline (**cho et al., 2006**).

Rank	Food	Percentage(%)
1	Red meat	14.26
2	Poultry	12.98
3	Milk	9.52
4	Eggs	7.57
5	Fish	5.22
6	Coffee	4.00
7	Beer	3.29
8	Potatos	4.03
9	Orange and orange juice	2.27
10	Broccoli	1.88

Furthermore, choline is widely available as a dietary supplement at an affordable cost as choline chloride (**Pitkin et al., 2000; Velazquez et al., 2020**).

III.2 Physiology of absorption, metabolism and excretion of choline

III.2.1 Absorption

When choline reaches to intestine, it is absorbed by the lumen of the small intestine before it reaches the areas of gut colonized with bacteria via transporter proteins in the enterocyte, and little amounts of choline reaches the colon (**Zeisel et al., 1989**).

Before choline can be absorbed by the gut, some is metabolized by bacteria to form betaine (which may be absorbed and used as a methyl donor) and methylamines (which are not methyl donors) (**Pitkin et al., 2000; Zeisel, 1981**).

No other component of the diet has been identified as competing with choline for transport by intestinal carriers (**Pitkin et al., 2000**).

III.2.2 Distribution and metabolism

Different forms of choline vary in how absorption and metabolism occur (**Wiedeman et al., 2018**). After absorption, water-soluble forms of choline (free choline) reach the liver through portal circulation while lipid soluble forms (phosphatidylcholine) are packaged into chylomicrons, which are absorbed and transported through lymphatic circulation and then choline metabolism can be divided into four main pathways which are involved in the synthesis of acetylcholine, TMA, betaine, and phospholipids (Figure 15) (**Wiedeman et al., 2018; Zeisel, 1981**).

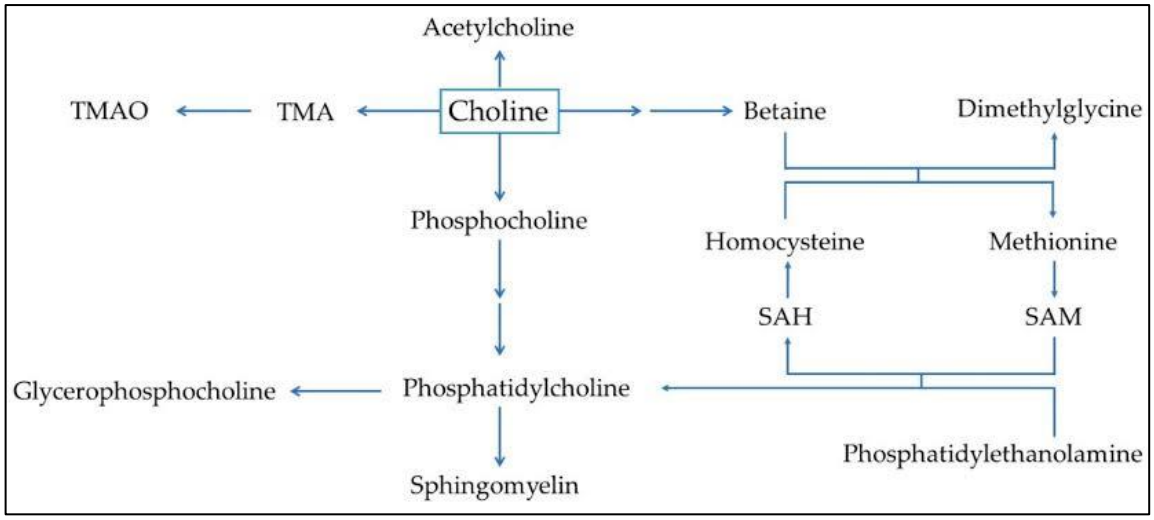

Figure 15: Simplified overview of choline metabolism. Abbreviations: SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; TMA, trimethylamine; TMAO, trimethylamine-N-oxide (**Wiedeman et al., 2018**).

Choline is used as the precursor for the synthesis of the neurotransmitter, acetylcholine, by choline acyltransferase in the cytosol of pre-synaptic cholinergic neurons (Figure 16) (**Sarter and Parikh, 2005**).

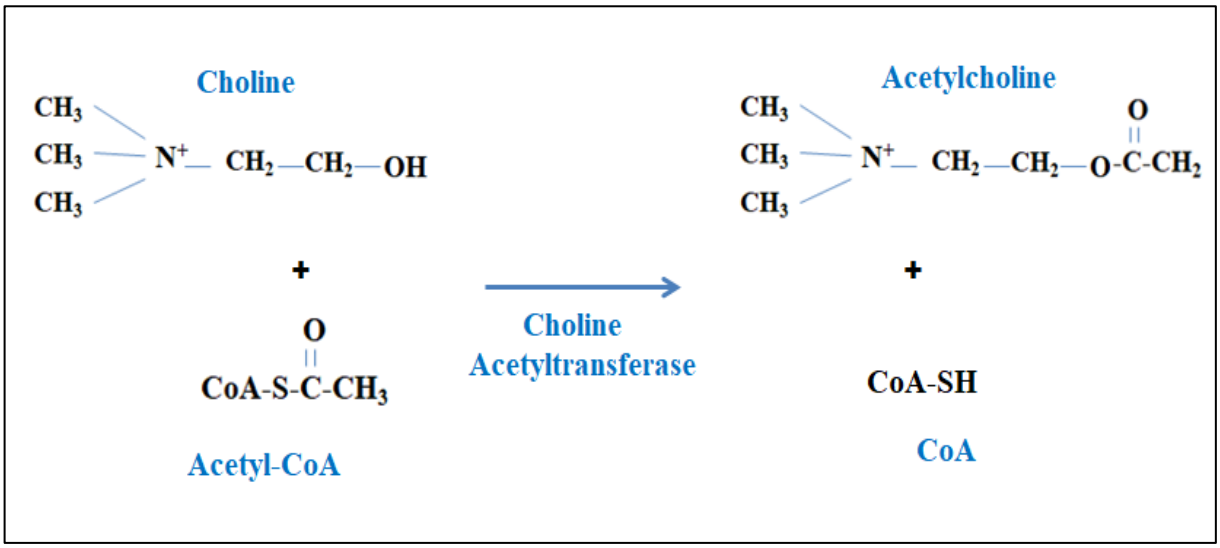

Figure 16: Structure of choline and acetylcholine and their reaction (**Blusztajn and Wurtman, 1983; Lockman and Allen, 2002**) (**modified**).

Acetylcholine is subsequently packaged into vesicles and released into the synaptic cleft, where it binds to receptors of the post-synaptic neuron in the central and peripheral nervous systems (**Lockman and Allen, 2002**).

In the large intestine, choline is metabolized to TMA by the gut microbiota prior to absorption. After the latter process, TMA is metabolized to TMAO by flavin monooxygenases (FMOs) in the liver (Figure 17) (**Baker and Chaykin, 1962; Zeisel et al., 1989**).

Choline is converted to betaine, which can donate a methyl group to homocysteine (**Cho et al., 2006**). It can be irreversibly oxidized to yield betaine in a two-step process catalyzed by choline dehydrogenase and betaine aldehyde dehydrogenase mainly in the liver and kidney (**Bianchi and Azzone, 1964; Rennick et al., 1977**).

Choline is a precursor for the synthesis of phosphatidylcholine, the most abundant form of phospholipid in the body (**Wiedeman et al., 2018**).

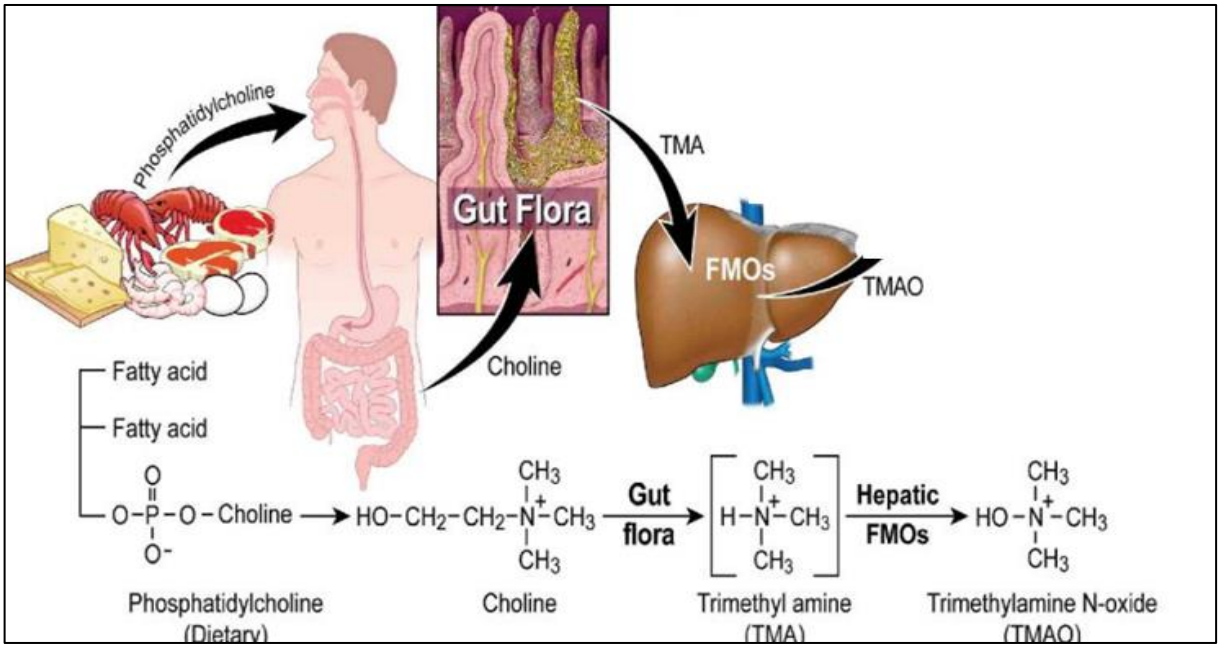

Figure 17: Schematic summary illustrating the pathway of choline in the human body (**Wang et al., 2011**).

A study by **Blusztajn and Wurtman,** (**1983**) and **Pitkin et al.,** (**2000**) has shown that radioactive choline injected systemically could be found in the brain as a specific amount of free choline, transports across the blood-brain barrier (BBB) at a rate that is proportional to the serum choline concentration **(Blusztajn and Wurtman, 1983; Pitkin et al., 2000**).

Another study by **Babb et al.,** (**2004**) demonstrated that in humans, as in animals, ingested choline does get into the brain. Thus choline obtained through the diet may be important for the normal synthesis of acetylcholine and phosphatidylcholine in the brain (**Babb et al., 2004**).

III.2.3 Excretion

All tissues accumulate choline by diffusion and mediated transport. The kidney accumulates choline (**Zeisel, 1981**) and some of this choline appears in the urine unchanged but most of it is oxidized within the kidney to form betaine (**Rennick et al., 1977**).

III.3 Functions and health effects of choline

In 1998, the National Academy of Sciences, in United States (USA) of America, issued a report identifying choline as a required nutrient for humans and recommended daily intake amounts (**Zeisel, 2000**).

The role of choline in the body is complex; it plays both a functional and a structural role in cells (**Penry and Manore, 2008**), it has many physiological functions throughout the body that are considered to be essential for (**Lockman and Allen, 2002**):

- The structural integrity of cell membranes; choline is a precursor for the synthesis of phospholipids such as phosphatidylcholine and sphingomyelin.

- In the process of methyl metabolism; choline is considered as a principal source for methyl groups in the body (**cho et al., 2006; Zeisel, 2000**).

- In cholinergic neurotransmission and transmembrane signaling; choline is a precursor of the synthesis of acetylcholine (neurotransmitter responsible for memory, muscle control and mood, it also builds cell membranes and plays a vital role in regulating gene expression) (**Velazquez et al., 2020; Zeisel, 2000**).

- Lipid and cholesterol transport and metabolism (**Pitkin et al., 2000; Zeisel, 2000**).

- Liver function; choline is a precursor of the formation of the methyl donor betaine. Betaine is required by renal glomerular cells, which use betaine and glycerophosphocholine as organic osmolytes to adapt to osmotic stress (**Moeckel et al., 2002; Rennick et al., 1977**).

- Prenatal memory development; decades of research have shown that supplementing the maternal (gestation and lactation) diet with choline produces profound benefits on the level of child's brain health and cognition, and it is really important for brain development. (**Velazquez et al., 2020; Zeisel, 2000**).

Also, the role of choline is not only the development of the brain but also protection; the results of **Freedman et al., (2020**) showed that higher choline levels obtained through diet or supplements protect fetal development and support infant early behavioral development, even if the mother contracts a viral infection as Corona Virus Disease 2019 (COVID-19), also in early gestation when the brain is first being formed (**Freedman et al., 2020**).

- The most important role for choline is that it serves as an effective and safe treatment for reducing the adverse consequences of prenatal alcohol exposure (**Thomas et al., 2000**).

III.4 Consequences of dietary choline deficiency in the human body

Previous data were not available for determining whether choline is essential in the human diet, and how much is required if it is essential (**Pitkin et al., 2000**) but now many studies confirmed that

choline is an essential nutrient for humans but with a specific focus; the dietary choline for adult women (425mg/day) and adult men (550mg/day) (**Wiedeman et al., 2018**). Its lack or overdose is likely to cause many problems such as:

- **Cardiovascular disease**

The overdose or lack of dietary choline intake might be correlated with cardiovascular disease risk (**Pitkin et al., 2000**).

- **Alzheimer's disease**

In Alzheimer's disease, the regulation of acetylcholine synthesis and cholinergic functioning is greatly diminished and disrupted. This cholinergic disruption supports a central hypothesis of Alzheimer's (**Lockman and Allen, 2002**).

- **Liver dysfunction**

The liver is damaged when humans consume an inadequate diet that is deficient in choline, resulting in elevated alanine aminotransferase levels in the blood (**Zeisel et al., 1991**), without an adequate supply of choline for phosphatidylcholine synthesis, triacylglycerides will accumulate which leads to fatty liver condition (**Corbin and Zeisel, 2012**).

The experiment of **Zeisel et al., (1991)** found that when healthy humans consume a diet deficient in choline for 3 weaks; they deplete stores of choline in plasma and develop signs that suggest incipient liver dysfunction. It is likely that the demand for choline in human bodies will be influenced by the availability of methionine and folate in the diet. This observation supports the conclusion that choline is an essential nutrient for humans when excess methionine and folate are not available in the diet (**Zeisel et al., 1991**).

- **Other**

As TMA, high doses choline has been associated with fishy body odor and sweating (**Arseculeratne et al., 2007**).

III.5 Relation between choline and alcohol

Choline and alcohol (Figure 18) are considered as two molecules that affect the mind oppositely. Recent data have shown that rat pups choline supplementation reduces the effects of ethanol on neurobehavior (**Tang et al., 2014**).

Prenatal choline availability has been shown to influence morphological, neurochemical, electrophysiological, and functional activity of the hippocampus and prefrontal cortex in developing rats (**Thomas et al., 2000; Thomas et al., 2010**).

One study examined the effects of choline supplementation on ethanol disruption of membrane function and focused on L1 cell adhesion molecule. This study determined that choline significantly reduces and prevents the effect of ethanol on L1 function.

L1 cell adhesion molecule is a transmembrane protein, critical for the development of the brain and neuroprotective of alcohol inducing cell death. L1 functions through signaling cascades and protein trafficking. Researchers have shown that ethanol interferes with this trafficking both in *vivo* and in *vitro*, and disrupts L1 signaling and they find that in the absence of ethanol exposure, choline supplementation does not alter L1 signaling (**Tang et al., 2014**).

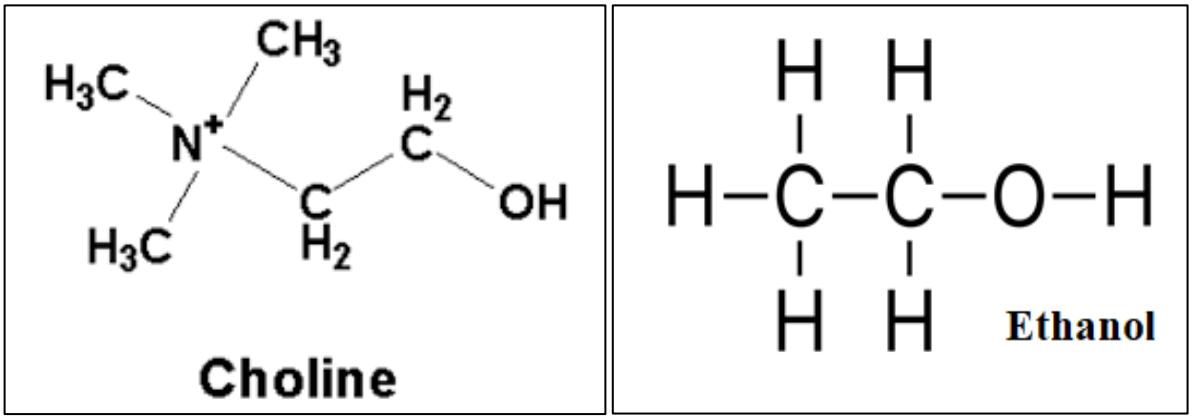

Figure 18: Chemical structures of choline and alcohol (**Gao et al., 2016; Peter and Richard, 2011**).

Another study conducted in 2007, indicated that choline supplementation ameliorates central nervous system functioning, even after ethanol damage is induced. it was the first study to show amelioration of ethanol-induced deficits in cognitive functioning following prenatal treatment with choline (**Thomas et al., 2007; Thomas et al., 2010**). After three years **Thomas et al., (2010)**, provided evidence supporting the fact that choline supplementation during prenatal alcohol exposure reduces the severity of fetal alcohol effects, particularly on alterations in tasks that require behavioral flexibility (**Thomas et al., 2010**).

Tang et al., (2014), pointed out to important information in his article, namely that deoxyribonucleic acid (DNA) methylation is reduced with choline supplementation following ethanol exposure, making it unlikely that the only role choline plays is as a direct methyl donor. Thus, more studies are needed to discover what is still hidden (**Tang et al., 2014**).

III.6 Relation between choline and trimethylamine

Choline and TMA are small molecules (Figure 19) that play central roles in biological processes in all living creatures (**Craciun and Balskus, 2012**). Through the attached figure, we notice a clear agreement between choline and TMA; through the figure (19) we can conclude that TMA is considered to be part of choline.

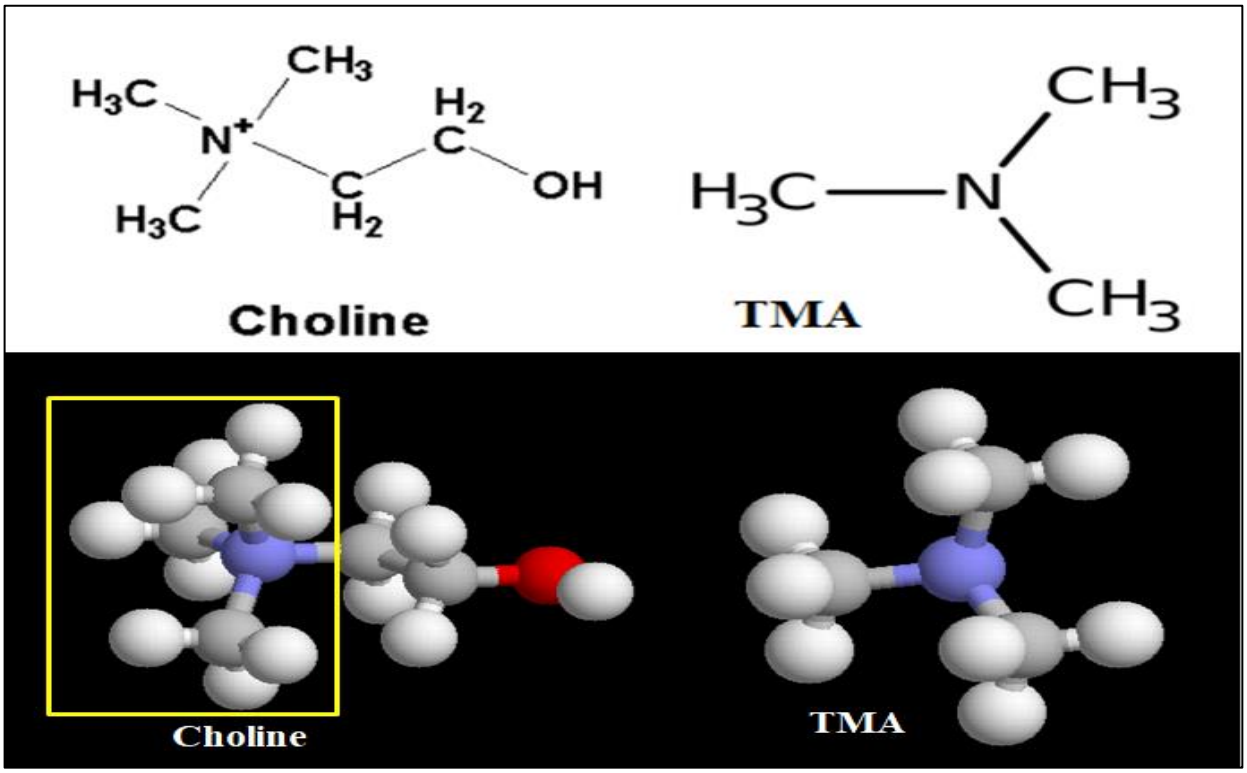

Figure 19: Tree-dimensional chemical structures in Rastop of choline and TMA (**Audebert, 2005; Chebi, 2016; Gao et al., 2016**) (**modified**).

As mention before, protonated TMA may combine with the same receptor of acetylcholine wish is synthesized by choline and acyltransferase. Interestingly, TMA competitively inhibits cholinesterase in the presence of acetylcholine chloride which is the second relation between choline and TMA (**Furukawa and furukawa, 1959; Hobbiger, 1956; MAKC, 2014; Sarter and Parikh, 2005**).

These ubiquitous metabolites are linked through a single biochemical transformation, the conversion of choline to TMA by anaerobic microorganisms (**Craciun and Balskus, 2012; Fennema et al., 2016**) and low proportion of choline intake derived from eggs is converted to TMAO (Figure 20) (**cho et al., 2006; Craciun and Balskus, 2012**).

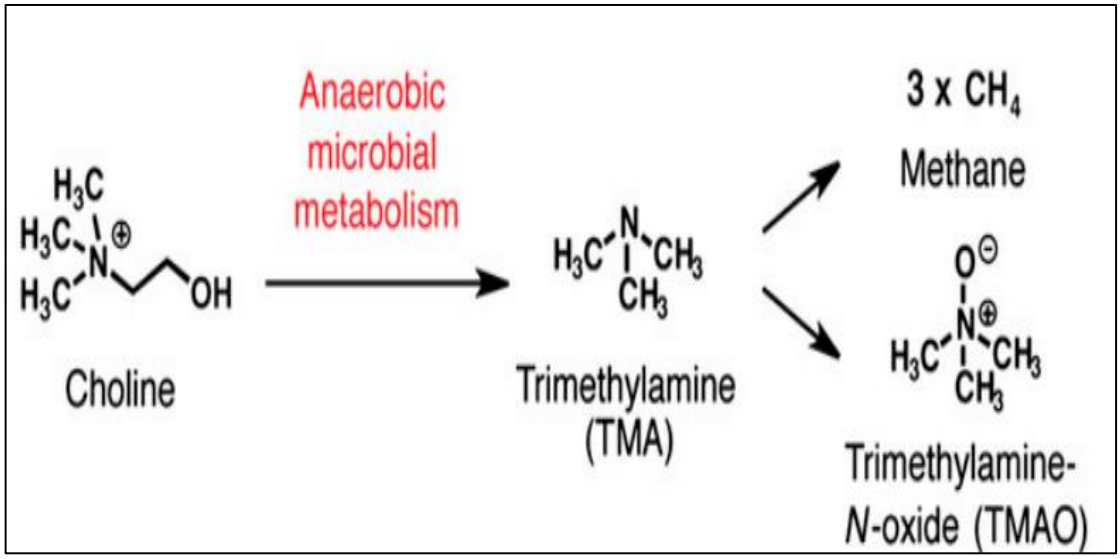

Figure 20: Generation of TMA from choline and its subsequent processing by other organisms (**Craciun and Balskus, 2012**).

Many studies have been conducted to evaluate the relationship between choline and TMA. They found mostly that orally administered choline increased the excretion of TMA and TMAO (Figure 21) (**Zeisel et al., 1989**), as shown by the following curve:

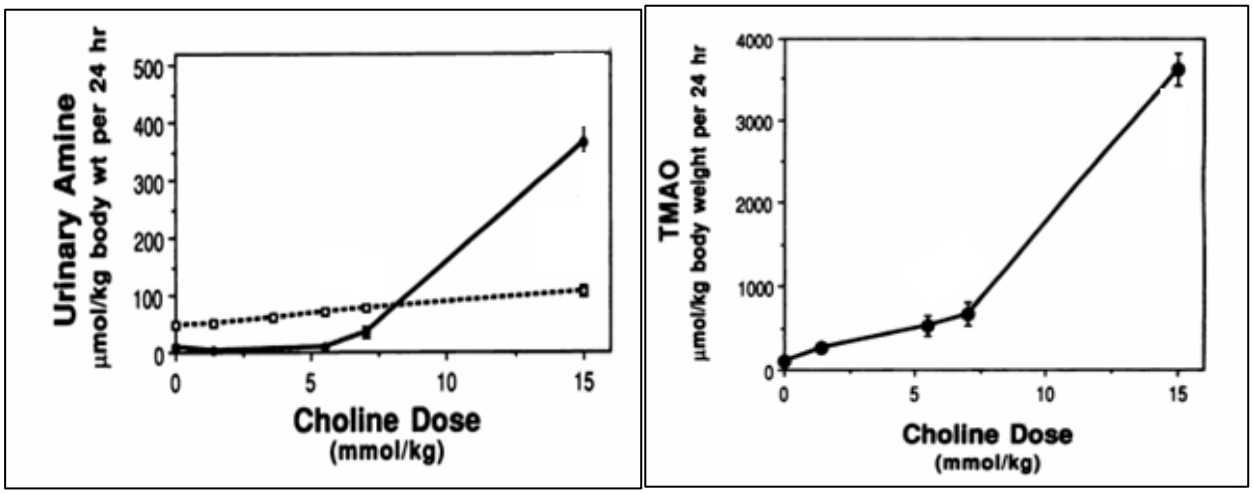

Figure 21: Dimethylamine (□...□), TMA (●...●) and TMAO formation from orally administered choline (**Zeisel et al., 1989**).

As mentioned before, high concentration of TMA is associated with fishy body odor, trimethylaminuria, and sweating and so do the high doses of choline when they are used as an aid to diagnosis. (**Arseculeratne et al., 2007; Marks et al., 1977**).

III.7 Possibility of using trimethylamine as an antagonist of the alcohol effect

We have gone beyond the experimental part which was to take place to test and prove in vivo effect of TMA on alcohol drinkers, and given the aforementioned facts, and due to the importance of our topic, we can suggest high probability of the effect for TMA on alcohol drinkers, as it has been known since ancient times, the smell of fish returns to the drunk his consciousness, as it is considered to be the source of TMA. Historically, according to Shihabuddin al halawani, if the person drunk and smell fish he will wake up, he said: if you are drunk, smell fish. So, her Shihabuddin al halawani meant that the smell of fish has an effect on alcohol drinker. Of course, this saying is not enough to propose that TMA has effect on alcohol, so we will try to relate between the effects of choline on alcohol and TMA basing on the structural similarity between TMA and choline.

TMA is considered to be part of choline and both have role in the human body, especially at the level of synapses; protonated TMA may combine with the same receptor of acetylcholine and TMA competitively inhibits cholinesterase in the presence of acetylcholine chloride (**Furukawa and furukawa, 1959; Hobbiger, 1956; MAKC, 2014**). TMA affect BBB and both TMAO and choline can cross it (**Del Rio et al., 2017; McArthur et al., 2018; Murakami et al., 2000**).

Not long ago, research has proven the effectiveness of choline on alcohol; it reduces and prevents the effect of alcohol on central nervous system, L1 cell adhesion molecule, the severity of fetal alcohol effects and its effects on neurobehavioral (**Tang et al., 2014; Thomas et al., 2007; Thomas et al., 2010**). So, starting from this relation between choline and TMA; -because choline reduces the effects of alcohol on neurobehavior and TMA is a metabolite of choline and it inhibits cholinesterase-, we propose that TMA can increase the level of acetylcholine so that it can enhances choline like effects on alcohol drinkers.

In otherwise, tetramethylammonium, which has similarity structure with TMA, attenuates significantly the evoked release of GABA, blocks the NMDA receptor and causes the release of cholinergic transmitter from excitatory nerve endings-releasing acetylcholine from nerve terminals. Moreover, both acetylcholine and tetramethylammonium combine with the same receptor, 'muscarinic' receptor (**Kuriyama et al., 1984; Mitchelson, 1971**; **Zarei and Dani, 1995**).

The importance of 'muscarinic' receptor in our work is that acetylcholine has long been implicated in reward and higher-order cognitive processes. Moreover, recent development of novel selective drugs targeting muscarinic acetylcholine receptors (mAChRs) also known as the cholinergic

receptor, has highlighted their therapeutic potential. **Walker et al., (2020)** identified mAChR as a potential therapeutic target for the treatment of alcohol use disorder (AUD). The researchers hypothesized that restoring cholinergic balance within the striatum through enhancement of mAChR signaling would reduce alcohol consumption (**Walker et al., 2020**). Taking in consideration that tetramethylammonium and TMA both combine with the same receptor of acetylcholine, 'muscarinic' receptor, and the researchers hypothesized that enhancement of mAChR signaling would reduce alcohol consumption; we can propose that TMA and TMAO could act on mAChR to reduce alcohol consumption.

Excitation in the brain is the key process for sensory perception, information transfer and consciousness. Alcohol inhibits the major excitatory neurotransmitter, glutamate, particularly at the NMDA glutamate receptor which is highly permeable to calcium figure (22) (A) (**Nutt, 1999; Zarei and Dani, 1995**).

TMA blocks endplate ionic currents and thus nerve signal transmission, and it can block NMDA receptor but its mechanism of action is not clear (**Dwyer et al., 1980; Zarei and Dani, 1995**). In the presence of alcohol (B) the latter blocks NMDA receptor and reverses the effects of glutamate. Otherwise, in the presence of TMA we think that probably it can interact with alcohol and inhibits it effects in synapses by two mechanisms; either fixes in NMDA receptor, inhibit alcohol to fixes and allow to calcium to enter inside the cell (C), or reacts directly with alcohol and binds it (D); allowing NMDA receptor to function properly and thus, the person regain its consciousness.

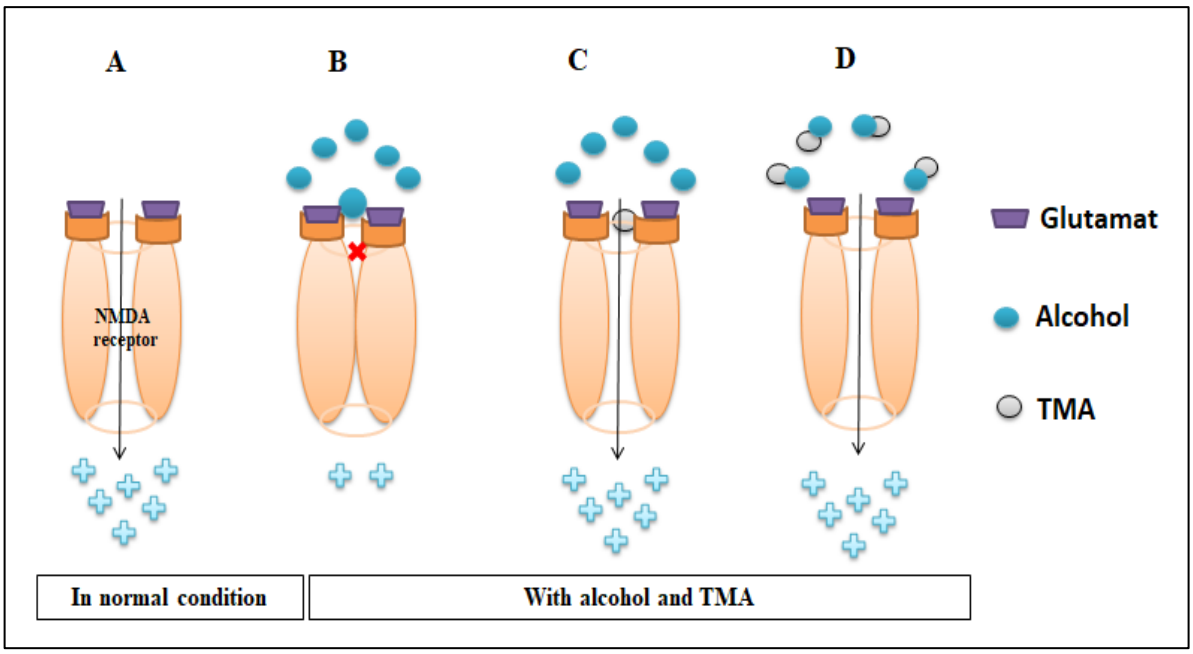

Figure 22: An estimate of how TMA inhibit alcohol at the NMDA receptor.

In an effort to bring the picture closer to the reader, we searched for drugs that works on awareness by the same mechanism that we are proposing for TMA. Flumazenil is among these drugs.

Flumazenil is a pharmacological antagonist of the central nervous system (CNS) effects of benzodiazepines. It is used to reverse sedation induced by benzodiazepine drugs. Thus, flumazenil provides a safe and effective means of attenuating or reversing the CNS-depressant effects of benzodiazepines (**Amrein et al., 1987; Votey et al., 1991**).

Flumazenil has been studied for awakening of comatose patients, reversal of sedation after surgery and in critically ill patients, and management of hepatic encephalopathy. It improves the level of consciousness in patients with benzodiazepine overdose (**Hoffman and Warren, 1993**).

Flumazenil interacts at the central benzodiazepine receptor to antagonize or reverse the behavioral, neurologic, and electrophysiologic effects of benzodiazepine agonists and inverse agonists (**Hoffman and Warren, 1993**). It acts by binding CNS benzodiazepine receptors and competitively blocking benzodiazepine activation of inhibitory GABAergic synapses. Flumazenil has been detected at the benzodiazepine receptor site of the GABA receptor in the cerebellum, the ventrolateral thalamus, and the lateral premotor cortex (**Boecker et al., 2010 Votey et al., 1991**).

Through the way flumazenil works, we can guess how TMA can works on alcohol, especially since structure similarities indicate that the interaction of TMA with alcohol is better than choline. As we look in figure (23) (A) GABA in normal condition dissociates from GABA receptor. In the presence of alcohol (B) the latter inhibits this dissociation and allows to chloride ions to enter the cell; which reduces the cell's excitability and thus, affects the conscience. Otherwise, in the presence of TMA we think that it can interact with alcohol and reverses its effects in the synapses by two mechanisms, either it fixes on GABA receptor and inhibit its action (C), or reacts directly with alcohol and binds it (D). By this way (C-D) alcohol probably wouldn’t be able to increase the ability of GABA to open the chloride channel and as a result, it won’t enhance the actions of the brain's main inhibitory system, leading to the preservation of consciousness (**Nutt, 1999**).

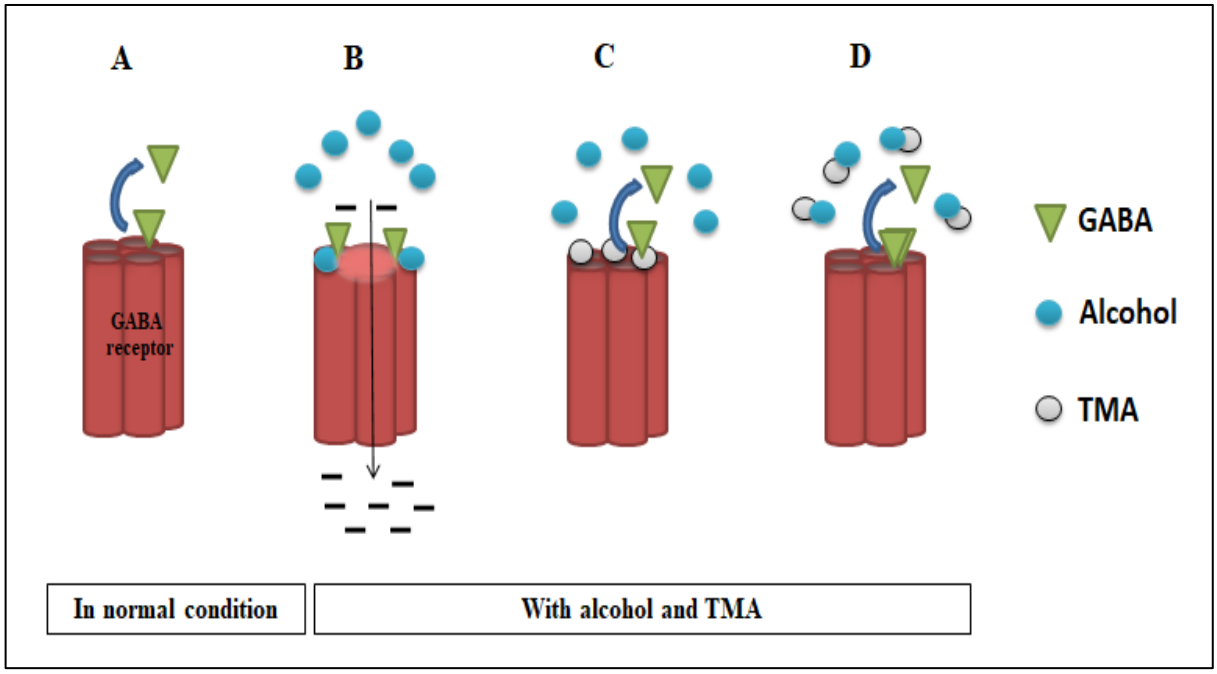

Figure 23: An estimate of how TMA inhibit alcohol at the GABA receptor.

The Possibility of using TMA as an antagonist on the alcohol effect remains uncertain as the research needs more in-depth elaboration and laboratory applications to prove the effectiveness of TMA on alcohol drinkers.

Conclusion

Conclusion

Marine food is a one source for animal protein and mineral salts, let alone, the commercial and economic value it carries around the world. Among this marine food, "sardines" (***Sardina pilchardus***) are considered the most consumed seafood in Algeria.

This study is interested in TMA which is considered to be the source of the smell of fish, especially ***S. pilchardus***. TMA is not considered as important nutrient in the human body but it may be turn more beneficial in future. In fact, it has been assumed historically, that it affects the alcohol drinker which is the biggest danger facing society. Alcohol does not only affect society, but also affects its consumer negatively. The metabolism of alcohol leads to the formation of toxic metabolites, affects especially the digestive tract, but also the liver, heart and brain.

Previously, in an attempt by researchers to reduce the harm effects of alcohol, choline, which has a similar structure as TMA, was studied as potential agent to fight the effects of alcohol.

Choline is found in a variety of food elements; moreover, limited quantities of it can be synthesized from endogenous sources. It has several biological functions in the human body but also has a great ability to interact with alcohol residues in the brain and reverse its effects on neurobehavior.

In this analytical study, we tried to prove that TMA also could reverse effect of alcohol on the brain, starting basically from the structural similarity between TMA and choline and also the fact that they both share the same receptor. Thus, experimental studies are required to verify this proposal.

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Viva date :07/10//2020

Theme

Sardine trimethylamine (***Sardina pilchardus***): a probable antagonist of the cerebral effect of alcohol

Abstract

Many people in various parts of the world suffer from the negative effect of alcohol, it lead to inappropriate ways of behavior in society, let alone, car accidents, crimes and social scourges.

This study sought to describe the relationship between alcohol, trimethylamine (TMA) and choline through recently published articles that demonstrated that choline has an effect on alcohol in the same way that the TMA can affect the alcohol drinker.

It is known that the smell of fish originates from TMA, which has a similar structure to choline, and from this basis we suggest this hypothesis. TMA is a part of the components of the human body that includes several functions, especially those related to synapses. Choline also has a greater importance in the human body, where it participates in its basic necessary processes, such as signal transmission and membrane formation, also, both choline and TMA are bound by metabolism processes.

In an attempt by us to establish the relationship between TMA and alcohol, we compared both structures, that of choline and that of TMA, which further can confirm the validity of the above-mentioned hypothesis.

Keywords: *Sardina pilchardus*, Trimethylamine, Choline, Alcohol.

Résumé

De nombreuses personnes dans diverses régions du monde souffrent des effets négatifs de l'alcool, qui conduisent à des comportements inappropriés dans la société, et ce en plus des accidents de voiture, des crimes et des fléaux sociaux.

Cette étude cherche à décrire une éventuelle relation entre l'alcool, la triméthylamine (TMA) et la choline à travers des articles récemment publiés qui ont démontré que la choline ressemble probablement la TMA concernant l'effet que peut affecter le buveur d'alcool.

C'est connu que l'odeur du poisson provient de TMA. Cette dernière a une structure similaire à celle de la choline, et à partir de cette particularité, nous suggérons cette hypothèse. La TMA fait partie des composants du corps humain et assurent plusieurs fonctions importantes, en particulier celles liées aux synapses. La choline a également une plus grande importance dans le corps humain, où elle participe aux ses processus vitaux, tels que la transmission du signal et la formation des biomembranes. De plus, la choline et la TMA sont liées par des processus métaboliques.

Dans notre tentative d'établir la relation entre la TMA et l'alcool, nous avons comparé les deux structures, celle de la choline et celle du TMA, ce qui peut contribuer à la validation de l'hypothèse susmentionnée.

Mots clés: *Sardina pilchardus*, Triméthylamine, Choline, Alcool.

الملخص

يعاني كثير من الناس في أنحاء مختلفة من العالم من التأثير السلبي للكحول، فهو يؤدي إلى سلوك غير لائق في المجتمع، ناهيك عن حوادث السيارات والجرائم والآفات الاجتماعية.

سعت هذه الدراسة إلى وصف العلاقة بين الكحول، ثلاثي ميثيل أمين والكولين من خلال المقالات المنشورة مؤخرًا والتي أظهرت أن للكولين تأثير على الكحول بنفس الطريقة التي يمكن أن يؤثر بها ثلاثي ميثيل أمين على شاربي الكحول.

من المعروف أن رائحة الأسماك تنبع من ثلاثي ميثيل أمين، التي لها بنية مشابهة لمادة الكولين، ومن هذا الأساس نقترح هذه الفرضية. يعتبر ثلاثي ميثيل أمين جزءًا من مكونات جسم الإنسان يتضمن وظائف عديدة، خاصة المتعلقة بالمشابك. للكولين أيضًا أهمية أكبر في جسم الإنسان، حيث يشارك في عملياته الأساسية الضرورية، مثل نقل الإشارات وتكوين الغشاء، ويرتبط كل من الكولين و ثلاثي ميثيل أمين بعمليات التمثيل الغذائي.

في محاولة منا لتأسيس العلاقة بين ثلاثي ميثيل أمين والكحول، قمنا بمقارنة بنيتي الكولين و ثلاثي ميثيل أمين، مما قد يساهم في تأكيد صحة الفرضية المذكورة أعلاه .

الكلمات المفتاحية: *Sardina pilchardus* ، ثلاثي ميثيل أمين، الكولين، الكحول.