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Spotlight

Fueling Ketone Metabolism Quenches Salt-Induced Hypertension

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Understanding the molecular mechanisms by which diet and exercise regulate disease has the potential to help identify new treatments. In their recent study, Chakraborty *et al*. (*Cell Reports* 2018;25:677–689) discovered that supplementation with a metabolic precursor produced β-hydroxybutyrate (BHB), counteracting the pathological effects of high-salt diet-induced hypertension, suggesting a new treatment modality.

In recent work by Chakraborty *et al.*, the authors discovered that the ketone body metabolite β-hydroxybutyrate (BHB) reduces hypertension-associated pathology induced by a high-salt diet [1]. While a high-salt diet led to a reduction in the protective metabolite BHB, the authors found that dietary supplementation with a BHB precursor, 1,3-butanediol, elevated BHB levels and protected mice from hypertension and kidney inflammation.

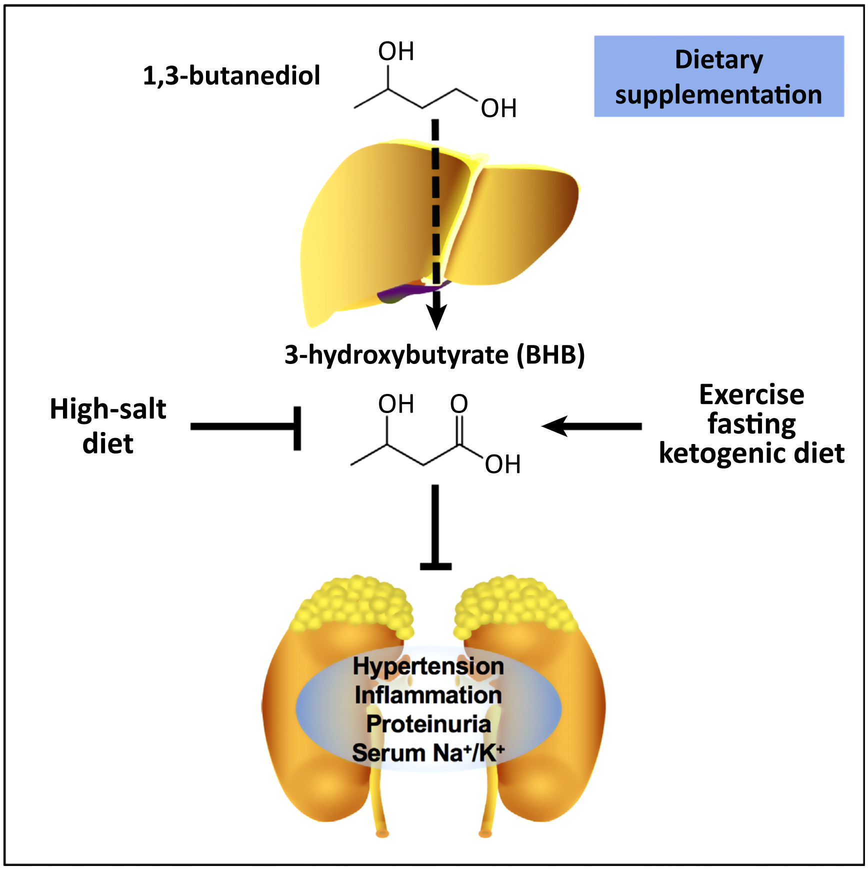
Hypertension, commonly known as high blood pressure, is a condition where blood flow is restricted, putting higher pressure on arterial walls. Increased blood pressure is a significant risk factor for morbidity and mortality worldwide and is associated with a greater risk of cardiovascular disease [2]. Many factors, such as genetics and diet, contribute to the onset of hypertension. Fortunately, hypertension can be treated with drugs that lower blood pressure, by changes in diet, and by increased physical activity. Drugs such as thiazide diuretics, angiotensin-converting enzyme inhibitors, or angiotensin II receptor blockers are commonly prescribed, but there is debate about the best first-line treatment [3].

Hypertension is also a feature of metabolic syndrome, a multifactorial disease associated with risk factors for heart disease, including abdominal obesity, low high-density lipoprotein (HDL) levels, high triglycerides, and high fasting blood sugar. Given the importance of exercise and changes in diet in improving the health of these patients, identifying biochemical pathways associated with these beneficial changes could lead to new therapeutic strategies. In the recent study by Chakraborty *et al.* [1], the authors used mass spectrometry to identify changes in the blood of salt-sensitive Dahl rats fed a high-salt diet, which induces hypertension [1]. In rats fed this high-salt diet, the most reduced metabolite was BHB, a ketone body generated primarily in the liver. This finding, coupled with earlier studies showing an increase in this metabolite in response to exercise and calorie restriction, suggests that BHB is a key molecule in regulating hypertension [4,5].

After identifying BHB as a potentially protective molecule, the authors proposed restoring BHB in high salt-fed rats to determine whether this metabolite protects against the deleterious effects of hypertension. Rats fed the precursor of BHB, 1,3-butanediol, had increased levels of BHB, despite being fed a high-salt diet. Treatment restored BHB levels in both fasting and fed rats, suggesting that nutritional supplementation bypasses the effects of a high-salt diet on BHB production. Supplementation also reduced the hallmarks of hypertension, including systolic, diastolic, and mean arterial blood pressure, in addition to other markers of kidney disease (Figure 1). Increased BHB is known to decrease inflammation, which could explain its ability to limit the features of hypertension and kidney dysfunction through effects on multiple organ systems, in particular endothelial cells or the kidneys [6,7].

To address the mechanism of how BHB reduces hypertension in high salt-fed mice, Chakraborty *et al.* examined inflammatory markers of kidney function and the effects of high salt and BHB on the microbiota. The authors found that expression of inflammatory Th17 genes (*Sgk1*, *Il23*, *Rorgt*, and *Il17r*), which are increased by a high-salt diet, were largely unaffected in rats supplemented with 1,3-butanediol, suggesting that the protective effect of BHB is independent of Th17 responses [6]. Changes in the microbiota, specifically a decrease in *Lactobacillus* spp., in rats fed a high-salt diet have also been implicated in driving the progression of hypertension [8]. The authors found that *Lactobacillus* spp. and *Proteobacteria* were reduced and *Prevotella* spp. were increased by a high-salt diet. The changes in the microbiota were associated with a decrease in gluconeogenesis and ketone metabolism, which was not restored by supplementation with 1,3-butanediol. Interestingly, after 1,3-butanediol treatment, *Proteobacteria* and *Prevotella* shifted back toward the low-salt relative abundance and also correlated with an increase in protective *Akkermansia* levels [9,10]. Despite this reversion to a more protective distribution of microbiota, the authors did not consider this supplementation a rescue of the high salt-induced changes to the microbiota. It will be important for future studies to examine how these bacterial communities change under different hypertension treatment regimens.

Relatively minor changes in the Th17 response and microbiota upon supplementation with 1,3-butanediol suggested that other pathways contribute to the observed protective effect. Given that BHB has been previously shown to inhibit the activation of the NLRP3



Trends in Endocrinology & Metabolism

Figure 1. 3-Hydroxybutyrate (BHB) Reduces High-Salt-Induced Hypertension and Kidney inflammation. In this study by Chakraborty *et al.* [1], the authors show that the ketone body BHB (or βOHB) reduces hypertension and markers of kidney inflammation in rats fed a high-salt diet. Dietary supplementation with 1,3-butanediol, which is processed in the liver to BHB, bypasses the effect of a high-salt diet on inhibiting the production of 3-hydroxybutyrate. Activities such as exercise, fasting, and a ketogenic diet are also known to increase production of BHB. The increased production of BHB in 1,3-butanediol-fed rats on a high-salt diet reduced the negative effects of the high-salt diet by lowering blood pressure, kidney inflammation, and other markers of kidney dysfunction.

inflammasome, which has a role in driving inflammation during hypertension, the authors examined the effect of 1,3-butanediol supplementation on kidney inflammation [7,11]. The NLRP3 inflammasome is a complex of proteins comprising the proteins NLRP3, an adaptor protein ASC, and the enzyme caspase-1. Upon NLRP3 activation by many cellular stressors, the inflammasome complex assembles and caspase-1 is activated to cleave its downstream substrates, including the proinflammatory cytokines IL-1β and IL-18 [12]. Chakraborty *et al.* found that supplementation with 1,3-butanediol reduced the expression of *Nlrp3*, *Casp1*, *Il1b*, and *Il18* in kidneys of rats fed a high-salt diet, with corresponding decreases in serum IL-1β and a marker of renal inflammation, lipocalin-2. Given that NLRP3 inflammasome activity has been implicated in the promotion of kidney disease, the authors further examined the effect of 1,3-butanediol on kidney tubulointerstitial fibrosis, protein casts, and urinary protein excretion. Overall, the authors observed reductions in these markers of renal injury. Inflammatory macrophages are one of the main sources of inflammasome-mediated inflammation and the authors found that supplementation with 1,3-butanediol reduced the macrophage abundance in the kidneys of rats fed a high-salt diet. Although the authors suggest a compelling mechanism where 1,3-butanediol supplementation increases BHB and directly inhibits the activity of the NLRP3 inflammasome, this study does not rule out that protection is indirectly due to reduced overall inflammation and macrophage recruitment to the kidney.

Together, these findings suggest an important role for BHB and dietary 1,3-butanediol in limiting hypertension. Given the popularity of ketogenic diets, this study importantly reveals that direct supplementation with a precursor molecule to BHB may be sufficient for some of the beneficial effects of the diet. Future studies will need to examine the effect of dietary supplementation with these metabolites in the context of other available treatment combinations and should take advantage of these metabolites as important biomarkers in patients and research studies.

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Forum

Metabolic Disease Epidemics: Emerging Challenges in Regenerative Medicine

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The interplay between cell/tissue damage caused by metabolic dysfunction and regenerative potential remains elusive. The tissue engineering and regenerative medicine (TERM) field is now facing a worldwide epidemic of obesity. This Forum article uncovers prospective questions to be addressed in TERM toward the development of effective regenerative therapies adjusted to these new demands.

At the Dawn of Metabolic Disease Epidemics

The field of TERM envisions the generation of therapeutic strategies for restoration of normal tissue function after damage. Over the past decades, advances in TERM have been leading to the development of new therapies, particularly targeting the modulation of cellular behavior. Several approaches are proposed, which can be roughly divided into two groups: (i) cellular strategies based on cell transplantation (either alone or embedded in biomaterials/*in vitro* engineered tissues); and (ii) acellular strategies targeting *in situ* cell modulation (through the use of smart biomaterials or biomolecules). Given that cells are in general the main targets, such therapies rely on knowing/predicting to some extent how cells will respond. TERM scientists often face the challenge of patient variability, but this frequently disregards patients with metabolic alterations as a result of overweight, obesity, and metabolic diseases. Notwithstanding, the worldwide epidemic of obesity is a serious public health problem as it is increasing the incidence of metabolic diseases (onset of metabolic syndrome, type 2 diabetes mellitus, and cardiovascular disease) and associated complications, including in young people [1,2] at an alarming rate, being a major driving force for research on metabolism-associated pathologies.

It is well known that metabolic dysfunctions, particularly diabetes, lead to nonhealing conditions and chronic wounding as a result of exacerbated local inflammatory responses. Therefore, alterations in wound healing and tissue regeneration in patients with metabolic disorders are often extrapolated as a consequence of immune/inflammatory dysregulation. Nonetheless, metabolic effects at the cellular level, as well as the regenerative capacity of other cells, rather than on the immunobiological microenvironment, are frequently neglected.

Here, the interplay between metabolic dysfunction and tissue regeneration is discussed along with novel questions arising in the field of TERM.

Inflammation and Tissue Repair: Uncovered Paradigms in TERM

Obesity and the metabolic syndrome are characterized by a subclinical form of chronic inflammation. In turn, inflammation is a strategic partner of tissue repair and regeneration [3]. The immune system/inflammation comes into play in virtually all systems of the human body; thus, the inflammatory dysregulation may have an impact not only locally but also systemically. The metabolic dysfunction and associated oxidative stress affects the pro-/anti-inflammatory balance of signaling molecules, resulting in a feed-forward loop of macrophage activation and altered polarization toward the proinflammatory phenotype as an adaptive physiological immune response. Hence, it is intriguing to speculate whether this subclinical/low-level chronic inflammation of obesity alters immune-sensing and infiltrating compartments in metabolism-associated pathologies, particularly those that are themselves triggered by inflammatory processes [e.g., osteoarthritis (OA), tendinopathies; Box 1]. Furthermore, the link between metabolism and cellular functions is not limited to immune cells and metabolic tissues, prospectively impacting the role of other cells orchestrating tissue repair. Diet-induced obesity triggers a proinflammatory state as the first-line response of adipose tissue to overnutrition, including an increase of proinflammatory cytokines and relative deficiency of anti-inflammatory ones, accumulation of advanced glycation end products (AGEs), a switch on macrophage phenotype, and increased production of reactive oxygen species (ROS), promoting a profibrotic program [1,2]. Altogether, these immunometabolic shifts challenge tissue regeneration. Nonetheless, there is a lack of understanding regarding the biological mechanisms underlying the differential repair processes between healthy/lean and metabolically altered tissues. Therefore,