COVID-19

https://doi.org/10.1038/s42255-022-00646-1

Fasting as key tone for COVID immunity

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SARS-CoV-2-induced anorexia triggers systemic metabolic alterations. In a study published in *Nature*, Karagiannis et al. show that the ketone body β-hydroxybutyrate (BHB) improves COVID-19 disease outcomes. Further, BHB metabolically and functionally reprograms CD4$^+$ T cells, highlighting immunometabolic tuning of immunity in COVID-19.

Infection-induced anorexia is an evolutionarily conserved sickness behaviour that can mediate protective or detrimental immune functions[1]. Fasting conditions, including anorexia, lead to distinct biochemical alterations in the metabolic fuel supply, including the generation of ketones, and this is associated with alleviated disease symptoms in cancer, cardiovascular disease, and neurodegenerative disease[2]. Under conditions of fasting, the ketone body β-hydroxybutyrate (BHB) is synthesized in the liver from β-oxidation of free fatty acids and absorbed as an alternative energy source by peripheral tissues, such as the muscles, heart, and brain. Accumulating evidence suggests that BHB not only serves as an energy fuel, but also is a multifunctional molecule with cellular signalling capability, exerting direct effects on immune cells[3]. Although BHB derived from a ketogenic diet is associated with improved T cell function in humans[4], the extent to which it contributes to the severity of SARS-CoV-2 infection, which is characterized by T cell lymphopenia and dysfunction[5], remains unclear. In a recent study published in *Nature*, Karagiannis et al. show that attenuated production of BHB is correlated with impaired CD4$^+$ T cell function in patients with severe COVID-19, and BHB supplementation through a ketogenic diet or oral administration of ketone esters enhances survival of CD4$^+$ T cells and their capacity to produce interferon-γ (IFNγ), thereby boosting the antiviral immune response[6] (Fig. 1).

By comparing peripheral blood from patients suffering from acute respiratory distress syndrome (ARDS) induced by SARS-CoV-2, influenza or bacterial respiratory infections, the authors found that patients infected with SARS-CoV-2 had substantially lower serum concentrations of BHB, indicative of dysregulated ketogenesis. While, as expected, serum concentrations of several pro-inflammatory cytokines were elevated in patients with SARS-CoV-2-induced ARDS as compared to those with moderate symptoms or uninfected control

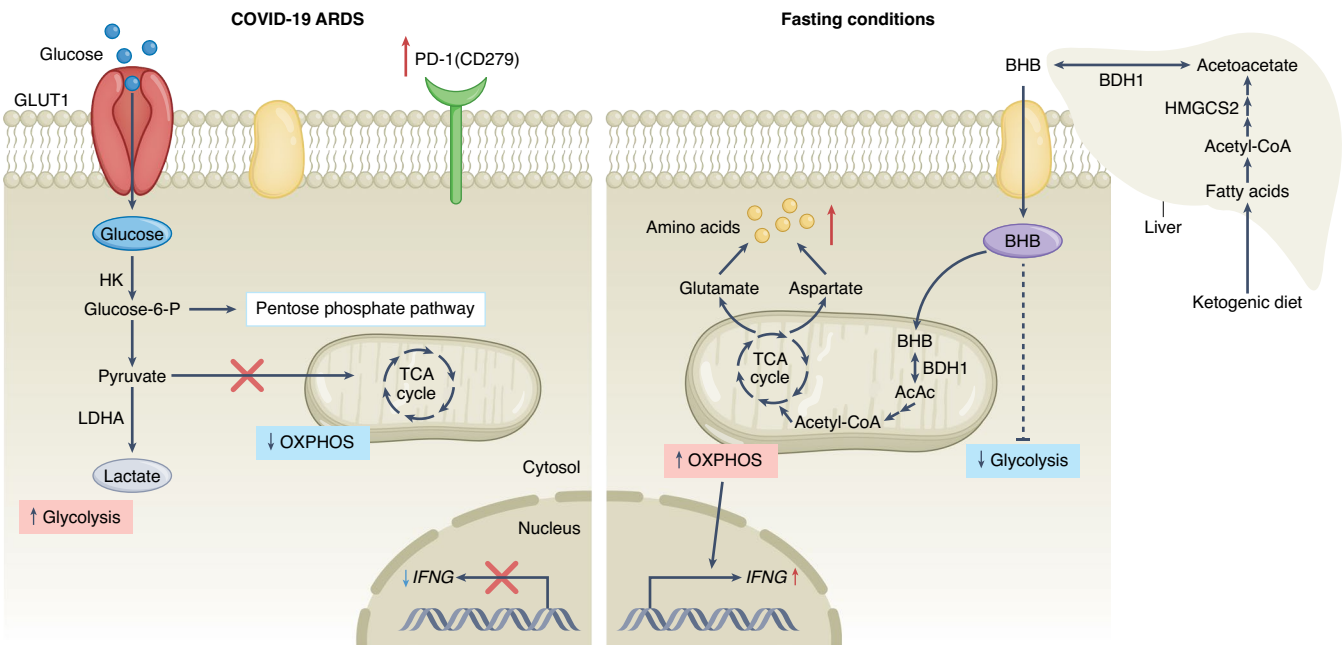

Fig. 1 | The ketone body β-hydroxybutyrate supports CD4$^+$ T cell functional fitness by fuelling mitochondrial OXPHOS to combat SARS-CoV-2 infection. Left, CD4$^+$ T cells from patients with severe COVID-19-associated ARDS express PD-1 and are metabolically skewed towards glycolysis. As the main carbon source, glucose is diverted into production of lactate and pentose phosphate pathway intermediates, rather than into the TCA cycle, resulting in reduced synthesis of amino acids and impaired capacity to produce IFNγ. Right, during fasting conditions (for instance, infection-induced anorexia or ketogenic diet), the ketone body β-hydroxybutyrate (BHB) is synthesized in the liver from fatty acids and serves as an alternative carbon source to fuel the TCA cycle, leading to enhanced mitochondrial OXPHOS and reduced glycolysis and thereby enhancing cellular capacity to produce IFNγ and boosting the antiviral immune response.

participants, interleukin-6 (IL-6) and IL-8 were significantly lower in SARS-CoV-2-induced ARDS than in influenza- or bacteria-induced ARDS, which suggests that cytokine storm might not be a primary driver of severe COVID-19. Previous studies have shown that moderate and severe COVID-19 are associated with altered cellular and systemic metabolism, including reduced levels of amino acids such as tryptophan and cysteine[7,8]. Therefore, the authors further explored the relationship between BHB and amino acid levels and observed that BHB supplementation increased cell numbers and enhanced IFNγ production in human and mouse type 1 helper (T_H1) cells under amino acid-deficient conditions in vitro[6]. Furthermore, in a preclinical model of SARS-CoV-2 infection, oral administration of ketone esters via drinking water or a ketogenic diet improved antiviral $CD4^+$ T cell functional fitness and viral clearance, resulting in reduced lung injury, faster recovery from weight loss and improved overall survival[6]. Collectively, this research suggests that BHB is a potential therapeutic target for the treatment of severe COVID-19.

Cellular metabolic alterations are emerging as drivers of T cell dysfunction in many diseases, including COVID-19[9], and dysfunctional mitochondria are characteristic of T cells in severely ill patients with COVID-19[10]. Using SCENITH (single-cell energetic metabolism by profiling translation inhibition)[11] for single-cell metabolic profiling analysis of $CD4^+$ and $CD8^+$ T cells from patients with COVID-19, the authors showed that T cells derived from bronchoalveolar lavage fluid displayed a markedly altered metabolic profile, favouring glycolysis with concomitantly dampened mitochondrial dependence. Further, peripheral blood-derived T cells from these patients had reduced capacity to oxidize both fatty acids and amino acids, which suggests that the overall capacity for mitochondrial oxidative phosphorylation (OXPHOS) is reduced in T cells from patients with COVID-19. Importantly, reduced cysteine levels might also contribute to an imbalanced redox state that could lead to impaired T cell survival in severe COVID-19, although this remains to be tested. Therefore, mitochondrial OXPHOS, which is required for T_H1 cell function[12], is impaired in severe COVID-19, thereby supporting the notion that both cell-extrinsic and cell-intrinsic metabolic alterations can affect T cell immunity and disease severity in COVID-19 and other diseases[9].

Given that BHB generation and T cell responses were impaired in patients with severe COVID-19 and that BHB supplementation improved disease outcomes, the authors next explored how BHB affects T cell metabolism. Upon its ketolysis, which is mediated by the enzyme BDH1 (β-hydroxybutyrate dehydrogenase), BHB serves as an alternative metabolic substrate for mitochondrial OXPHOS[13]. Accordingly, cell-intrinsic deficiency of BDH1 reverted the BHB-induced improvements in T cell survival and cytokine production, suggesting that the promotion of $CD4^+$ T cell function by BHB is largely dependent on BDH1-mediated ketolysis. The authors then performed a series of in vitro experiments to demonstrate that BHB improves mitochondrial function. First, they showed that BHB-supplemented T_H1 cells had elevated basal and maximal mitochondrial respiration and spare respiratory capacity, indicative of improved mitochondrial OXPHOS. Second, using SCENITH analysis, the authors showed that cultured T_H1 cells had increased mitochondrial dependence and compromised glycolytic capacity after the addition of BHB. In addition, the ability of $CD4^+$ T cells to metabolize fatty acids and amino acids was enhanced in the presence of BHB. Third, using kinetic tracing experiments with ^{13}C-labelled BHB, the authors showed that BHB was integrated into tricarboxylic acid (TCA) cycle intermediates in T_H1 cells. Furthermore, carbons from ^{13}C-labelled BHB were also incorporated into bioenergetic amino acids (for example, glutamate and aspartate) and oxidized glutathione (GSSG), consistent with the upregulated expression of genes encoding enzymes involved in cellular metabolic pathways, such as *Cpt1* (fatty acid oxidation), *Got1* (amino acid metabolism), and *Ndufs8* (OXPHOS). By contrast, carbons from ^{13}C-labelled glucose were entirely found in glycolytic intermediates, including lactate, and the pentose phosphate pathway. Therefore, these results collectively identify BHB as an alternative carbon source in $CD4^+$ T cells that alters cellular metabolism to support mitochondrial function in nutrient-deprived environments.

The disparity in ketosis observed between patients with severe COVID-19 and those with influenza remains to be determined. The synthesis of BHB is tightly regulated with temporal and spatial precision by the expression and catalytic activities of BDH1 and HMGCS2 (ref. [14]), which may be underlying factors for impaired BHB production in patients with severe COVID-19. Furthermore, lower serum concentrations of BHB in patients with newly diagnosed COVID-19 might also serve as a predictive risk factor for the development of severe COVID-19, and those patients could be recommended to adopt a ketogenic diet as a therapeutic strategy to enhance antiviral immunity. Finally, the authors showed that PD-1 expression was enhanced on T cells from peripheral blood and bronchoalveolar lavage fluid from patients with COVID-19-associated ARDS and that BHB supplementation reduced PD-1 expression on T cells in vitro and in vivo. As PD-1 is associated with T cell exhaustion or dysfunction in many clinical settings[9], it will be interesting to determine whether BHB similarly affects the expression of PD-1 or other exhaustion- or dysfunction-related molecules in other diseases, including chronic infections and cancer.

Overall, this study has identified the ketone body BHB as an alternative carbon source to fuel mitochondrial OXPHOS, thereby metabolically reprogramming T_H1 cells and improving antiviral immunity in conditions of infection-induced anorexia. Considering the diverse cellular signalling activities of BHB[3], it is possible that BHB alters $CD4^+$ T cell function through additional means such as transcriptional regulation or epigenetic modifications, consistent with a role for BHB in controlling $CD8^+$ memory T cell development via epigenetic regulation[15]. As metabolic programs are crucial regulators of $CD4^+$ and $CD8^+$ T cell plasticity and heterogeneity[9], additional studies are required to address whether BHB and other metabolites induced by a ketogenic diet have similar effects on other types of T cell during viral infection or in other nutrient-deprived contexts, including the tumour microenvironment. In summary, these important findings broaden our knowledge of dietary influence on antiviral immunity and provide new insights into and understanding of the variable morbidity associated with COVID-19.

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Published online: 30 September 2022

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Competing interests

H.C. is a consultant for Kumquat Biosciences, Inc. Y.W. declares no competing interests.