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TRANSLATIONAL MEDICINE: BENCH TO BEDSIDE

Quelling Inflammation with Ketosis and Steric Chemistry

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Exploring the clinical relationship of diet and inflammatory disorders of the gastrointestinal tract has been a challenging landscape of investigation.[1] Recent studies have shed light on the contribution of specific carbohydrates to fat accumulation and inflammation in animal models of non-alcoholic fatty liver disease. Rodents fed *ad libitum* diets that include high-fructose corn syrup in their chow, develop profound fatty infiltration of the liver and necroinflammatory changes.[2, 3] In the process of absorption through the gut with passage to the liver, where nutrition, metabolism, and immunity are intimately balanced, the wrong substrates in food could set inflammatory mechanisms awry. Research efforts and epidemiological reports in this line of investigation have emboldened many of us to dissuade our patients from consuming concentrated sources of fructose.[4]

Long before high-fructose corn syrup became a pariah, however we well understood alcohol to be a key culprit in liver injury. Oxidation of alcohol by alcohol dehydrogenase generates acetaldehyde in hepatocytes. Acetaldehyde flogs hepatic destruction forward by generating protein adducts, stimulating lipid peroxidation, and nucleic acid oxidation.[5] Less known is the role alcohol plays in directly stimulating the inflammasome and triggering immune cell cascades in the liver after the initial insult.[6] The inflammasome is a cytosolic complex of proteins inside immune cells and hepatocytes, which converts extracellular signals into an inflammatory response.[7] Five inflammasome complexes have been described: NLRP3, NLRP1, NLRP6, NLRC4, and AIM2. The inflammasome is initially spurred into formation by so-called "group 1" signals: typically TOLL-like receptor agonists, such as the TLR4 agonist lipopolysaccharide (LPS) or TLR9 agonistic CpG DNA fragments. These prime the inflammasome by upregulating transcription of its components and ramping up production of pro-cytokines. This prepares the inflammasome to respond to diverse "group 2" signals which include metabolic danger signals, such as ATP and uric acid (both of which are key signals driving inflammasome activation in alcoholic liver disease).[8] The end result is component protein oligomerization and conversion of pro-caspase-1 to caspase-1 and secretion of mature IL-1β and IL-18 along with elaboration of a host of chemokines that recruit additional immune effectors to the injured liver.[9, 10] Genetic manipulation of the pathway by deleting group 1 signal sensing or direct blockade of group 2 signals leads to an attenuated inflammation, and in the case of liver disease, protection from inflammatory injury and fibrosis.[10–13] Overall, the inflammasome has come to be recognized as a central driver in many autoimmune and autoinflammatory diseases including gout, obesity, multiple sclerosis, and atherosclerosis. In the GI tract, inflammation in the liver, pancreas, and bowel are all regulated in part by inflammasome activation.[14–16] We know that we need to get our alcoholic liver disease patients to stop drinking, and we may choose to advise them against concentrated sources of fructose, but what other diet or lifestyle recommendations can we offer to our patients struggling with inflammation?

Recently, two groups published complementary articles identifying means of quelling inflammasome activation that may lead to new management approaches in GI inflammatory disorders. Youm *et al.*[17] showed ketone production could quiet inflammasome signaling through *in vitro* demonstrations with murine macrophages and human monocytes as well as *in vivo* measures of inflammasome activation with a mouse model of Muckle-Wells syndrome. The authors first stimulated bone marrow-derived macrophages (BMDMs) with LPS (a group 1 signal) followed by ATP (a group 2 signal) in the presence or absence of β-hydroxybutyrate (BHB). They demonstrated inhibition of caspase-1 activation at serum concentrations of BHB that are regularly achieved by strenuous exercise or a 2-day fast.

Next, they utilized the same experimental design but primed the BMDMs with either *Salmonella typhimurium* infection to stimulate NLRC4 or *Franciscella tularensis* to activate AIM2. In both cases, NLRC4 and AIM2 inflammasome pathways remained intact and cultured cells produced IL-1β regardless of the presence of BHB in the supernatant. Thus demonstrating BHB specifically inhibits the NLRP3 inflammasome but not its relatives NLRC4 or AIM2. What follows is a long parade of molecular pathway work carefully demonstrating just what BHB-mediated NLRP3 inflammasome inhibition is not: it is not signaling through the G-protein-coupled receptor GPR109a, it is not due to transcriptional regulation via inhibition of histone deacetylation, nor is it due to reduced mitochondrial stress given the increased energetic efficiency of ketone body metabolism. The authors ultimately show that BHB turns off NLRP3 activation of caspase-1 by inhibiting potassium efflux from cells, similar to its putative active function in quieting neuronal excitability in epilepsy. They wrap up their work with an elegant demonstration *in vivo* using a

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ketogenic diet to blunt inflammation and limit end-organ damage in a mouse model of Muckle-Wells syndrome. Paleo diet, anyone?

In parallel, Coll *et al.*[18] demonstrate an alternate means of inhibiting NLRP3 using the molecule MCC950, a compound screened from a panel of IL-1β-processing inhibitors. MCC950 inhibits the NLRP3 inflammasome directly and more broadly than BHB, shutting down both canonical (group 1+2 signals above) and non-canonical (caspase-11-driven) NLRP3-mediated production of IL-1β. This team from Dublin used similar cell culture techniques to Youm's group from Yale. They stimulated murine BMDMs with LPS, pre-treated with MCC950 and challenged with ATP, measuring IL-1β production as their readout. MCC950 blocked IL-1β release, but did not alter TNF-α production. MCC950 inhibited intracellular NLRP3 component protein oligomerization, and ultimately appears to work downstream of cellular potassium efflux, distinguishing its effects on the pathway from BHB. Coll's group closeout their study with a mouse model of multiple sclerosis and employ MCC950 to protect animals from clinical disease as well as effector cell accumulation in the brain. Ultimately, their work may lead to pharmacologic options for inflammasome modulation given the anticipated challenges with diet interventions and the limitations of long-term ketotic diets.

Should we recommend extreme low-carb diets to our patients with inflammatory diseases or wait for an inhibitor to make it through the trials and tribulations of, well, trials? The American cultural anthropologist Margaret Mead once declared, "It is easier to change a man's religion than to change his diet." If this is the case, let us pray that food becomes the new religion or at least grant us pharmacologic inhibition of the inflammasome, which may protect us from danger signals, forgive us our dietary sins and repair our inflammatory injuries.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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