KAPITEL 7 / *CHAPTER 7* [7]
THE POTENTIAL OF TRIMETHYLGLYCINE AS A MEANS OF THE COMPLEX REGULATION OF CARBOHYDRATE METABOLISM IN HYPERGLYCEMIA AND OBESITY

DOI: 10.30890/2709-2313.2024-32-00-006

Introduction

Since 2021, we have been researching means of regulating metabolic processes with the help of bio-protectors—substances of different chemical natures that can stabilize the body's biochemical processes under the influence of aggressive exogenous factors.

Trimethylglycine showed the most promising results among several substances. Trimethylglycine is a donor of methyl groups -CH_3, which determines its ability to neutralize chemically active radicals. Our experiment on animal models demonstrated a pronounced antioxidant effect of trimethylglycine in alcohol-induced oxidative stress [1]. Brief results of this experiment showing the hepatoprotective effect of trimethylglycine are presented below (Table 1).

Table 1 - Activity of transferases (AST, ALT and GGT) in the blood of rats, U/L (in control group, 2 group (EtOH) and 5 group (EtOH + trimethylglycine) of animals; *M*±*m*, *n*=7)

Groups of animals → / Enzymatic activity ↓	Control	2	5
AST, U/L	98.1±4.9	197.6±10.9**	110.1±7.1##
ALT, U/L	79.2±4.7	169.3±8.8**	91.3±6.9##
GGT, U/L	6.9±0.7	19.7±1.1**	9.2±0.6*##

*Note: data are statistically significant (*p<0.05 and **p<0.001) compared with the control group and # p<0.05, and ##p<0.001 compared with the 2nd experimental group, respectively*

At first, we thought that trimethylglycine's hepatoprotective effect was due to its antioxidant properties, which are explained by its chemical structure (Table 2).

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However, further study of its properties demonstrated its anti-inflammatory properties, which are discussed below.

Table 2 - Activity of oxidoreductases (LDH, SOD and catalase) and the content of malonic dialdehyde (MDA) in the blood serum and liver tissue of rats (in control group, 2 group (EtOH) and 5 group (EtOH + trimethylglycine) of animals; *M*±*m*, *n*=7)

Groups of animals → / Biochemical parameters ↓	Control	2	5
LDH, U/L	489±18.2	990±28.3**	610±19.1**##
SOD, U/mg of protein/min	260±21.2	148.5±15.3**	220.2±14.2#
Catalase, U/mg of protein/min	239.8±11.3	139.3±9.1**	179.7±12.1*#
MDA, nmol/mg of protein	40.9±2.3	56.3±4.1*	42.1±1.8#

*Note: data are statistically significant (*p<0.05 and **p<0.001) compared with the control group and # p<0.05, and ##p<0.001 compared with the 2nd experimental group, respectively*

The COVID-19 epidemic has also awakened our interest in the role of inflammation in the dysfunction of endothelial cells, which are the target cells for the SARS-CoV-19 virus. For this reason, we focused on the anti-inflammatory effects of trimethylglycine, particularly its ability to inhibit the NF-κB signaling pathway, which is responsible for the secretion of IL-1β, and its ability to regulate TLR4.

Our second discovery was the effect of trimethylglycine on the culture of porcine aortic endothelial cells. The addition of trimethylglycine to the culture solution promoted cell division, which increased the volume of cell mass by 20% compared to the control group [2].

Also, under the influence of trimethylglycine, endothelial cells began to form more complex morphological structures, presumably precapillary (Figure 1).

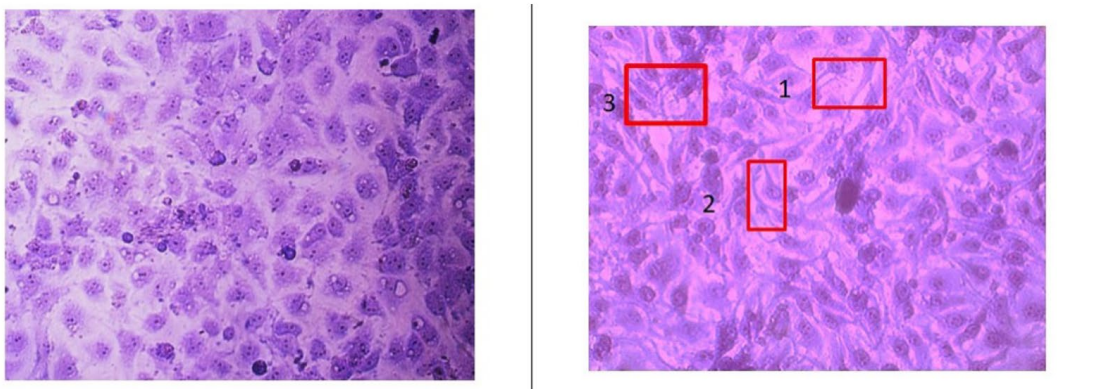

Figure 1 - Confirmation of signs of cell differentiation (cell elongation (2) and formation of precapillary structures (1,3)) of the endothelium under the action of trimethylglycine.

Thus, we have a biologically active substance (trimethylglycine) that:

✓ has antioxidant properties by neutralizing free radicals;

✓ has anti-inflammatory properties by regulating the NF-κB signaling pathway;

✓ has a beneficial effect on endothelial cells, promoting their proliferation and complicating their morphological structure.

Also, the main mechanisms of the development of non-alcoholic fatty liver disease:

- energy stress of the cell caused by excess calories (excess free radicals);
- violation of the intestinal barrier (dysfunction of endothelial cells);
- inflammatory process as a result of the two previous factors.

Thus, this article will consider how trimethylglycine can correct metabolic processes in non-alcoholic fatty liver disease and type 2 diabetes mellitus.

7.1. Potential of trimethylglycine for mitochondria, oxidative stress and development of non-alcoholic fatty liver disease

It is necessary to clarify that non-alcoholic liver disease is a complex of structural and functional changes in the liver that develop in stages:

1. Steatosis - accumulation of lipids in hepatocytes without their structural and functional changes.

2. Non-alcoholic steatohepatitis - occurrence of inflammation as a result of functional changes.

3. Fibrosis and cirrhosis - occurrence of irreversible structural changes.

Since direct metabolic disturbances occur in the early stages, we will consider these clinical conditions i

Steatosis is an adaptive response of hepatocytes to caloric stress and is an excessive accumulation of intracellular triglyceride stores. Lipid accumulation in hepatocytes should be considered as a balance between the metabolic processes of adipose tissue lipolysis, an increase in free lipids in the blood and their absorption, *de novo* lipogenesis on the one hand, and fatty acid oxidation and the secretion of very low-density lipoproteins on the other hand (Figure 2).

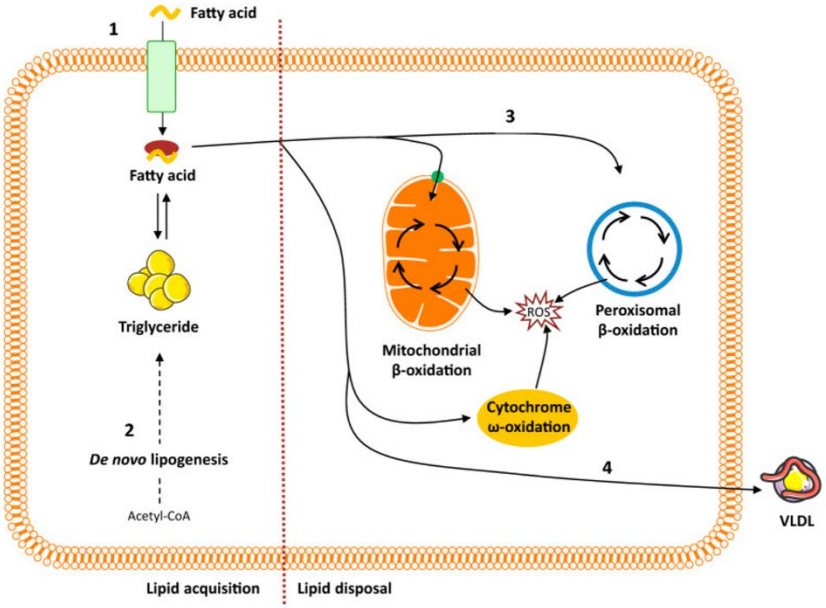

Figure 2 - Simplified diagram of lipid metabolism in the liver [3]. Although the absorption of free fatty acids (1) and their extraction from hepatocytes by secretion of very low-density lipoproteins (4) affect the number of intracellular lipids, the development of oxidative stress is associated with the processes of lipogenesis *de novo* (2) and lipid oxidation (3).

The primary protective reaction to an increase in the level of intracellular lipids is

an increase in the intensity of β-oxidation of fatty acids, with subsequent induction of tricarboxylic acid cycle and stimulation of oxidative phosphorylation—at least due to the substrate acetyl-CoA, which is formed as a result of β-oxidation of fatty acids. Although the process occurs primarily in the mitochondria, lipid overload, and impaired mitochondrial function cause higher levels of fatty acid oxidation in peroxisomes and cytochromes, generating reactive oxygen species (ROS) [3-4].

It should also be noted that chronic free fatty acids overload of hepatocytes leads to a decrease in their transport rate into the mitochondria. This, in turn, reduces the activity of the mitochondrial respiratory chain, which reduces ATP synthesis. Decreased ATP levels may be responsible for the induction of endoplasmic reticulum stress and activation of the unfolded protein response, which stimulates *de novo* lipogenesis pathways and further aggravates liver steatosis [5].

In parallel, there is an accumulation of mitochondrial cholesterol, which leads to changes in the inner mitochondrial membrane and depletion of mitochondrial glutathione (GSH) [6].

In fact, trimethylglycine can utilize ROS by donating a methyl group -CH_3 and participating in the methionine cycle [1,7] (Figure 3).

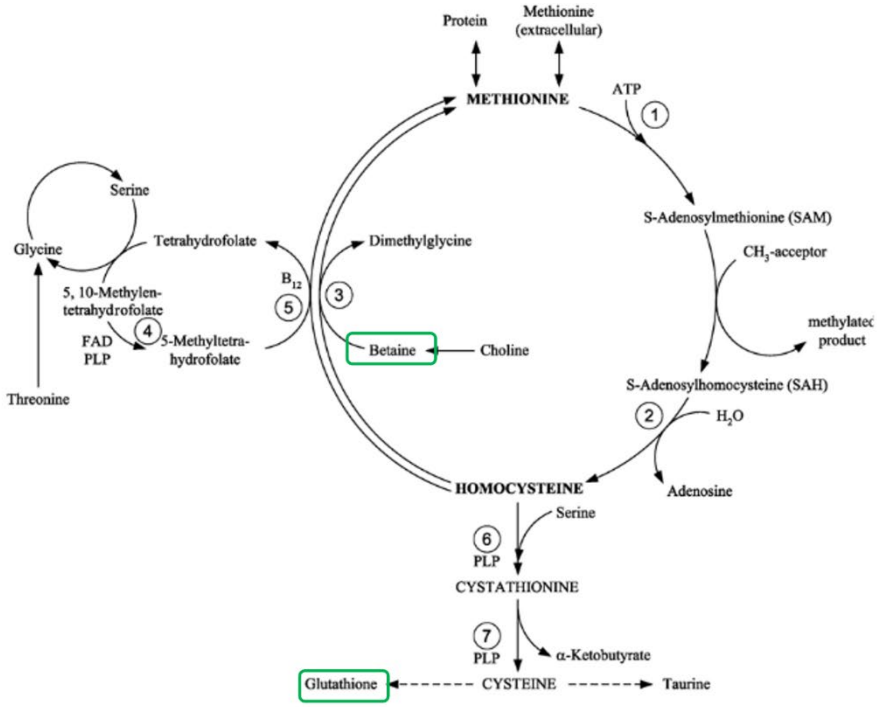

Figure 3 - Methionine cycle and intermediates (adapted [8])

7.2. Potential of trimethylglycine in intestinal barrier dysfunction, inflammation and non-alcoholic fatty liver disease

As mentioned above, the protective mechanism consists of the adaptation of adipose tissue to excessive fat intake. The first stage of this adaptation is an increase in the number of adipocytes (hypertrophy). This is followed by proliferative processes leading to an increase in the number of adipocytes (hyperplasia). At this stage, the adaptation process takes place without violating the structural and functional integrity of the organism, but up to a certain limit. In the second stage, a critical adaptation of the adipocytes to the excessive accumulation of triglycerides occurs by activating inflammatory signaling pathways. The most important of these is the NF-κB kinase inhibitor/nuclear factor κB pathway, in which free fatty acids activate receptors of the toll-like receptor TLR-4, which is located on the surface of macrophages and adipocytes. This results in the release of interleukins IL-1 and tumor necrosis factor TNF-α. At the same time, insulin is inhibited in the presence of TNF-α tumor necrosis factor [8].

One of the strategies for treating insulin resistance is to use drugs that block the synthesis of TNF-α. For example, berberine is a natural isocholone alkaloid that inhibits MEKK1 and MEK1/2 [9], through which TNF-α affects the DNA of cells and consequently their insulin resistance (Figure 4).

It should be noted that the canonical mechanism of anti-inflammatory action of trimethylglycine is the suppression of NF-κB activity and IL-1β expression by inhibiting MAPK and NIK/IKK - it suppresses the expression of HMGB1 mRNA and protein, which regulates the activation of TLR4, which is involved in the activation of NF-κB, as well as HDAC3, which binds to IκBα to activate NF-κB [10]. This suggests that the therapeutic potential of trimethylglycine may be similar to that of berberine.

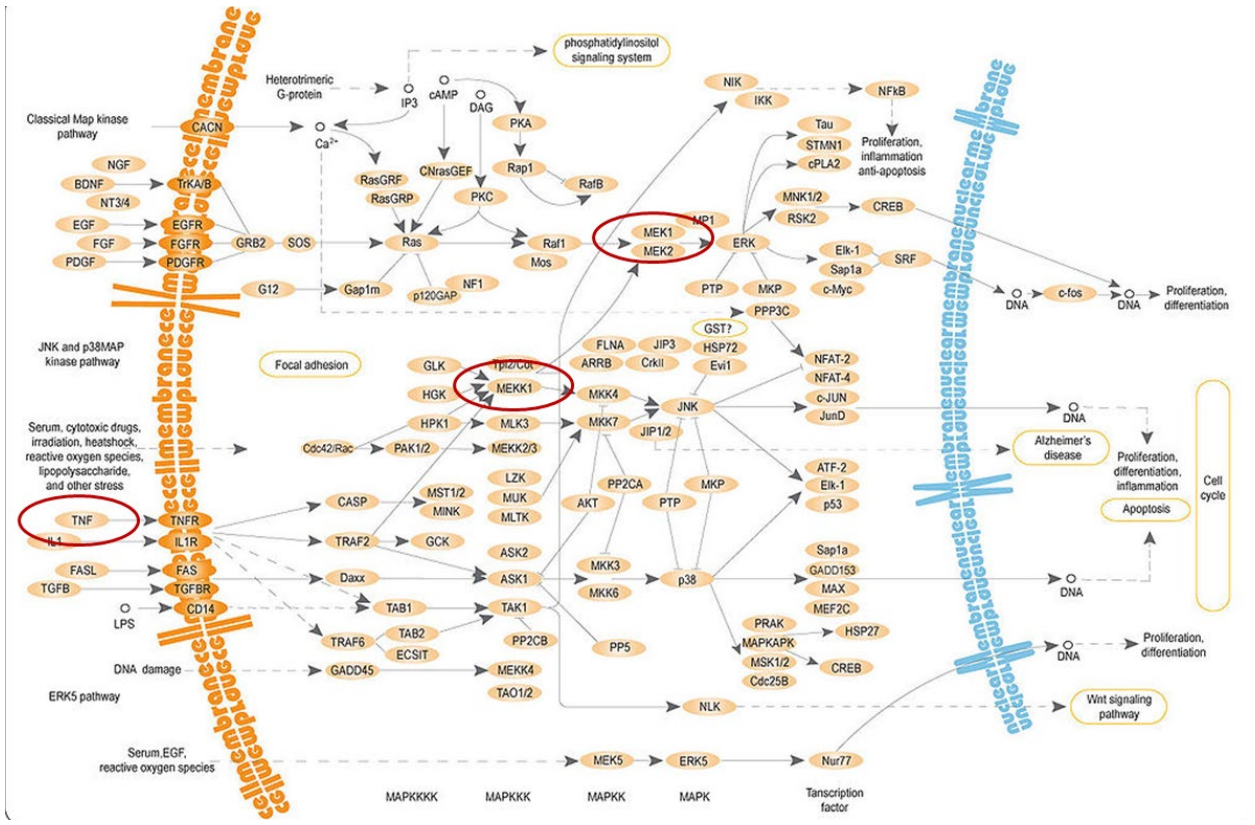

Figure 4 - MAPK pathway and berberine therapeutic targets (circled red)

At the same time, the role of the intestinal barrier in the development of an inflammatory response and the subsequent cascade of biochemical changes in the metabolism must be taken into account. Obesity is often associated with an active growth of bacteria in the small intestine. As a result, the permeability of the intestinal barrier is disrupted and bacterial products, lipopolysaccharides (LPS), enter the liver from the intestine via the biliary system or the portal vein. Products and metabolites of the intestinal microbiota reach Kupffer cells and cause inflammation via LPS/Toll-like receptor TLR4, releasing cytokines, including TNF-α [11] (Figure 5).

Considering the ability of trimethylglycine to inhibit the receptor TLR4, as well as its effect on the morphology of endothelial cell cultures, we believe that trimethylglycine can reduce the manifestation of the inflammatory process when the intestinal barrier is disrupted.

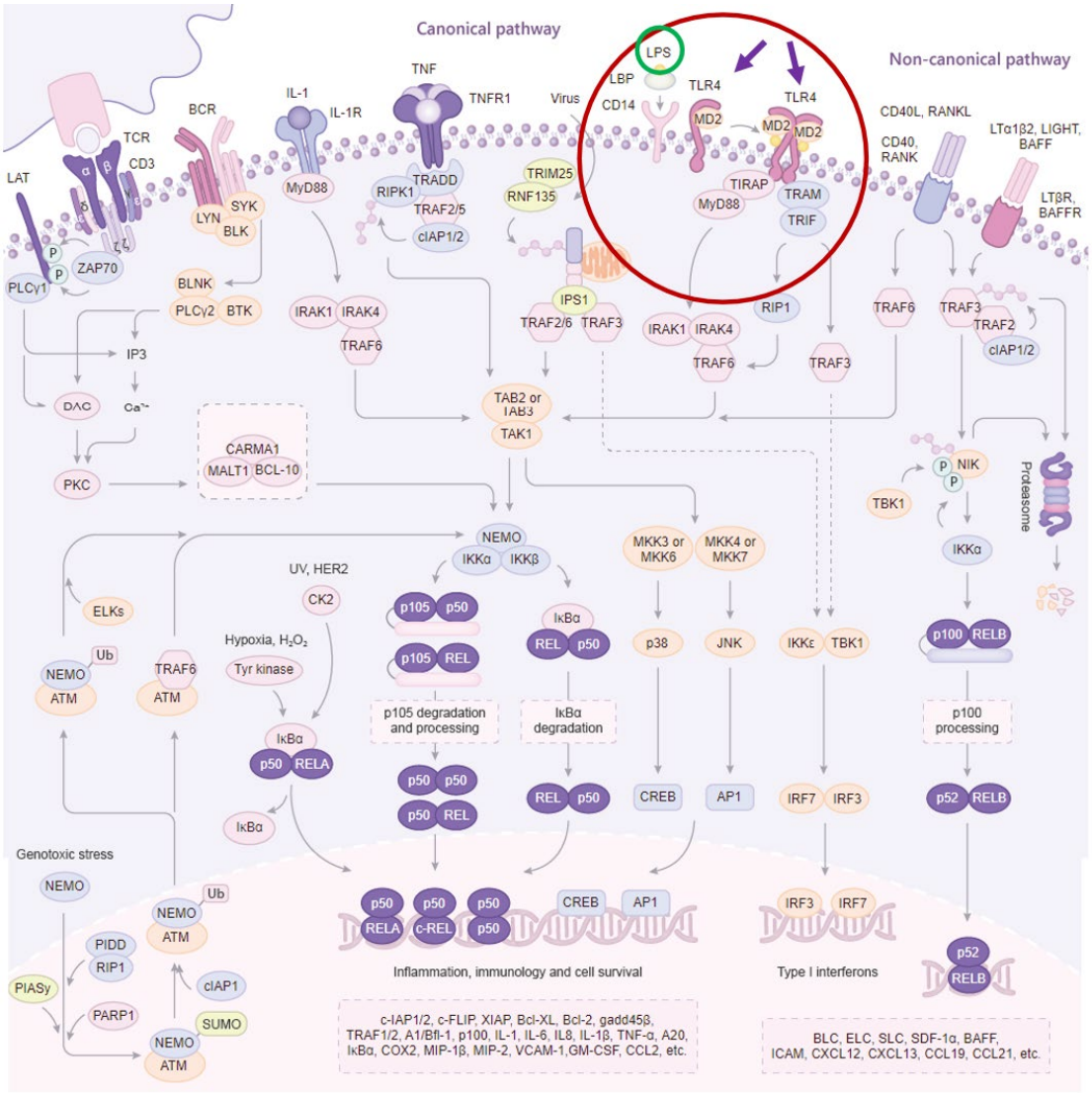

Figure 5 - LPS/Toll-like receptor TLR4 mediated inflammation by gut microbiota products and metabolites (adapted [12])

Summary and conclusions

Type 2 diabetes mellitus should be viewed as a persistent morphofunctional change caused by a complex of cascading biochemical abnormalities. Considering that the causes of these biochemical derangements persist even when the clinical picture of type 2 diabetes mellitus occurs, we believe that by influencing these causes we will be able to stabilise the metabolism. In particular, by analogy with berberine, we believe that trimethylglycine will be able to influence hyperglycaemia based on its ability to influence key factors in the development of hyperglycaemia (cellular stress, intestinal barrier disruption, inflammatory reactions and insulin inhibition).