

Gut bacterial metabolite TMA induces hepatic metabolic stress and inflammation via mediation of A20

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Choline can be found in food products such as eggs, milk, liver, red meat, poultry, shellfish and fish⁽¹⁾. Ingested choline is processed by gut commensal bacteria into trimethylamine (TMA)⁽²⁾ which is subsequently transferred to the liver through blood circulation where it is enzymatically converted into trimethylamine N-oxide (TMAO) by a family of enzymes, Flavin-containing monooxygenases (FMOs)⁽³⁾. FMO3 is one of the members that is strongly associated with lipid metabolism, while its substrate (TMA) and product (TMAO) are linked with metabolic syndrome and cardiovascular diseases^(4–7).

We employed a cell culture model of mouse hepatocytes AML12 and treated the cells with TMA to investigate the pathophysiological effect of this metabolite in lipid metabolism. We further used quantitative polymerase chain reaction (qPCR) and immunoblotting analysis to determine the change in expression of lipid metabolism associated genes when exposed to TMA. Lipid synthesis and concentrations were determined by enzymatic assays.

Our studies showed that treatment with TMA promoted lipogenic processes which are associated with upregulation of *fmo* genes expression comparing to untreated control hepatocytes ($p < 0.01$) (Figure 1A). TMA treated AML12 cells had increased mRNA expression of key regulators in cholesterol and triglycerides synthesis, *srebp2* and *srebp1c* ($p < 0.01$) as well as their downstream target genes such as *acc*, *fasn*, *scd1* ($p < 0.05$) (Figure 1A). Increased lipid synthesis in AML12 cells induced by TMA was further reflected in the assembly and secretion of very-low-density lipoproteins (VLDL). This was evidenced by the upregulated expression of VLDL structural protein ApoB, *mtp*, *apob* and *crebh* mRNA content ($p < 0.001$) as well as secreted triglycerides and cholesterols ($p < 0.0001$) (Figure 1B). More intriguingly, we found that protein expression of A20 (Figure 1C), an anti-inflammatory gene against the NFkB inflammatory signalling⁽⁸⁾, was inhibited by the activation of lipid and lipoprotein overproduction suggesting TMA induced lipotoxicity.

In conclusion, we demonstrated that TMA delivers its pro-lipogenic and pro-inflammatory activity by inducing expression of genes in hepatic lipid and lipoprotein metabolism. The subsequent lipotoxicity activates metabolic inflammation via the mediation of anti-inflammatory factor A20.

References

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