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Review

NAD metabolic therapy in metabolic dysfunction-associated steatotic liver disease: Possible roles of gut microbiota

Xinyi Lu,[1,2] Rui Yang,[2] Yu Chen,[2,*] and Daozhen Chen[1,2,3,*]

SUMMARY

Metabolic dysfunction–associated steatotic liver disease (MASLD), formerly named non-alcoholic fatty liver disease (NAFLD), is induced by alterations of hepatic metabolism. As a critical metabolites function regulator, nicotinamide adenine dinucleotide (NAD) nowadays has been validated to be effective in the treatment of diet-induced murine model of MASLD. Additionally, gut microbiota has been reported to have the potential to prevent MASLD by dietary NAD precursors metabolizing together with mammals. However, the underlying mechanism remains unclear. In this review, we hypothesized that NAD enhancing mitochondrial activity might reshape a specific microbiota signature, and improve MASLD progression demonstrated by fecal microbiota transplantation. Here, this review especially focused on the mechanism of Microbiota-Gut-Liver Axis together with NAD metabolism for the MASLD progress. Notably, we found significant changes in *Prevotella* associated with NAD in a gut microbiome signature of certain MASLD patients. With the recent researches, we also inferred that *Prevotella* can not only regulate the level of NAD pool by boosting the carbon metabolism, but also play a vital part in regulating the branched-chain amino acid (BCAA)-related fatty acid metabolism pathway. Altogether, our results support the notion that the gut microbiota contribute to the dietary NAD precursors metabolism in MASLD development and the dietary NAD precursors together with certain gut microbiota may be a preventive or therapeutic strategy in MASLD management.

INTRODUCTION

MASLD prevails as a leading chronic liver condition globally, with an anticipated impact on 190 million individuals in the United States by 2030.[1] The rising prevalence in China introduces distinctive features, including notable youthfulness among patients.[2] MASLD manifests across a spectrum, ranging from mild steatosis to severe metabolic steatohepatitis (MASH), potentially progressing to liver fibrosis, cirrhosis, and hepatocellular carcinoma.[3] The intricate connection between metabolic syndrome and MASLD, extending beyond liver pathology to impact extrahepatic organs, underscores the complexity of this condition.[4] Despite ongoing research into metabolic targets, drug therapy for MASLD remains inconclusive.

As a central metabolite, nicotinamide adenine dinucleotide (NAD) plays a vital role in DNA repair, protein deacetylation, energy, and redox homeostasis.[5] The association between NAD metabolism and MASLD has been explored extensively, with NAD deficiency identified as a contributor to MASLD in murine models.[6] NAD precursors such as nicotinamide riboside (NR) have shown promise in mitigating MASLD in animal models. However, the precise mechanisms linking NAD metabolic abnormalities in MASLD and gut microbiota remain elusive.

In this review, we provide an overview of the relationship between NAD metabolism and MASLD, with a focus on the role of the gut microbiota and its metabolites. Notably, the potential of specific gut microbiota, including *Prevotella*, in influencing NAD levels and MASLD progression is explored. Additionally, we discuss the therapeutic implications of manipulating gut microbiota for MASLD treatment, emphasizing the need for personalized approaches considering regional, dietary, and individual variations.

THE EFFECT OF NAD METABOLISM ON MASLD

NAD metabolism, mainly controlled by various metabolic enzymes, may regulate MASLD. In one way, NAD can be consumed by key metabolic enzymes, such as poly(ADP-ribose) polymerase (PARP), CD38, and Sirtuins, which have been reported in different metabolic disorders associated with MASLD.[7] In another, NAD levels in the liver are typically increased by the NAD synthesis pathway, which primarily includes the nicotinamide phosphoribosyltransferase (NAMPT)-mediated salvage pathway (nicotinamide (NAM) as the precursor), the nicotinate

[1]Wuxi Medical Center, Nanjing Medical University, Jiangsu 211166, China
[2]Wuxi Maternity and Child Health Care Hospital, Wuxi School of Medicine, Jiangnan University, Jiangsu 214002, China
[3]Department of Laboratory, Haidong Second People's Hospital, Haidong 810699, China
*Correspondence: cy-78@hotmail.com (Y.C.), chendaozhen@163.com (D.C.)
https://doi.org/10.1016/j.isci.2024.109174

Table 1. Reported associations between levels of NAMPT and MASLD and its high-risk factors

Study	Experimental grouping	Changes in levels of NAMPT and related indicators	High-risk factors related to NAMPT
Hosseinzadeh-Attar et al.[14]	Severely obese patients (n = 35) after 6-week weight reduction	Decreased levels of serum NAMPT	None
Dahl et al.[15]	Patients with MASLD (n = 58) vs. healthy controls (n = 27)	Decreased levels of serum NAMPT and hepatic mRNA levels of Nampt	PPAR α, glucose
Gaddipati et al.[16]	Patients with MASLD combined with moderate steatosis (n = 77) vs. non MASLD with or without simple steatosis (n = 38)	Decreased levels of visceral NAMPT	None
Qiu et al.[17]	Patients with MASLD (14 females and 86 males) vs. healthy controls (20 females and 91 males)	Decreased levels of circulating NAMPT	None
Auguet et al.[18]	Women with obesity and MASLD (n = 69) vs. women with obesity without MASLD (n = 19)	Higher levels of serum NAMPT	BMI, resistin, CRP, IL-6, TNF α
Motawi et al.[19]	Patients with T2DM (44 without CVD and 46 with CVD) vs. healthy controls (n = 60)	Higher levels of serum NAMPT	T2DM, CVD, resistin gene
Akbal et al.[20]	MASLD patients with increased liver enzymes (n = 30) vs. healthy controls (n = 27)	Higher levels of serum NAMPT	None
Elkabany et al.[21]	Children with obesity and MASLD (n = 31) vs. children with obesity without MASLD (n = 49)	Higher levels of serum NAMPT	Age, BMI, waist circumference, waist/hip ratio, ALT, total cholesterol, liver stiffness, CAP
Martos-Moreno et al.[22]	Prepubertal obese Caucasian children (n = 100) vs. healthy controls (n = 42)	Higher levels of serum NAMPT	Age, BMI, waist circumference, the surrogate markers of body fat, resistin, IL-6[a]

[a]PPAR α, peroxisome proliferator-activated receptor α; BMI, body mass index; CRP, C-reaction protein; CVD, cardiovascular disease; ALT, alanine aminotransferase; CAP, controlled attenuation parameters.

phosphoribosyltransferase (NAPRT)-mediated Preiss-Handler pathway (nicotinic acid (NA) as the precursor), and the quinolinate phosphoribosyltransferase (QPRT)-mediated *De novo* pathway (relatively rare).

NAMPT, which is an intracellular enzyme catalyzes the first step in the biosynthesis of NAD from NAM, is a core regulator of the intracellular NAD pool. Low hepatic NAD levels have reportedly been associated with the onset of MASLD, however, the NAMPT levels have been found to be diversity in a number of MASLD patients. (Table1) The levels of NAMPT may be influenced by multiple factors, including suffering from other metabolic diseases, age, gender,[8] exercise,[9] etc., which could partly be attributed to differences in basal metabolism. High expression of NAMPT is associated with other high-risk factors in most studies and is more suitable as a joint judgment indicator, while low expression of NAMPT is usually a single factor and may serve as a monitoring indicator for MASLD. Therefore, in-depth stratified research should be conducted to expand understanding of individual differences in NAMPT levels in MASLD patients. Moreover, after weight loss, NAMPT was accompanied by an increase in Sirtuin1 (SIRT1) levels and a decrease in PARP activity in healthy obese subjects.[10] In fact, the NAMPT-SIRT1-AMPK axis reportedly plays a role in inducing lipolysis,[11] alleviating liver steatosis,[12] and improving vascular repair after ischemia.[13] Consequently, the level of NAMPT is expected to become a comprehensive metabolic assessment indicator, and single factor analysis, as animal experiments have demonstrated, can better help understand its role in evaluating MASLD.

Correspondingly, in HFD-induced MASLD mice models with a single factor, supplementation with NAD precursor or NAMPT activator P7C3 has been shown to be significantly effective, with the potential to partially reverse the symptoms of MASLD. (Table 2) NAD precursor supplementation can improve glucose tolerance and insulin sensitivity and reduce body weight and liver damage in mice. Significantly, several experiments have reported that NAD can alleviate liver steatosis and inflammation, however, it has not been found in other experiments, which might be closely related to the type of mice, the purpose of induction, and the type and dosage of precursors. Anyway, animal experiments have shown promising prospects.

However, although in several clinical trials, NAD supplementation did increase the circulating concentration of NAD and its metabolites,[30] and a time-dependent decrease in liver enzyme ALT and γ-glutamyl transferase (GGT) circulating levels was observed, other significant effects have not been observed during a certain trial period compared with animal experiments.[31] But hopefully, in another clinical trial targeting postmenopausal women with prediabetes who were overweight or obese, supplementing with NMN enhances muscle insulin sensitivity, insulin signaling, and remodeling,[32] while similar experiments conducted in middle-aged and elderly men did not yield consistent results.[33] Moreover, the carnitine metabolic pathway and its derivatives metabolic pathway, which transferred BCAA, short-chain fatty acid and hydroxy fatty acid esters to mitochondria, is up-regulated in skeletal muscle by different NAD precursors in several researches.[34,35] Meanwhile, it is reported that the ratio of NAD^+/NADH significantly affected the irreversible oxidation reaction mediated by branched-chain a-keto acid

Table 2. The therapeutic effects of supplementing NAD on diet-induced MASLD mice

Study	Study design	Results
X. Pham et al.[23]	Male C57BL/6 J mice: (i) low-fat control , (ii) HF/high-sucrose/high-cholesterol control (iii) HF diet supplemented with NR at 400 mg/kg/day for 20 weeks.	NR supplementation significantly reduced body weight and collagen accumulation in the liver, and inhibited the activation of hepatic stellate cells, but did not attenuate serum ALT levels, liver steatosis, or liver inflammation.
Manickam et al.[24]	(i) C57Bl/6J wild-type male mice (WT) (ii) the type 2 diabetic B6.BKS(D)-Leprdb/J (db/db) male mice with P7C3 10 mg/kg body weight for 4 weeks, daily, i/p.	P7C3 treatment alleviates the fasting blood glucose, insulin resistance and glucose intolerance and increases the number of pancreatic β cells in db/db mice. Forthermore, P7C3 treatment increases the circulating HDL levels and decreases the inflammatory lipid mediators.
Cantó et al.[25]	(i) HFD-fed C57Bl/6J (ii) control mice. NR/MNM/NA was treated at the dose of 400 mg/kg/day for one week.	NR increases intracellular and mitochondrial NAD content in mammalian cells and tissues and enhances sirtuin activity. Foremore, NR lowers HFD-induced body weight gain by enhancing energy expenditure and make mice more sensitive to insulin.
A.J. Trammell et al.[26]	Male C57Bl/6J mice: (i) normal chow (ii) HFD (iii) HFD plus two low doses of streptozotocin. Half of the three groups were supplemented with 3g of NR chloride per kg of their diet.	NR improved glucose tolerance, reduced weight gain, liver damage and the development of hepatic steatosis both in prediabetic and T2DM mice.
Lee et al.[27]	Male, 8-week-old KK/HlJ mice were allocated to the control or NR group. NR (100 mg/kg/day) was administrated by an osmotic pump for 7 days.	NR treatment lowers the total cholesterol concentration in the liver and improves glucose control and levels of serum insulin and adiponectin. Hepatic proinflammatory markers such as TNF α and IL-6 are significantly improved, with no change in body weight gain, food intake, and liver function.
Yoshino et al.[28]	HFD-induced T2D model. NMN was treated at the dose of 500 mg/kg body weight/day	NMN ameliorates NAD biosynthesis and glucose metabolism and hepatic insulin sensitivity and restores gene expression related to oxidative stress, inflammatory response, and circadian rhythm.
Mitchell et al.[29]	male C57BL/6J mice: (i) synthetic low-fat diet (ii) HFD. Both were supplemented with two doses of NAM (0.5 and 1.0 g/kg of diet).	NAM supplementation is associated with reduced hepatic steatosis and inflammation concomitant with increased glycogen deposition and flux through the pentose phosphate and glycolytic pathways without extending lifespan.[a]

[a]HF, high-fat; HDL, high-density lipoprotein; NMN, nicotinamide mononucleotide.

dehydrogenase (BCKDH) in BCAA metabolism,[36] highlighting the possible role of NAD precursors in promoting the metabolism of BCAA and short-chain fatty acid. Importantly, the supplementation of 1000mg NR per day has not reported any adverse reactions in long-term trials, and only a few acute experiments have reported some mild adverse reactions such as flushing reactions.[34,37] Therefore, the poor efficacy of NAD precursor supplements is an urgent issue that needs to be addressed.

Currently, NAD precursors are mainly supplemented orally. In one way, the availability of NAD precursor supplements in the liver may be much lower than oral doses due to the complex digestive system and different absorption efficiency of humans, so that appropriate adjustments should be made to the drug dosage and administration method under the premise of safety. In another, MASLD patients often suffer from gut microbiota disorders, with lower diversity of gut microbiota compared to healthy subjects. Numerous studies have shown that the gut microbiota participates in human metabolic function, and the disorders of the microbiota may disrupt the interaction network with humans, thereby potentially reducing the conversion efficiency and therapeutic efficacy of NAD precursor supplements. Finally, it should be emphasized that supplementing NAD may greatly increase the level of NAMPT, which may be a risk factor for MASLD patients with elevated NAMPT expression. Activation of the eNAMPT/TLR4 inflammatory pathway have been reported to contribute to the progression of MASLD, MASH, and liver fibrosis.[38]

Overall, considering factors such as gut microbiota, drug dosage, administration method, gender and age, the treatment efficiency of NAD precursor supplements in future is still promising, which requires more clearly grouped clinical trials.

NAD METABOLISM CAN REGULATE THE COMPOSITION OF GUT MICROBIOTA IN MASLD

Recent research has linked the gut microbiota and its metabolites to the occurrence and development of MASLD. In one way, unhealthy dietary habits and lifestyles affect the composition and homeostasis of gut microbiota. The disruption of signal homeostasis between bacteria

Table 3. studies on gut microbiota associating with MASLD in animals and human

Study	Study design	Results
Zhang et al.[40]	C57BL/6 male mice: (i) High-fat/high-cholesterol (ii) high-fat/low-cholesterol (iii) normal chow diet for 14 months.	*Mucispirillum*, *Desulfovibrio*, *Anaerotruncus* and *Desulfovibrionaceae* were sequentially increased along stages of MASLD–HCC formation, and *Bifidobacterium* and *Bacteroides* were depleted in high-fat/high-cholesterol-fed mice.
Schwimmer et al.[41]	Children with biopsy-proven MASLD (n = 87) vs. children with obesity without MASLD (n = 37).	High abundance of *Prevotella copri* was associated with more severe fibrosis. *Proteobacteria* and *TM7* were higher in patients with MASH while *Fusobacteria*, *Verrucomicrobia*, and *Lentisphaerae* were higher in patients with MASLD but not MASH.
Boursier et al.[42]	Biopsy-proven MASLD patients: (i)Fibrosis F0/1 (n = 30, 10 with MASH) (ii)Fibrosis F $\geq$ 2 (n = 27).	*Bacteroidaceae* increased with the severity of liver lesions, whereas *Prevotellaceae* and *Erysipelotrichaceae* decreased. Patients with MASH had higher abundance of *Bacteroides* and lower abundance of *Prevotella* campared with those without MASH.
Jiang et al.[43]	MASLD patients (n = 53) vs. healthy subjects (n = 32)	*Alistipes* and *Prevotella* were significantly decreased in MASLD patients while *Escherichia*, *Anaerobacter*, *Lactobacillus* and *Streptococcus* were increased.
Shen et al.[44]	MASLD patients (n = 25) vs. healthy subjects (n = 22)	*Proteobacteria* and *Fusobacteria* phyla were more abundant in MASLD patients, however, there was a lower abundance of *Prevotella* in the MASLD group.
Moran-Ramos et al.[45]	Well-characterized patients with obesity and biopsy-proven MASLD: non-alcoholic fatty liver controls (n = 11) vs. fatty liver (n = 11) vs. MASH (n = 23).	*Parabacteroides distasonis* and *Alistipes putredenis* were enriched in fatty liver but not in MASH patients. *Prevotella copri* dominant cluster was associated with a greater risk of developing MASH.[a]

[a]HCC, hepatocellular carcinoma.

and host leads to a break in intestinal barrier function, thereby promoting bacterial translocation to the liver. Exposure of the liver to gut derived bacterial products can cause chronic endotoxemia and related changes in the gut-liver axis, leading to metabolic disorders.[39] Luckily, with the development of omics technology, gut microbes related to MASLD are gradually being discovered. Similar to the results of NAMPT, the composition of gut microbiota also exhibits diversity in MASLD patients. (Table 3)

In another, diet is also a powerful regulator of the gut microbiota. Peluso et al.[46] reported that supplementing NR in HFD-induced rats not only reproduced the improvement results in mice but reshaped the microbiota composition of the lower digestive tract (cecum, proximal colon, and distal colon). Increased α-diversity was observed in HFD-induced animals treated with NR at 4, 8, and 12 weeks. NR was found to modulate the rat fecal microbiota by inducing a sustained increase in *Erysipelotrichaceae* and *Ruminococcaceae* species. Moreover, a significant increase in the relative abundance of a species of *Prevotellaceae* was also observed in the lower gastrointestinal tract. Interestingly, the regulatory effect of NR on gut microbiota showed similar results in another group of HFD-induced mice experiments.[47]

Additionally, NAD-dependent histone deacetylase SIRT1 seems to serve as a core enzyme in regulating gut microbiota, epithelial biology, and inflammation.[48] Mice with gut-specific SIRT1 deficiency have more Paneth and Goblet cells and reshape the gut microbiota,[49] which may be the key point for the connection between MASLD and gut microbiota.

In general, NAD metabolism can regulate the composition of gut microbiota in MASLD animal models, but the role of reshaped gut microbiota is still unknown, which needs more research to demonstrate the complex relationship between NAD metabolism and gut microbiota.

THE EFFECT OF GUT MICROBIOTA ON MASLD BY REGULATING NAD METABOLISM

The gut microbiota has unique NAD metabolic pathways that may help enhance host metabolic flexibility. In comparison with the liver, the gut microbiota converted NAD precursors into NAD through a specific *De novo* synthesis pathway via the key enzyme NaMNAT and NADS. *Fusobacterium*, *Firmicutes*, and *Proteobacterium* synthesized NAD through this pathway. Notably, some *Firmicutes* possessed alternative synthetic pathways as well. This pathway converts quinolinic acid (QA) into NaMN and synthesizes NAD through NMNS and NMNAT. In addition, the supplementation of NAD precursor NAM, NA, and NR by gut microbiota also plays an important role in the synthesis of NAD. Only *Actinomycetes*, *Firmicutes*, and *Proteobacterium* are capable of uptaking NAM and NA, while the salvage of NR is only present in *Firmicutes* and *Proteobacteria*.[50] (Figure 1)

Different from NAMPT, a homologous nicotinamide enzyme was found in *Escherichia coli* and *Saccharomyces cerevisiae*.[51] Nicotinamide deamidase (PncA) can convert NAM into NA, thus up-regulating the Preiss-Handler pathway. The recovery routes of NAM in different microbiota and eukaryotes are different. Vertebrates carry a nadV-like nicotinamide phosphoribosyltransferase gene and no nicotinamidase gene, while the pncA-like nicotinamidase gene is more common in microbiota.[52]

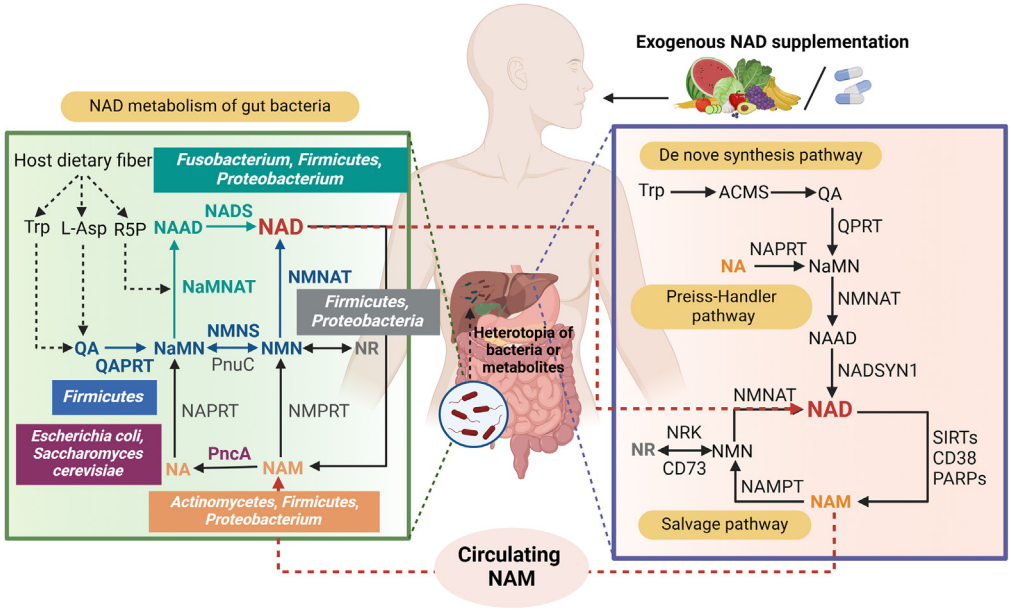

Figure 1. The metabolic pathway of nicotinamide adenine dinucleotide (NAD) and the interaction between gut microbiota and host

When the imbalance of gut microbiota leads to damage to the intestinal barrier, the microbiota or metabolites can translocate to the liver through the gut-liver axis, affecting liver NAD metabolism. On the one hand, the host utilizes amino acids or vitamins from dietary to synthesize NAD and its precursors, and the host derived circulating NAM is one of the main sources of microbial NAD synthesis. On the other hand, the gut microbiota can utilize dietary fiber that the host cannot metabolize to synthesize NAD and supplement the liver's NAD pool. In summary, exogenous NAD supplements or dietary can promote gut microbiota and host NAD metabolism and their crosstalk, jointly promoting liver NAD pool levels. L-Asp, L-Aspartic acid; Trp, tryptophan; R5P, ribose 5-phosphate; QA, quinolinic acid; NaMN, nicotinic acid mononucleotide; NAAD, nicotinate adenine dinucleotide; NA, niacin; NAM, nicotinamide; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; ACMS, aminocarboxymuconate semialdehyde decarboxylase. (Created with BioRender.com).

In particular, the NAD deamidation metabolites synthesized by gut microbiota using PncA contribute to most of the synthesized liver NAD.[53] Chellappa et al.[54] revealed that gut microbiota, together with the host's NA synthesis pathway bypass the intestinal salvage pathway, suggesting a unique bidirectional interaction between the host and gut microbiota when sharing NAD biosynthesis precursors. Gut microbiota also endows the host with resistance to NAMPT inhibitors. The deamidated NAD precursors are sufficient to salvage the NAD reduction and energy consumption induced by NAMPT inhibitors, highlighting the equally important role of the Preiss-Handler pathway in the liver NAD synthesis. Moreover, overexpression of the pncA gene in *Escherichia coli* could affect the liver NAD metabolism. Further optimization of the PncA sequence of *Escherichia coli* for direct expression in mammals resulted in significantly higher NAD levels compared to the control group, even about five times higher than the normal diet group, and improved diet-induced MASLD mice. However, due to the limitation of liver cell absorption efficiency, the widely used and efficient NAD precursors NR and NMN only increased liver NAD levels by 1.5– to 2-fold.[55]

Therefore, direct incorporation into the NAD through NR is insufficient to significantly raise the total NAD pool in the liver without the effect of gut microbiota, which highlighting the role of gut microbiota in absorption of NR supplements into the liver NAD levels, even if there isn't much evidence to support this. Particularly, gut microbiota with high pncA gene expression may greatly increase NAD levels in the liver, thereby enhancing the therapeutic effect of NAD precursor supplements on MASLD.

FACTORS AFFECTING HOST-BACTERIA NAD METABOLISM: PHYSIOLOGICAL STRUCTURE AND DIETARY HABITS

The obvious vertical structure, horizontal structure, pH, oxygen concentration and reduction potential of the intestinal tract shape the colonization, distribution and density of bacterial community in the intestinal tract.[56]

The small intestine maintains a high intracellular oxygen concentration, and the main use of oxygen or nitrate as electron acceptors drives the microbial community to be composed mainly of facultative anaerobic bacteria and aerobic bacteria, such as *Streptococcus*, *Lactobacillus*, and *Enterobacterium*.[57] Meanwhile, dietary habits dynamically affect the composition of gut microbiota. Dietary amino acids are the main precursors of NAD synthesis. Since they are primarily absorbed in the upper digestive tract, microbes colonized in the distal sections of the small and large intestine cannot use them. Bacteria in the small intestine can almost rely on host derived NAM for microbial NAD synthesis, with circulating NAM accounting for 80–90% of microbial NAD synthesis.[54]

The colon is where the majority of intestinal bacteria colonize. Long retention time of intestinal contents, adequate nutrition supply, slow intestinal peristalsis, and low anaerobic electrification *in situ* promote colonization and reproduction of bacteria. Anaerobic bacteria, such as

Bacteroides, *Bifidobacterium*, *Bacteroides*, can outnumber aerobic bacteria by a factor of 100–1000 due to restrictions on oxygen and nitrate uptake.[58]

Carbohydrate fermentation is the core activity of gut microbiota, driving the energy and carbon economy of the colon.[59] Colonic bacteria synthesize NAD by decomposing and fermenting food dietary fiber that cannot be absorbed by the small intestine. The abundance of *Bacteroides* and *Prevotella* is significantly related to fiber rich diet.[60–62] Dietary fiber mainly promotes the synthesis of microbial NAD in large intestine through Aspartic acid and ribose 5-phosphate. About half of the colon microbial NAD is produced by *De novo* synthesis passway from fiber, with the other half made up of circulating NAM.[54]

Importantly, diet is also a key source of BCAA. On the one hand, a higher NAD^+/NADH ratio is the main factor promoting the irreversible oxidation reaction of BCAA, on the other hand, specific gut microbiota also encode genes that mediate the degradation of BCAA.[63] Correspondingly, BCAA metabolism participates in mitochondrial energy metabolism by producing acetyl CoA and succinyl CoA, and regulates the NAD^+/NADH ratio. Therefore, gut microbiota also plays a significant role in regulating the balance of NAD and BCAA metabolism in host.

Additionally, Litvak et al.[64] proposed that colonocyte metabolism functions as a control switch, mediating a shift between homeostatic and dysbiotic communities. Specifically, the oxidative phosphorylation metabolism of colon cells induces the epithelial hypoxia environment, which helps maintain the colonization of specific anaerobic bacteria. In turn, anaerobic bacteria provide benefits for the host through fermentation fibers, which supports a new strategy to promote the stability of gut microbiota by restoring the intestinal environment.[65,66] However, there is limited research on the NAD metabolism of gut microbiota, focusing on the NAD metabolism of gut microbiota may help to better understand the metabolic interactions between hosts and microbiota.

PROBIOTICS MAY ENHANCE THE EFFICACY OF NAD AND ITS PRECURSORS IN MASLD

Based on the relationship between gut microbiota and MASLD, researchers have conducted numerous researches on NAD regulated dominant microbiota to improve MASLD. For example, after supplementing HFD-induced C57BL/6 male mice with NR, fecal matter was transferred to another mouse to establish an NR-conditioned microbiota. NR conditioned fecal microbiota transplantation (FMT) reshaped the gut microbiota and reduced HFD-induced weight gain by increasing energy expenditure, similar to the effect of NR supplements.[47] Moreover, in HFD-induced mice with methylation-controlled J protein knockout microbiota, intestinal NAMPT and SIRT1 expression as well as total NAD levels and NAD^+/NADH ratio were significantly increased, and the availability of NAD in the liver was improved, which slowed the progression of MASH.[67] Consequently, it is speculated that the NAD regulated probiotics can influence MASLD favorably independently of NAD precursor supplements.

Interestingly, Roy et al.[68] proposed that gut microbiota influences MASLD susceptibility; thus, a healthier gut microbiota is beneficial for both treating and preventing MASLD. However, in a latest research, NR supplementation failed to change the α and β diversity of fecal microflora of 40 non-diabetes and obese men within 12 weeks,[46] which may be due to the complexity and diversity of the human microbiota. Therefore, the limited effect of NAD precursor supplements may be due to the lack of synergistic effects with NAD regulated probiotics, thus enhancing the proportion and diversity of probiotics is necessary.

Currently, some probiotic supplements have shown good effects *in vitro* and *vivo* models. For instance, in HepG46 cells stimulated by oleic acid and cholesterol, *Lactobacillus sakei MJM60958* significantly inhibited 79.2% of lipid accumulation. Furthermore, in HFD-induced mice, *Lactobacillus sakei MJM60958* and *ADM14* both reduced weight, total blood cholesterol, and protein expression related to lipid accumulation, At the same time, the ratio of *Firmicutes*/*Bacteroides* was restored, and the relative abundance of specific microbiota was increased, which had a positive impact on metabolic disorders such as obesity and fatty liver.[69–71] Therefore, probiotic supplements combined with NR may be a more promising treatment option for MASLD. However, which microbiota may become NAD regulated probiotics and the specific mechanisms by which they respond to NAD precursor supplements to improve MASLD are still unclear.

NAD-METABOLIZED WITH *PREVOTELLA* MIGHT HAVE POTENTIAL EFFECTS ON THE TREATMENT OF MASLD

Prevotella is a kind of gram-negative obligate anaerobe, which has always been reported to be related to human infections such as caries and periodontal disease, as well as other diseases such as chronic osteomyelitis, bite related infections, rheumatoid arthritis and intestinal diseases.[72]

However, *Prevotella* is also associated with vegetarian or high fiber, which is consistent with the prevention and intervention of MASLD. D. Corbin et al.[73] observed that the microbiome enhancer diet (MBD) reduced the host's metabolizable energy by increasing gut microbiota fermentation and metabolites without changing energy intake and physical activity. The high abundance microbial communities detected in MBD are mainly fiber degrading or butyrate producers, including *Prevotella copri* and *Lachnospira pectinoschiza*. This study supports enhancing the body's energy metabolism system and shaping the microbiota through a fiber-rich diet and fewer processed foods, which may serve as a potential future treatment for the global obesity problem. In fact, the Mediterranean diet has been reported to be significantly associated with the increase of *Prevotella* and some fiber-degrading *Firmicutes*.[74] Hence the intervention of dietary fiber on intestinal microbiota can also be used as a novel protective therapy for diabetes nephropathy.[75] In addition, the use of *Lactobacillus plantarum* Dad-13 powder in Indonesia significantly reduced average weight and BMI in 30 overweight adults after 90 days of treatment. Gut microbiota showed a significant increase in *Bacteroides*, especially *Prevotella*, while *Firmicutes* significantly decreased.[76] Moreover, in nervous system disease, whether probiotic therapy or microbiota transfer therapy, *Prevotella* is significantly increased and participates in reshaping the gut microbiota.[77,78] To conclude, the metabolic relationship between *Prevotella* and the host, as well as its potential as a probiotic, are highlighted by these studies.

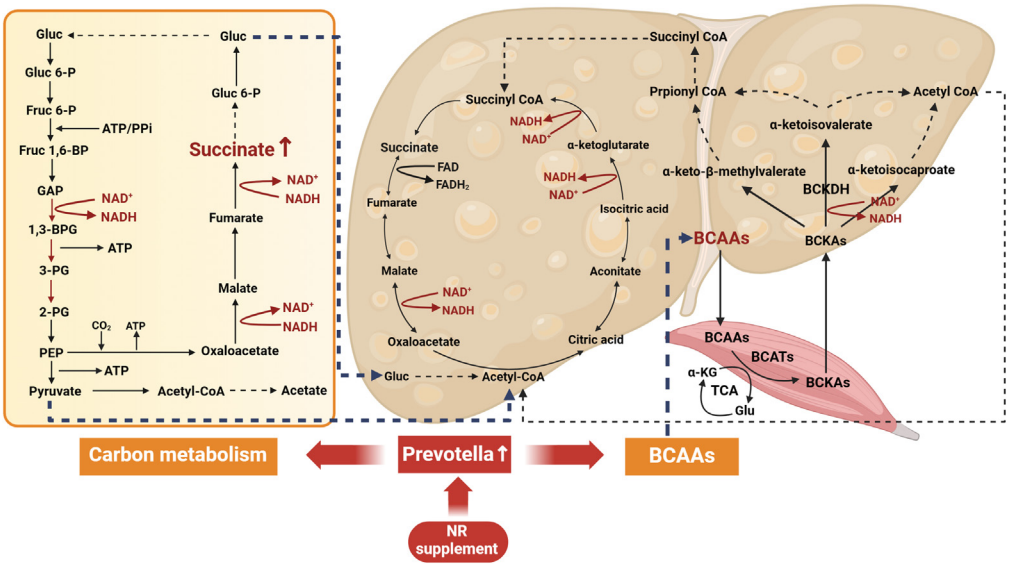

Figure 2. The hypothesis that *Prevotella* may improve MASLD by enhancing carbon metabolism and BCAA metabolism after supplementation with NAD

On the one hand, Prevotella encodes all the enzymes required for the Embden meyerhof pathway. The succinate produced by enhanced carbon metabolism can undergo glucose neogenesis through *Prevotella*, while Phosphoenolpyruvate (PEP) or Pyruvate can participate in the liver TCA cycle and regulate the NAD^+/NADH ratio. On the other hand, Prevotella contains BCAA biosynthesis genes, which can be converted into metabolic intermediates in the TCA cycle through skeletal muscle and liver metabolism, and further regulate the NAD^+/NADH ratio. Therefore, specific microorganisms such as *Prevotella* may play a unique role in using NAD supplements to improve MASLD. (Created with BioRender.com).

Moreover, in MASLD mice, NAD supplements regulate the composition of several intestinal bacteria, including *Prevotella*. *Prevotella* is reported to possess a Vitamin B3 biosynthesis pathway that contributes to an increase in the source of NAD precursors.[79] Likewise, by regulating ammonium assimilation, carbon metabolism, and BCAA metabolism, *Prevotella* also regulates the ratio of NAD^+/NADH and enhance the level of NAD in accordance with host.

Therefore, *Prevotella* may serve as NAD regulatory microbiota, who is crucial for preserving metabolic balance in MASLD. The NAD related carbon metabolism and BCAA metabolic pathway regulated by Prevotella are closely related to MASLD, and in-depth study of its abundance and function may be of great significance for NAD supplementation therapy.

Prevotella might improve MASLD by enhancing carbon metabolism and regulating NAD metabolism pool

As an important intermediate product of energy metabolism, NAD plays an important role in carbon metabolism. Franke et al.[80] predicted the central energy and carbon metabolism of *Prevotella copri* through bioinformatics and found that *Prevotella copri*'s genome encodes all the enzymes required for the Embden-Meyerhof pathway but lacks the key enzymes of the oxidative branch of the pentose phosphate pathway and the 2-keto-3-deoxy-6-phosphogluconate aldolase and 6-phosphogluconate dehydratase of the Entner–Doudoroff pathway, so the glycolysis pathway is the central process in the cytoplasmic sugar degradation of *Prevotella copri*. Glucose is metabolized into phosphoenolpyruvate, and the phosphoenolpyruvate pathway is divided into two branches. (Figure 2)

The first branch of phosphoenolpyruvate is carboxylated to oxaloacetate and produces adenosine 5′-triphosphate (ATP). Oxaloacetate participates in the TCA cycle and finally converts into succinate. Succinate, a new perspective of metabolites derived from the gut microbiota, is essential in regulating intestinal homeostasis and energy metabolism.[81] For example, in inflammatory intestinal epithelial cells, activated hypoxia inducible factor-1 α (HIF-1 α) is believed to alleviate inflammation by improving epithelial barrier function and reducing epithelial cell apoptosis. Prolyl hydroxylase domain (PHD) enzyme converts oxygen and alpha-ketoglutarate (α-KG) into succinate and CO_2. High levels of succinate slowed down PHD and inhibited HIF-1 α hydroxylation by inhibiting the product, thus contributing to HIF-1 α Stability and activation. In fact, the accumulation of succinate itself also triggered HIF-1 α activation.[82,83]

More importantly, succinic acid can be used as a substrate for gluconeogenesis to improve glucose homeostasis. Succinate supplementation not only reduced liver glucose production and enhanced glucose and insulin tolerance in mice by intestinal gluconeogenesis, but it also reduced weight gain in mice by boosting energy consumption.[84] Since increased hepatic glucose release is thought to be one of the contributors of insulin resistance and type 2 diabetes (T2DM),[85] thus inhibiting this mechanism can help prevent obesity and diabetes. What's more, after feeding barley kernel bread, the feces of responders with improved glucose metabolism were enriched with *Prevotella* and showed the potential for increased fermentation of complex polysaccharides. Moreover, compared with the nonresponders, the proximal colon biopsy of the responders showed a significant increase in glucose-6-phosphatase catalytic subunit (G6pc) expression but a decrease

in glycogen phosphorylase L (Pygl).[61] G6pc is the main enzyme for glucose homeostasis and matters in gluconeogenesis and glycogenolysis, while Pygl mediates the conversion of glycogen to glucose.[86] Therefore, *Prevotella* not only improves metabolism by producing succinates, but it may also improve glucose metabolism in some people by promoting increased glycogen storage.[61] Additionally, succinate is essential for promoting host immunity. Through SUCNR1 as a negative feedback signal, it can stimulate macrophages' anti-inflammatory responses, although its dynamic level variations are tightly correlated with those of other metabolites. As a consequence, it is necessary to further determine the concentration of succinate that affects the polarization direction of macrophage polarization.[87]

In the second branch phosphoenolpyruvate is converted to pyruvate and pyruvate is further converted to acetyl CoA and can finally be to acetate.[80] Acetate mediates the activation of FFAR2 through G (i/o) βγ-PLC-PKC-PTEN to inhibit insulin signaling and promote energy consumption, thereby inhibiting fat accumulation in adipose tissue while promoting lipid and glucose metabolism in other tissues, which prevents the development of MASLD/MASH.[88,89] What's more, Alisol A 24-Acetate may improve liver steatosis through adiponectin, which activates the AMPK α signaling pathway to downregulate sterol regulatory element-binding protein (SREBP)-1c, acetyl-CoA carboxylase (ACC), and factor-related apoptosis (FAS) and inhibit inflammation, potentially serving as a therapeutic medicine for MASLD.[90] In addition, the preservation of mucosal homeostasis is another critical function of acetate. By regulating the interaction between immunological and epithelial cells, it induces the production of T-cell-dependent IgA, which alters the position of particular bacteria in the colon.[91] In summary, the increased *Prevotella* may improve MASLD by promoting the carbon metabolism with NAD supplementation.

BCAAs: Potential targets for *Prevotella* to improve MASLD

More importantly, NAD can upregulate BCAA-related fatty acid metabolism, while *Prevotella*, strongly correlated with BCAA levels, contains BCAA biosynthesis gene.[92,93] BCAAs involve three essential amino acid, including Leucine, Isoleucine and Valine, which can promote the repair of liver injury and restore its detoxification function. Given that branched-chain aminotransferase (BCAT) activity is strong in the colon, kidneys, and skeletal muscles but low in the liver, BCAA typically avoid the liver's first pass catabolism and instead convert into branched-chain α-keto acid (BCKA) in tissues like skeletal muscles.[94] BCATs catalyze the transamination reaction of amino groups from BCAAs to α-KG, producing BCKAs and glutamate. BCAAs transamination matters more than oxidation, and effective transamination produces alanine and glutamine respectively, which are important gluconeogenesis substrates in the liver and kidney.[95] The conversion of BCKAs by BCKDH is the next step in BCAAs catabolism, which is a rate limiting step in the BCAAs catabolism pathway. BCKDH, regulated by BCKDH kinase (BCKDK), is most active in the liver and brain, but lower in resting skeletal muscles, so the liver is responsible for the extraction and decomposition of BCKAs.[95] BCKAs are decomposed into α-ketoisocaproate, α-ketoisovalerate and α-keto-β-methylvalerate and further metabolized into acetyl CoA and succinyl CoA, participating in mitochondrial TCA cycle.[96] *Prevotella ruminicola 23* encodes two different redox dependent glutamate dehydrogenase (GDH) and three different glutamine synthetase (GS) (GSI, GSIII-1 and GSIII-2).[97] GDH controls the assimilation of ammonium in the nitrogen cycle. The coupling of GDH and glutamate synthase can catalyze the direct conversion of ammonium into α-KG and ATP dependent glutamine synthetase, supporting the transamination of BCAAs. GSIII-1 participates in the recovery of ammonia and the maintenance of intracellular glutamate pools, providing amine groups to synthesize other N-containing cellular components such as amino acids, purines, pyrimidines, and polyamines.[98] Amino acid metabolism is essential to regulate intestinal epithelial barrier function, intestinal endocrine system and glucose metabolism homeostasis[99]; The deoxyhypusine synthase-deoxyhypusine hydroxylase-eukaryotic translation initiation factor 5A (DHPS-DOHH-EIF5AH) pathway associated with polyamines can increase the liver protein synthesis and mitochondrial fatty acid oxidation, making it a therapeutic target to prevent the progression of MASH.[100] Therefore, *Prevotella* is of great significance in promoting BCAAs metabolism.

One of the characteristics of MASLD is that BCKDH activity is inhibited because of the high expression level of BCKDK, leading to the accumulation of BCKAs in plasma and dysfunction of the TCA cycle and mitochondria.[101,102]

Interestingly, BCAAs supplement has been proven effective to improve some symptoms *in vitro and vivo* models. (Table 4) In animal models, supplementing with BCAA can reduce weight gain and lower hepatic triglycerides while improving the function of muscle. More delightfully, AXA1125, an orally administered endogenous metabolic modulator comprised of five amino acids (leucine, isoleucine, valine, arginine, glutamine) and N-acetylcysteine, may have significant therapeutic effects on MASLD patients, and its biologic activity tends to be more pronounced in those with comorbid T2D.[103] After treatment, the liver fat content, insulin resistance, liver enzymes, liver necrosis, and liver fibrosis levels in MASLD patients were significantly improved within 16 weeks, and its further improvement on MASLD requires long-term observation. Significantly, AXA1125 showed good safety and tolerability. Consequently, with NAD supplementation, the increased *Prevotella* may also improve the symptoms and delay the progression of MASLD by supplementing BCAAs.

BCAA supplement may improve MASLD through multiple passways. (Figure 3) By activating AMPK, BCAAs upregulate PPAR α mediated liver uncoupling protein2 (UCP2) and muscle UCP3 levels, thereby affecting fatty acid oxidation and reducing tissue TG accumulation.[105,108] UCP2 is a mitochondrial anion membrane transport protein, which not only regulates the production of mitochondrial ATP and reactive oxygen species (ROS), but is essential in regulating cell metabolism, cell proliferation and cell death. Recently, UCP2 is considered as a potential biomarker for tumor management.[109] In addition, leucine supplement increased the expression of SIRT1 and NAMPT and increased the level of intracellular NAD. Activation of SIRT1 leads to a decrease in the degree of acetylation of PGC1 α and FoxO1.[110] The deacetylation of FoxO1 inhibits the expression of tribbles homologous protein 3 (TRB3) and prevents the interaction between TRB3 and Akt, thereby enhancing insulin sensitivity.[111] PGC1 α Deacetylation not only increases genes that regulate mitochondrial biogenesis, but also activates signal transduction that controls fatty acid oxidation, thereby reducing subcutaneous and visceral fat content and preventing lipid accumulation in the liver. SIRT1 may be an important target for BCAAs supplement in treating HFD-induced obesity, insulin resistance, and metabolic disorders.[110] In

Table 4. The improvement effects of supplementing BCAA on MASLD

Study	Experimental subjects	Study design	Results
Wang et al.[104]	The human-hepatoma-derived cell line HepG2	pretreated with adenosylmethionine betaine, taurine, and BCAA for 24 h, followed by treatments of a high concentration of glucose (50 mM) or palmitic acid (0.5 mM)	Pretreatment of BCAA inhibited the fat accumulation, and the mRNA expression of lipogenic enzymes was decreased while the PPAR-γ expression was increased.
Nishimura et al.[105]	Male C57Bl/6J mouse	45% high-fat food for 6 weeks. In last 4 weeks, a concentration of 2.5% Ile in 0.5% methylcellulose or 0.5% methylcellulose alone was added to the drinking water.	Improve weight gain, signficantly decreased levels of hepatic and skeletal muscle triglyceride and hyperinsulinemia
Honda et al.[106]	Male C57BL/6J mice	(1) choline-sufficient HF diet (2) HF plus 2% BCAA in drinking water (3) CDHF diet (4) CDHF-BCAA for 8 weeks.	Hepatic steatosis and liver injury associated with MASH were alleviated along with significantly lower levels of serum ALT, hepatic TG, lipid droplet areas, hepatic total and free cholesterol.
Lee et al.[107]	Male and female C57BL/6J wild-type mice	high-fat high-sucrose or high-fat diet with free access to deiodonized water supplemented with or without BCAAs	BCAA supplementation raised circulating BCAA and branched-chain α-keto acid levels and C5-OH/C3-DC acylcarnitines in muscle without worsening insulin resistance or glucose tolerance. Valine oxidation was increased in muscle while leucine oxidation did not change.
Harrison et al.[103]	102 MASLD patients (40 with type 2 diabetes)	Subjects were randomized 2:2:2:1 to receive twice-daily AXA1125 24 g (22.6-g free AA); AXA1957 (13.5 g or 20.3 g, the latter isocaloric and isonitrogenous to AXA1125); or a placebo 24 g orally for 16 weeks.	Reduced liver fat content and a reduction of MRI-PDFF; a reduction in HOMA-IR and improved insulin sensitivity; ALT levels continue to decrease throughout the treatment; soluble markers of hepatic fibrogenesis cT1 and plasma Pro-C3 were reduced; K-18 M65 was significantly reduced.[a]

[a]CDHF, choline-deficient high-fat; TG, triglyceride.

addition, BCAAs inhibit the activation of Akt through mTORC1 and mTORC2 dependent pathways, leading to downregulation of adipogenic genes such as SREBP1, FAS, and ACC.[112] The activation of AMPK and SIRT1 is involved in inhibiting SREBP1 as well.[113] What's more, experiments in AML1 liver cells have shown that SREBP1 inhibition can reduce the expression of BCKDK mRNA.[101] Inhibition of BCKDK activity can enhance the activity of BCKAs rate limiting enzyme BCKDH, alleviating the problem of plasma BCKAs accumulation. BCKAs catabolites can participate in the liver TCA cycle and gluconeogenesis to improve glucose homeostasis. Moreover, after treatment, Acetate derived from gut microbiota can improve MASLD by downregulating the expression of FAS and ACC in the liver, and induce the proliferation of gut microbiota like *R. flavefaciens*.[114] Importantly, BCAAs treatment of cirrhotic rats also improved glucose tolerance, possibly by inducing GLUT4 and GLUT1 translocation to the plasma membrane to promote glucose uptake in skeletal muscles.[115] A systematic evaluation and meta-analysis showed that supplementing BCAAs improved prognostic factors in patients with liver cirrhosis, with a potential positive impact on mortality.[116]

The Contradictions of *Prevotella* in MASLD

However, it must be acknowledged that the potential therapeutic effects of *Prevotella* may be specific to a particular population. As the multiple studies listed, the abundance of *Prevotella* among different MASLD patients is contradictory. A study on fatty liver disease among Hispanics in Texas suggests that, the high abundance of *Prevotella copri* seems to be significantly correlated with Hispanics, which is closely related to their dietary and lifestyle habits.[117] For example, although the Mediterranean diet is advocated in Mediterranean countries, the amount of alcohol consumed by Hispanics in Texas increases, which is positively correlated with the *Prevotellaceae* and *Prevotella copri*. In addition, long-term ethanol feeding of mice also resulted in an increase in *Prevotella* not only in the mucous layer of the ileum, but also in liver samples,[118] all of which emphasizing it is the changes in dietary structure that reshape the gut microbiota and lead to diversity of results in the population.

Equally important, *prevotella* has two sides, and its potential therapeutic effects may depend on the individual. Patients with MASLD and relatively high abundance of *P. copri* are more likely to suffer from impaired glucose metabolism,[119] and the accumulation of TCA related products are positively correlated with circulating total cholesterol, low-density lipoprotein cholesterol, and IL-1β.[120] Moreover, *Prevotella* has been associated with individuals having elevated trimethylamine N-oxide (TMAO), a potential cardiovascular biomarker,[121] suggesting a potential correlation between *Prevotella* and cardiovascular disease. Therefore, a joint evaluation of gut microbiota and its metabolites with

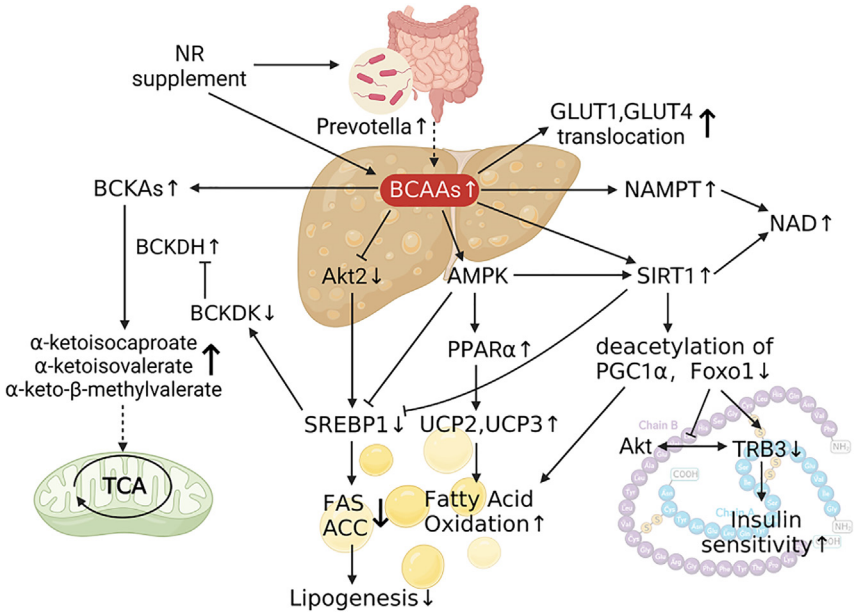

Figure 3. *Prevotella* along with NR supplements improving the signaling pathway of BCAA metabolism in MASLD

NAD precursor supplements can affect fatty acid oxidation and lipid synthesis as well as the TCA cycle and insulin sensitivity. Simultaneously, *Prevotella* may mediate the improvement of BCAA metabolism and corresponding signaling pathways after NR supplementation, playing a synergistic role with NR supplementation in improving BCAA metabolism in MASLD. (Created with BioRender.com).

TMAO should be conducted to screen patients who may benefit from NAD regulated microbiota or have risk factors. On the whole, it is more feasible to supplement dietary fiber along with NAD precursor to increase the abundance of *Prevotella* and achieve better treatment outcomes for those who experience a decrease in *Prevotella* abundance due to a high fat and high protein diet.

Meanwhile, high levels of BCAAs in MASLD patients have a negative impact on the occurrence and development of MASLD, despite its effectiveness in improving MASLD.[122–124] Yu et al. found that the adverse metabolic effects of BCAAs in diet-induced obese mice may be mediated by Isoleucine and Valine while Leucine plays a beneficial role in the treatment of various liver and tumor diseases.[125,126] Consequently, further research is necessary to determine whether the conflicting roles served by BCAAs in the pathophysiology and therapeutic strategies for MASLD are due to differences in the kinds of amino acids.

To sum up, dietary habits, genetic factors, age, etc. may all have a significant impact on how well MASLD is improving as a result of increased *Prevotella* abundance. Therefore, selective and specific therapy should be given to MASLD patients.

FUTURE PERSPECTIVE

In conclusion, the gut microbiota may be a key factor in improving MASLD with NAD supplements as demonstrated by the MASLD mouse model. Interventions through diet or exercise may enhance the absorption efficiency of NAD supplements by promoting metabolic interactions between gut microbiota and the host, thereby enhancing the liver NAD pool. In addition, diet also affects the BCAAs metabolism of host and intestinal microbiota, which further regulates the NAD^+/NADH ratio. Focusing on the metabolic balance between NAD and BCAAs may be a future research direction.

NAD precursor supplements have demonstrated considerable therapeutic effects in MASLD animal models, bringing high expectations. The results of numerous clinical researches, however, need additional validation. The precise mechanism of the beneficial effects of NAD and its metabolites on MASLD is still elusive. The renaming of MASLD once again emphasizes the necessity of stratified treatment for patients. The therapeutic dose and preferred route of administration of NAD supplements require to be optimized according to the etiology and unique characteristics of MASLD patients. Overall, more research is needed, but NAD supplements may be a promising treatment option for MASLD patients. Fortunately, the current research work still brings us many noteworthy research highlights.

(1) Gut microbiota, particularly those with high pncA gene expression, may synergistically improve NAD levels by upregulating NAPRT to fully utilize the ignored Preiss-Handler pathway, making NAD supplements more effective in improving the liver NAD availability.
(2) Probiotic therapy has shown promise in animal models, but due to the difficulty in reshaping the human gut microbiota through single supplementation, we suggest a combination therapy approach that includes adding to the NR conditioned microbiota through oral or fecal microbiota transplantation.

(3) *Prevotella* has demonstrated potential in the treatment of MASLD, and NR supplement in combination with a high-fiber diet may be more efficient in increasing the diversity of this microbiota as well as the expression of NAMPT, thereby restoring the ratio of *Firmicutes*/*Bacteroides* and *Bacteroides*/*Prevotella* and exerting greater therapeutic effects.

(4) The abundance of *Prevotella* varies throughout populations tested, which could be attributable to BMI, MASLD progression, gender, or other factors, and further research is needed.

(5) Although BCAAs supplement can successfully prevent cirrhosis, but the development and treatment of MASLD may involve various roles for each of the three amino acids in BCAAs. By identifying these roles, BCAAs may be more effective in treating MASLD.

(6) NAD is affected by the metabolic pool and circadian rhythm. It is difficult to accurately and repeatedly monitor the dynamic NAD and its metabolites in patients, which calls for an effective monitoring indicator. Luckily, the metabolites of gut microbiota might serve as a more stable indicator and could be combined with corresponding clinical indicators to provide patients for specific treatment through clearer stratification.

ACKNOWLEDGMENTS

This study was supported by Qinghai Province Key Research and Development and Transformation Plan Specific fund of Science and Technology Assistance to Qinghai (No. 2022-QY-216) and Jiangsu Province Graduate Research and Practice Innovation Plan Project (KYCX23_1938).

AUTHOR CONTRIBUTIONS

C.D.Z., C.Y., and L.X.Y.: conceptualization. L.X.Y. and Y.R.: wrote the original manuscript. C.Y. and L.X.Y.: edited the manuscript. All authors read and approved the final manuscript. Figures were created with BioRender software.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Friedman, S.L., Neuschwander-Tetri, B.A., Rinella, M., and Sanyal, A.J. (2018). Mechanisms of NAFLD development and therapeutic strategies. Nat. Med. *24*, 908–922. https://doi.org/10.1038/s41591-018-0104-9.
2. Zhou, J., Zhou, F., Wang, W., Zhang, X.J., Ji, Y.X., Zhang, P., She, Z.G., Zhu, L., Cai, J., and Li, H. (2020). Epidemiological Features of NAFLD From 1999 to 2018 in China. Hepatology *71*, 1851–1864. https://doi.org/10.1002/hep.31150.
3. Pouwels, S., Sakran, N., Graham, Y., Leal, A., Pintar, T., Yang, W., Kassir, R., Singhal, R., Mahawar, K., and Ramnarain, D. (2022). Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. BMC Endocr. Disord. *22*, 63. https://doi.org/10.1186/s12902-022-00980-1.
4. Byrne, C.D., and Targher, G. (2015). NAFLD: a multisystem disease. J. Hepatol. *62*, S47–S64. https://doi.org/10.1016/j.jhep.2014.12.012.
5. Abdellatif, M., Sedej, S., and Kroemer, G. (2021). NAD(+) Metabolism in Cardiac Health, Aging, and Disease. Circulation *144*, 1795–1817. https://doi.org/10.1161/CIRCULATIONAHA.121.056589.
6. Dall, M., Hassing, A.S., Niu, L., Nielsen, T.S., Ingerslev, L.R., Sulek, K., Trammell, S.A.J., Gillum, M.P., Barrès, R., Larsen, S., et al. (2021). Hepatocyte-specific perturbation of NAD+ biosynthetic pathways in mice induces reversible nonalcoholic steatohepatitis–like phenotypes. J. Biol. Chem. *297*, 101388. https://doi.org/10.1016/j.jbc.2021.101388.
7. Szántó, M., Gupte, R., Kraus, W.L., Pacher, P., and Bai, P. (2021). PARPs in lipid metabolism and related diseases. Prog. Lipid Res. *84*, 101117. https://doi.org/10.1016/j.plipres.2021.101117.
8. Amirkalali, B., Sohrabi, M.R., Esrafily, A., Jalali, M., Gholami, A., Hosseinzadeh, P., Keyvani, H., Shidfar, F., and Zamani, F. (2017). Association between Nicotinamide Phosphoribosyltransferase and de novo Lipogenesis in Nonalcoholic Fatty Liver Disease. Med. Princ. Pract. *26*, 251–257. https://doi.org/10.1159/000455862.
9. Costford, S.R., Bajpeyi, S., Pasarica, M., Albarado, D.C., Thomas, S.C., Xie, H., Church, T.S., Jubrias, S.A., Conley, K.E., and Smith, S.R. (2010). Skeletal muscle NAMPT is induced by exercise in humans. Am. J. Physiol. Endocrinol. Metab. *298*, E117–E126. https://doi.org/10.1152/ajpendo.00318.2009.
10. Rappou, E., Jukarainen, S., Rinnankoski-Tuikka, R., Kaye, S., Heinonen, S., Hakkarainen, A., Lundbom, J., Lundbom, N., Saunavaara, V., Rissanen, A., et al. (2016). Weight Loss Is Associated With Increased NAD+/SIRT1 Expression But Reduced PARP Activity in White Adipose Tissue. J. Clin. Endocrinol. Metab. *101*, 1263–1273. https://doi.org/10.1210/jc.2015-3054.
11. Imi, Y., Amano, R., Kasahara, N., Obana, Y., and Hosooka, T. (2023). Nicotinamide mononucleotide induces lipolysis by regulating ATGL expression via the SIRT1-AMPK axis in adipocytes. Biochem. Biophys. Rep. *34*, 101476. https://doi.org/10.1016/j.bbrep.2023.101476.
12. Wang, L.-F., Wang, X.-N., Huang, C.-C., Hu, L., Xiao, Y.-F., Guan, X.-H., Qian, Y.-S., Deng, K.-Y., and Xin, H.-B. (2017). Inhibition of NAMPT aggravates high fat diet-induced hepatic steatosis in mice through regulating Sirt1/AMPKα/SREBP1 signaling pathway. Lipids Health Dis. *16*, 82. https://doi.org/10.1186/s12944-017-0464-z.
13. Wang, P., Li, W.-L., Liu, J.-M., and Miao, C.-Y. (2016). NAMPT and NAMPT-controlled NAD Metabolism in Vascular Repair. J. Cardiovasc. Pharmacol. *67*, 474–481.
14. Hosseinzadeh-Attar, M.J., Golpaie, A., Janani, L., and Derakhshanian, H. (2013). Effect of Weight Reduction Following Bariatric Surgery on Serum Visfatin and Adiponectin Levels in Morbidly Obese Subjects. Obes. Facts *6*, 193–202. https://doi.org/10.1159/000351162.
15. Dahl, T.B., Haukeland, J.W., Yndestad, A., Ranheim, T., Gladhaug, I.P., Damås, J.K., Haaland, T., Løberg, E.M., Arntsen, B., Birkeland, K., et al. (2010). Intracellular Nicotinamide Phosphoribosyltransferase Protects against Hepatocyte Apoptosis and Is Down-Regulated in Nonalcoholic Fatty Liver Disease. J. Clin. Endocrinol. Metab. *95*, 3039–3047. https://doi.org/10.1210/jc.2009-2148.
16. Gaddipati, R., Sasikala, M., Padaki, N., Mukherjee, R.M., Sekaran, A., Jayaraj-Mansard, M., Rabella, P., Rao-Guduru, V., and Reddy-Duvvuru, N. (2010). Visceral adipose tissue visfatin in nonalcoholic fatty liver disease. Ann. Hepatol. *9*, 266–270.
17. Qiu, Y., Wang, S.-F., Yu, C., Chen, Q., Jiang, R., Pei, L., Huang, Y.-L., Pang, N.-Z., Zhang, Z., Ling, W., and Yang, L. (2019). Association of Circulating Adipsin, Visfatin, and Adiponectin with Nonalcoholic Fatty Liver Disease in Adults: A Case-Control Study. Ann. Nutr. Metab. *74*, 44–52. https://doi.org/10.1159/000495215.
18. Auguet, T., Terra, X., Porras, J.A., Orellana-Gavaldà, J.M., Martinez, S., Aguilar, C., Lucas, A., Pellitero, S., Hernández, M., Del

Castillo, D., and Richart, C. (2013). Plasma visfatin levels and gene expression in morbidly obese women with associated fatty liver disease. Clin. Biochem. *46*, 202–208. https://doi.org/10.1016/j.clinbiochem.2012.11.006.

19. Motawi, T.M.K., Shaker, O.G., El-Sawalhi, M.M., Abdel-Nasser, Z.M., and Civetta, A. (2014). Visfatin −948G/T and resistin −420C/G polymorphisms in Egyptian type 2 diabetic patients with and without cardiovascular diseases. Genome *57*, 259–266. https://doi.org/10.1139/gen-2014-0022.
20. Akbal, E., Koçak, E., Taş, A., Yüksel, E., and Köklü, S. (2012). Visfatin Levels in Nonalcoholic Fatty Liver Disease. J. Clin. Lab. Anal. *26*, 115–119. https://doi.org/10.1002/jcla.21491.
21. Elkabany, Z.A., Hamza, R.T., Ismail, E.A.R., Elsharkawy, A., Yosry, A., Musa, S., Khalaf, M.A., Elgawesh, R.M., and Esmat, G. (2020). Serum visfatin level as a noninvasive marker for nonalcoholic fatty liver disease in children and adolescents with obesity: relation to transient elastography with controlled attenuation parameter. Eur. J. Gastroenterol. Hepatol. *32*, 1008–1016. https://doi.org/10.1097/meg.0000000000001608.
22. Martos-Moreno, G.Á., Kratzsch, J., Körner, A., Barrios, V., Hawkins, F., Kiess, W., and Argente, J. (2011). Serum visfatin and vaspin levels in prepubertal children: effect of obesity and weight loss after behavior modifications on their secretion and relationship with glucose metabolism. Int. J. Obes. *35*, 1355–1362. https://doi.org/10.1038/ijo.2010.280.
23. Pham, T.X., Bae, M., Kim, M.-B., Lee, Y., Hu, S., Kang, H., Park, Y.-K., and Lee, J.-Y. (2019). Nicotinamide riboside, an NAD+ precursor, attenuates the development of liver fibrosis in a diet-induced mouse model of liver fibrosis. Biochim. Biophys. Acta, Mol. Basis Dis. *1865*, 2451–2463. https://doi.org/10.1016/j.bbadis.
24. Manickam, R., Tur, J., Badole, S.L., Chapalamadugu, K.C., Sinha, P., Wang, Z., Russ, D.W., Brotto, M., and Tipparaju, S.M. (2022). Nampt activator P7C3 ameliorates diabetes and improves skeletal muscle function modulating cell metabolism and lipid mediators. J. Cachexia Sarcopenia Muscle *13*, 1177–1196. https://doi.org/10.1002/jcsm.12887.
25. Yoshino, J., Mills, K.F., Yoon, M.J., and Imai, S.-i. (2011). Nicotinamide Mononucleotide, a Key NAD+ Intermediate, Treats the Pathophysiology of Diet- and Age-Induced Diabetes in Mice. Cell Metabol. *14*, 528–536. https://doi.org/10.1016/j.cmet.2011.08.014.
26. Cantó, C., Houtkooper, R.H., Pirinen, E., Youn, D.Y., Oosterveer, M.H., Cen, Y., Fernandez-Marcos, P.J., Yamamoto, H., Andreux, P.A., Cettour-Rose, P., et al. (2012). The NAD+ Precursor Nicotinamide Riboside Enhances Oxidative Metabolism and Protects against High-Fat Diet-Induced Obesity. Cell Metabol. *15*, 838–847. https://doi.org/10.1016/j.cmet.2012.04.022.
27. Trammell, S.A.J., Weidemann, B.J., Chadda, A., Yorek, M.S., Holmes, A., Coppey, L.J., Obrosov, A., Kardon, R.H., Yorek, M.A., and Brenner, C. (2016). Nicotinamide Riboside Opposes Type 2 Diabetes and Neuropathy in Mice. Sci. Rep. *6*, 26933. https://doi.org/10.1038/srep26933.
28. Lee, H.J., Hong, Y.-S., Jun, W., and Yang, S.J. (2015). Nicotinamide Riboside Ameliorates Hepatic Metaflammation by Modulating NLRP3 Inflammasome in a Rodent Model of Type 2 Diabetes. J. Med. Food *18*, 1207–1213. https://doi.org/10.1089/jmf.2015.3439.
29. Mitchell, S.J., Bernier, M., Aon, M.A., Cortassa, S., Kim, E.Y., Fang, E.F., Palacios, H.H., Ali, A., Navas-Enamorado, I., Di Francesco, A., et al. (2018). Nicotinamide Improves Aspects of Healthspan, but Not Lifespan, in Mice. Cell Metabol. *27*, 667–676.e4. https://doi.org/10.1016/j.cmet.2018.02.001.
30. Pencina, K.M., Valderrabano, R., Wipper, B., Orkaby, A.R., Reid, K.F., Storer, T., Lin, A.P., Merugumala, S., Wilson, L., Latham, N., et al. (2023). Nicotinamide Adenine Dinucleotide Augmentation in Overweight or Obese Middle-Aged and Older Adults: A Physiologic Study. J. Clin. Endocrinol. Metab. *108*, 1968–1980. https://doi.org/10.1210/clinem/dgad027.
31. Dellinger, R.W., Holmes, H.E., Hu-Seliger, T., Butt, R.W., Harrison, S.A., Mozaffarian, D., Chen, O., and Guarente, L. (2023). Nicotinamide riboside and pterostilbene reduces markers of hepatic inflammation in NAFLD: A double-blind, placebo-controlled clinical trial. Hepatology *78*, 863–877. https://doi.org/10.1002/hep.32778.
32. Yoshino, M., Yoshino, J., Kayser, B.D., Patti, G.J., Franczyk, M.P., Mills, K.F., Sindelar, M., Pietka, T., Patterson, B.W., Imai, S.-I., and Klein, S. (2021). Nicotinamide mononucleotide increases muscle insulin sensitivity in prediabetic women. Science *372*, 1224–1229.
33. Dollerup, O.L., Christensen, B., Svart, M., Schmidt, M.S., Sulek, K., Ringgaard, S., Stødkilde-Jørgensen, H., Møller, N., Brenner, C., Treebak, J.T., and Jessen, N. (2018). A randomized placebo-controlled clinical trial of nicotinamide riboside in obese men: safety, insulin-sensitivity, and lipid-mobilizing effects. Am. J. Clin. Nutr. *108*, 343–353. https://doi.org/10.1093/ajcn/nqy132.
34. Li, X., Yang, H., Jin, H., Turkez, H., Ozturk, G., Doganay, H.L., Zhang, C., Nielsen, J., Uhlén, M., Borén, J., and Mardinoglu, A. (2023). The acute effect of different NAD+ precursors included in the combined metabolic activators. Free Radic. Biol. Med. *205*, 77–89. https://doi.org/10.1016/j.freeradbiomed.2023.05.032.
35. Remie, C.M.E., Roumans, K.H.M., Moonen, M.P.B., Connell, N.J., Havekes, B., Mevenkamp, J., Lindeboom, L., de Wit, V.H.W., van de Weijer, T., Aarts, S.A.B.M., et al. (2020). Nicotinamide riboside supplementation alters body composition and skeletal muscle acetylcarnitine concentrations in healthy obese humans. Am. J. Clin. Nutr. *112*, 413–426. https://doi.org/10.1093/ajcn/nqaa072.
36. Holeček, M. (2020). Why Are Branched-Chain Amino Acids Increased in Starvation and Diabetes? Nutrients *12*, 3087. https://doi.org/10.3390/nu12103087.
37. Nadeeshani, H., Li, J., Ying, T., Zhang, B., and Lu, J. (2022). Nicotinamide mononucleotide (NMN) as an anti-aging health product – Promises and safety concerns. J. Adv. Res. *37*, 267–278. https://doi.org/10.1016/j.jare.2021.08.003.
38. Sun, B.L., Sun, X., Kempf, C.L., Song, J.H., Casanova, N.G., Camp, S.M., Reyes Hernon, V., Fallon, M., Bime, C., Martin, D.R., et al. (2023). Involvement of eNAMPT/TLR4 inflammatory signaling in progression of non-alcoholic fatty liver disease, steatohepatitis, and fibrosis. Faseb. J. *37*, e22825. https://doi.org/10.1096/fj.202201972RR.
39. Lian, C.-Y., Zhai, Z.-Z., Li, Z.-F., and Wang, L. (2020). High fat diet-triggered non-alcoholic fatty liver disease: A review of proposed mechanisms. Chem. Biol. Interact. *330*, 109199. https://doi.org/10.1016/j.cbi.2020.109199.
40. Zhang, X., Coker, O.O., Chu, E.S., Fu, K., Lau, H.C.H., Wang, Y.X., Chan, A.W.H., Wei, H., Yang, X., Sung, J.J.Y., and Yu, J. (2021). Dietary cholesterol drives fatty liver-associated liver cancer by modulating gut microbiota and metabolites. Gut *70*, 761–774. https://doi.org/10.1136/gutjnl-2019-319664.
41. Schwimmer, J.B., Johnson, J.S., Angeles, J.E., Behling, C., Belt, P.H., Borecki, I., Bross, C., Durelle, J., Goyal, N.P., Hamilton, G., et al. (2019). Microbiome Signatures Associated With Steatohepatitis and Moderate to Severe Fibrosis in Children With Nonalcoholic Fatty Liver Disease. Gastroenterology *157*, 1109–1122. https://doi.org/10.1053/j.gastro.2019.06.028.
42. Boursier, J., Mueller, O., Barret, M., Machado, M., Fizanne, L., Araujo-Perez, F., Guy, C.D., Seed, P.C., Rawls, J.F., David, L.A., et al. (2016). The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. Hepatology *63*, 764–775. https://doi.org/10.1002/hep.28356.
43. Jiang, W., Wu, N., Wang, X., Chi, Y., Zhang, Y., Qiu, X., Hu, Y., Li, J., and Liu, Y. (2015). Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. Sci. Rep. *5*, 8096. https://doi.org/10.1038/srep08096.
44. Shen, F., Zheng, R.-D., Sun, X.-Q., Ding, W.-J., Wang, X.-Y., and Fan, J.-G. (2017). Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. Hepatobiliary Pancreat. Dis. Int. *16*, 375–381. https://doi.org/10.1016/s1499-3872(17)60019-5.
45. Moran-Ramos, S., Cerqueda-García, D., López-Contreras, B., Larrieta-Carrasco, E., Villamil-Ramírez, H., Molina-Cruz, S., Torres, N., Sánchez-Tapia, M., Hernández-Pando, R., Aguilar-Salinas, C., et al. (2023). A metagenomic study identifies a Prevotella copri enriched microbial profile associated with non-alcoholic steatohepatitis in subjects with obesity. J. Gastroenterol. Hepatol. *38*, 791–799. https://doi.org/10.1111/jgh.16147.
46. Peluso, A.A., Lundgaard, A.T., Babaei, P., Mousovich-Neto, F., Rocha, A.L., Damgaard, M.V., Bak, E.G., Gnanasekaran, T., Dollerup, O.L., Trammell, S.A.J., et al. (2023). Oral supplementation of nicotinamide riboside alters intestinal microbial composition in rats and mice, but not humans. NPJ Aging *9*, 7. https://doi.org/10.1038/s41514-023-00106-4.
47. Lozada-Fernández, V.V., deLeon, O., Kellogg, S.L., Saravia, F.L., Hadiono, M.A., Atkinson, S.N., Grobe, J.L., and Kirby, J.R. (2022). Nicotinamide Riboside-Conditioned Microbiota Deflects High-Fat Diet-Induced

Weight Gain in Mice. mSystems *7*, e0023021.

48. Wellman, A.S., Metukuri, M.R., Kazgan, N., Xu, X., Xu, Q., Ren, N.S.X., Czopik, A., Shanahan, M.T., Kang, A., Chen, W., et al. (2017). Intestinal Epithelial Sirtuin 1 Regulates Intestinal Inflammation During Aging in Mice by Altering the Intestinal Microbiota. Gastroenterology *153*, 772–786. https://doi.org/10.1053/j.gastro.2017.05.022.
49. Lo Sasso, G., Ryu, D., Mouchiroud, L., Fernando, S.C., Anderson, C.L., Katsyuba, E., Piersigilli, A., Hottiger, M.O., Schoonjans, K., Auwerx, J., and Auwerx, J. (2014). Loss of Sirt1 Function Improves Intestinal Anti-Bacterial Defense and Protects from Colitis-Induced Colorectal Cancer. PLoS One *9*, e102495. https://doi.org/10.1371/journal.pone.0102495.
50. Magnúsdóttir, S., Ravcheev, D., de Crécy-Lagard, V., and Thiele, I. (2015). Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. Front. Genet. *6*, 148.
51. Ghislain, M., François, J.M., and François, J.M. (2002). Identification and functional analysis of the Saccharomyces cerevisiae nicotinamidase gene. Yeast *19*, 215–224.
52. Gazzaniga, F., Stebbins, R., Chang, S.Z., McPeek, M.A., and Brenner, C. (2009). Microbial NAD metabolism: lessons from comparative genomics. Microbiol. Mol. Biol. Rev. *73*, 529–541. https://doi.org/10.1128/MMBR.00042-08.
53. Shats, I., Williams, J.G., Liu, J., Makarov, M.V., Wu, X., Lih, F.B., Deterding, L.J., Lim, C., Xu, X., Randall, T.A., et al. (2020). Bacteria Boost Mammalian Host NAD Metabolism by Engaging the Deamidated Biosynthesis Pathway. Cell Metabol. *31*, 564–579.e7. https://doi.org/10.1016/j.cmet.2020.02.001.
54. Chellappa, K., McReynolds, M.R., Lu, W., Zeng, X., Makarov, M., Hayat, F., Mukherjee, S., Bhat, Y.R., Lingala, S.R., Shima, R.T., et al. (2022). NAD precursors cycle between host tissues and the gut microbiome. Cell Metabol. *34*, 1947–1959.e5. https://doi.org/10.1016/j.cmet.2022.11.004.
55. Feng, S., Guo, L., Wang, H., Yang, S., and Liu, H. (2023). Bacterial PncA improves diet-induced NAFLD in mice by enabling the transition from nicotinamide to nicotinic acid. Commun. Biol. *6*, 235. https://doi.org/10.1038/s42003-023-04613-8.
56. de Vos, W.M., Tilg, H., Van Hul, M., and Cani, P.D. (2022). Gut microbiome and health: mechanistic insights. Gut *71*, 1020–1032. https://doi.org/10.1136/gutjnl-2021-326789.
57. Lee, J.Y., Tsolis, R.M., and Bäumler, A.J. (2022). The microbiome and gut homeostasis. Science *377*, eabp9960. https://doi.org/10.1126/science.abp9960.
58. Luo, W., Guo, S., Zhou, Y., Zhao, J., Wang, M., Sang, L., Chang, B., and Wang, B. (2022). Hepatocellular Carcinoma: How the Gut Microbiota Contributes to Pathogenesis, Diagnosis, and Therapy. Front. Microbiol. *13*, 873160. https://doi.org/10.3389/fmicb.2022.873160.
59. Marchesi, J.R., Adams, D.H., Fava, F., Hermes, G.D.A., Hirschfield, G.M., Hold, G., Quraishi, M.N., Kinross, J., Smidt, H., Tuohy, K.M., et al. (2016). The gut microbiota and host health: a new clinical frontier. Gut *65*, 330–339. https://doi.org/10.1136/gutjnl-2015-309990.
60. Zeng, X., Xing, X., Gupta, M., Keber, F.C., Lopez, J.G., Lee, Y.C.J., Roichman, A., Wang, L., Neinast, M.D., Donia, M.S., et al. (2022). Gut bacterial nutrient preferences quantified in vivo. Cell *185*, 3441–3456.e19. https://doi.org/10.1016/j.cell.2022.07.020.
61. Kovatcheva-Datchary, P., Nilsson, A., Akrami, R., Lee, Y.S., De Vadder, F., Arora, T., Hallen, A., Martens, E., Björck, I., and Bäckhed, F. (2015). Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of Prevotella. Cell Metabol. *22*, 971–982. https://doi.org/10.1016/j.cmet.2015.10.001.
62. Ma, W., Nguyen, L.H., Song, M., Wang, D.D., Franzosa, E.A., Cao, Y., Joshi, A., Drew, D.A., Mehta, R., Ivey, K.L., et al. (2021). Dietary fiber intake, the gut microbiome, and chronic systemic inflammation in a cohort of adult men. Genome Med. *13*, 102. https://doi.org/10.1186/s13073-021-00921-y.
63. Qiao, S., Liu, C., Sun, L., Wang, T., Dai, H., Wang, K., Bao, L., Li, H., Wang, W., Liu, S.-J., and Liu, H. (2022). Gut Parabacteroides merdae protects against cardiovascular damage by enhancing branched-chain amino acid catabolism. Nat. Metab. *4*, 1271–1286. https://doi.org/10.1038/s42255-022-00649-y.
64. Litvak, Y., Byndloss, M.X., and Bäumler, A.J. (2018). Colonocyte metabolism shapes the gut microbiota. Science *362*, eaat9076. https://doi.org/10.1126/science.aat9076.
65. Shelton, C.D., and Byndloss, M.X. (2020). Gut Epithelial Metabolism as a Key Driver of Intestinal Dysbiosis Associated with Noncommunicable Diseases. Infect. Immun. *88*, e00939-19. https://doi.org/10.1128/IAI.
66. Rivera-Chávez, F., Lopez, C.A., and Bäumler, A.J. (2017). Oxygen as a driver of gut dysbiosis. Free Radic. Biol. Med. *105*, 93–101. https://doi.org/10.1016/j.freeradbiomed.2016.09.022.
67. Juárez-Fernández, M., Goikoetxea-Usandizaga, N., Porras, D., García-Mediavilla, M.V., Bravo, M., Serrano-Maciá, M., Simón, J., Delgado, T.C., Lachiondo-Ortega, S., Martínez-Flórez, S., et al. (2023). Enhanced mitochondrial activity reshapes a gut microbiota profile that delays NASH progression. Hepatology *77*, 1654–1669. https://doi.org/10.1002/hep.32705.
68. Le Roy, T., Llopis, M., Lepage, P., Bruneau, A., Rabot, S., Bevilacqua, C., Martin, P., Philippe, C., Walker, F., Bado, A., et al. (2013). Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut *62*, 1787–1794. https://doi.org/10.1136/gutjnl-2012-303816.
69. Nguyen, H.T., Gu, M., Werlinger, P., Cho, J.-H., Cheng, J., and Suh, J.-W. (2022). Lactobacillus sakei MJM60958 as a Potential Probiotic Alleviated Non-Alcoholic Fatty Liver Disease in Mice Fed a High-Fat Diet by Modulating Lipid Metabolism, Inflammation, and Gut Microbiota. Int. J. Mol. Sci. *23*, 13436. https://doi.org/10.3390/ijms232113436.
70. Won, S.-M., Seo, M.J., Kwon, M.J., Park, K.W., and Yoon, J.-H. (2021). Oral Administration of Latilactobacillus sakei ADM14 Improves Lipid Metabolism and Fecal Microbiota Profile Associated With Metabolic Dysfunction in a High-Fat Diet Mouse Model. Front. Microbiol. *12*, 746601. https://doi.org/10.3389/fmicb.2021.746601.
71. Won, S.-M., Chen, S., Lee, S.Y., Lee, K.E., Park, K.W., and Yoon, J.-H. (2020). Lactobacillus sakei ADM14 Induces Anti-Obesity Effects and Changes in Gut Microbiome in High-Fat Diet-Induced Obese Mice. Nutrients *12*, 3703. https://doi.org/10.3390/nu12123703.
72. Sharma, G., Garg, N., Hasan, S., and Shirodkar, S. (2022). Prevotella: An insight into its characteristics and associated virulence factors. Microb. Pathog. *169*, 105673. https://doi.org/10.1016/j.micpath.2022.105673.
73. Corbin, K.D., Carnero, E.A., Dirks, B., Igudesman, D., Yi, F., Marcus, A., Davis, T.L., Pratley, R.E., Rittmann, B.E., Krajmalnik-Brown, R., and Smith, S.R. (2023). Host-diet-gut microbiome interactions influence human energy balance: a randomized clinical trial. Nat. Commun. *14*, 3161. https://doi.org/10.1038/s41467-023-38778-x.
74. De Filippis, F., Pellegrini, N., Vannini, L., Jeffery, I.B., La Storia, A., Laghi, L., Serrazanetti, D.I., Di Cagno, R., Ferrocino, I., Lazzi, C., et al. (2016). High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. Gut *65*, 1812–1821. https://doi.org/10.1136/gutjnl-2015-309957.
75. Li, Y.J., Chen, X., Kwan, T.K., Loh, Y.W., Singer, J., Liu, Y., Ma, J., Tan, J., Macia, L., Mackay, C.R., et al. (2020). Dietary Fiber Protects against Diabetic Nephropathy through Short-Chain Fatty Acid–Mediated Activation of G Protein–Coupled Receptors GPR43 and GPR109A. J. Am. Soc. Nephrol. *31*, 1267–1281. https://doi.org/10.1681/asn.2019101029.
76. Rahayu, E.S., Mariyatun, M., Putri Manurung, N.E., Hasan, P.N., Therdtatha, P., Mishima, R., Komalasari, H., Mahfuzah, N.A., Pamungkaningtyas, F.H., Yoga, W.K., et al. (2021). Effect of probiotic Lactobacillus plantarum Dad-13 powder consumption on the gut microbiota and intestinal health of overweight adults. World J. Gastroenterol. *27*, 107–128. https://doi.org/10.3748/wjg.v27.i1.107.
77. Kang, D.-W., Adams, J.B., Coleman, D.M., Pollard, E.L., Maldonado, J., McDonough-Means, S., Caporaso, J.G., and Krajmalnik-Brown, R. (2019). Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. Sci. Rep. *9*, 5821. https://doi.org/10.1038/s41598-019-42183-0.
78. Wang, L., Zhao, Z., Zhao, L., Zhao, Y., Yang, G., Wang, C., Gao, L., Niu, C., and Li, S. (2022). Lactobacillus plantarum DP189 Reduces α-SYN Aggravation in MPTP-Induced Parkinson's Disease Mice via Regulating Oxidative Damage, Inflammation, and Gut Microbiota Disorder. J. Agric. Food Chem. *70*, 1163–1173. https://doi.org/10.1021/acs.jafc.1c07711.
79. Hong, Y., Ning, X., Liang, Y.-y., Li, X.-l., Cui, Y., Wu, W., Cai, Y., Zhao, S., Zhu, M., Zhong, T.-x., et al. (2023). Colonic mechanism of serum NAD+ depletion induced by DEHP during pregnancy. Sci. Total Environ. *872*, 162188. https://doi.org/10.1016/j.scitotenv.2023.162188.
80. Franke, T., and Deppenmeier, U. (2018). Physiology and central carbon metabolism of the gut bacteriumPrevotella copri. Mol. Microbiol. *109*, 528–540. https://doi.org/10.1111/mmi.14058.
81. de Vadder, F., and Mithieux, G. (2018). Gut-brain signaling in energy homeostasis: the unexpected role of microbiota-derived

succinate. J. Endocrinol. *236*, R105–R108. https://doi.org/10.1530/joe-17-0542.
82. Connors, J., Dawe, N., and Van Limbergen, J. (2018). The Role of Succinate in the Regulation of Intestinal Inflammation. Nutrients *11*, 25. https://doi.org/10.3390/nu11010025.
83. Mills, E., and O'Neill, L.A.J. (2014). Succinate: a metabolic signal in inflammation. Trends Cell Biol. *24*, 313–320. https://doi.org/10.1016/j.tcb.2013.11.008.
84. De Vadder, F., Kovatcheva-Datchary, P., Zitoun, C., Duchampt, A., Bäckhed, F., and Mithieux, G. (2016). Microbiota-Produced Succinate Improves Glucose Homeostasis via Intestinal Gluconeogenesis. Cell Metabol. *24*, 151–157. https://doi.org/10.1016/j.cmet.2016.06.013.
85. Warner, S.O., Yao, M.V., Cason, R.L., and Winnick, J.J. (2020). Exercise-Induced Improvements to Whole Body Glucose Metabolism in Type 2 Diabetes: The Essential Role of the Liver. Front. Endocrinol. *11*, 567. https://doi.org/10.3389/fendo.2020.00567.
86. Hatting, M., Tavares, C.D.J., Sharabi, K., Rines, A.K., and Puigserver, P. (2018). Insulin regulation of gluconeogenesis. Ann. N. Y. Acad. Sci. *1411*, 21–35. https://doi.org/10.1111/nyas.13435.
87. Wei, Y.-h., Ma, X., Zhao, J.-c., Wang, X.-q., and Gao, C.-q. (2023). Succinate metabolism and its regulation of host-microbe interactions. Gut Microb. *15*, 2190300. https://doi.org/10.1080/19490976.2023.2190300.
88. Kimura, I., Ozawa, K., Inoue, D., Imamura, T., Kimura, K., Maeda, T., Terasawa, K., Kashihara, D., Hirano, K., Tani, T., et al. (2013). The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nat. Commun. *4*, 1829. https://doi.org/10.1038/ncomms2852.
89. Aoki, R., Onuki, M., Hattori, K., Ito, M., Yamada, T., Kamikado, K., Kim, Y.-G., Nakamoto, N., Kimura, I., Clarke, J.M., et al. (2021). Commensal microbe-derived acetate suppresses NAFLD/NASH development via hepatic FFAR2 signalling in mice. Microbiome *9*, 188. https://doi.org/10.1186/s40168-021-01125-7.
90. Zeng, L., Tang, W., Yin, J., Feng, L., Li, Y., Yao, X., and Zhou, B. (2016). Alisol A 24-Acetate Prevents Hepatic Steatosis and Metabolic Disorders in HepG2 Cells. Cell. Physiol. Biochem. *40*, 453–464. https://doi.org/10.1159/000452560.
91. Takeuchi, T., Miyauchi, E., Kanaya, T., Kato, T., Nakanishi, Y., Watanabe, T., Kitami, T., Taida, T., Sasaki, T., Negishi, H., et al. (2021). Acetate differentially regulates IgA reactivity to commensal bacteria. Nature *595*, 560–564. https://doi.org/10.1038/s41586-021-03727-5.
92. Dhakan, D.B., Maji, A., Sharma, A.K., Saxena, R., Pulikkan, J., Grace, T., Gomez, A., Scaria, J., Amato, K.R., and Sharma, V.K. (2019). The unique composition of Indian gut microbiome, gene catalogue, and associated fecal metabolome deciphered using multi-omics approaches. GigaScience *8*, giz004. https://doi.org/10.1093/gigascience/giz004.
93. Hayashi, T., Yamashita, T., Takahashi, T., Tabata, T., Watanabe, H., Gotoh, Y., Shinohara, M., Kami, K., Tanaka, H., Matsumoto, K., et al. (2021). Uncovering the Role of Gut Microbiota in Amino Acid Metabolic Disturbances in Heart Failure Through Metagenomic Analysis. Front. Cardiovasc. Med. *8*, 789325. https://doi.org/10.3389/fcvm.2021.789325.
94. Brosnan, J.T., and Brosnan, M.E. (2006). Branched-Chain Amino Acids: Enzyme and Substrate Regulation. J. Nutr. *136*, 207S-11S. https://doi.org/10.1093/jn/136.1.207S.
95. Biswas, D., Duffley, L., and Pulinilkunnil, T. (2019). Role of branched-chain amino acid–catabolizing enzymes in intertissue signaling, metabolic remodeling, and energy homeostasis. Faseb. J. *33*, 8711–8731. https://doi.org/10.1096/fj.201802842RR.
96. Lo, E.K.K., Felicianna, Xu, J.-H., Xu, J.H., Zhan, Q., Zeng, Z., and El-Nezami, H. (2022). The Emerging Role of Branched-Chain Amino Acids in Liver Diseases. Biomedicines *10*, 1444. https://doi.org/10.3390/biomedicines10061444.
97. Kim, J.N., Méndez-García, C., Geier, R.R., Iakiviak, M., Chang, J., Cann, I., and Mackie, R.I. (2017). Metabolic networks for nitrogen utilization in Prevotella ruminicola 23. Sci. Rep. *7*, 7851. https://doi.org/10.1038/s41598-017-08463-3.
98. Kim, J.N., Cann, I.K.O., and Mackie, R.I. (2012). Purification, Characterization, and Expression of Multiple Glutamine Synthetases from Prevotella ruminicola 23. J. Bacteriol. *194*, 176–184. https://doi.org/10.1128/jb.05916-11.
99. Roager, H.M., and Licht, T.R. (2018). Microbial tryptophan catabolites in health and disease. Nat. Commun. *9*, 3294. https://doi.org/10.1038/s41467-018-05470-4.
100. Zhou, J., Pang, J., Tripathi, M., Ho, J.P., Widjaja, A.A., Shekeran, S.G., Cook, S.A., Suzuki, A., Diehl, A.M., Petretto, E., et al. (2022). Spermidine-mediated hypusination of translation factor EIF5A improves mitochondrial fatty acid oxidation and prevents non-alcoholic steatohepatitis progression. Nat. Commun. *13*, 5202. https://doi.org/10.1038/s41467-022-32788-x.
101. Grenier-Larouche, T., Coulter Kwee, L., Deleye, Y., Leon-Mimila, P., Walejko, J.M., McGarrah, R.W., Marceau, S., Trahan, S., Racine, C., Carpentier, A.C., et al. (2022). Altered branched-chain α-keto acid metabolism is a feature of NAFLD in individuals with severe obesity. JCI Insight *7*, e159204. https://doi.org/10.1172/jci.insight.159204.
102. Sunny, N.E., Kalavalapalli, S., Bril, F., Garrett, T.J., Nautiyal, M., Mathew, J.T., Williams, C.M., and Cusi, K. (2015). Cross-talk between branched-chain amino acids and hepatic mitochondria is compromised in nonalcoholic fatty liver disease. Am. J. Physiol. Endocrinol. Metab. *309*, E311–E319. https://doi.org/10.1152/ajpendo.00161.2015.
103. Harrison, S.A., Baum, S.J., Gunn, N.T., Younes, Z.H., Kohli, A., Patil, R., Koziel, M.J., Chera, H., Zhao, J., and Chakravarthy, M.V. (2021). Safety, Tolerability, and Biologic Activity of AXA1125 and AXA1957 in Subjects With Nonalcoholic Fatty Liver Disease. Am. J. Gastroenterol. *116*, 2399–2409. https://doi.org/10.14309/ajg.0000000000001375.
104. Wang, S., Jung, S., and Ko, K.S. (2022). Effects of Amino Acids Supplementation on Lipid and Glucose Metabolism in HepG2 Cells. Nutrients *14*, 3050. https://doi.org/10.3390/nu14153050.
105. Nishimura, J., Masaki, T., Arakawa, M., Seike, M., and Yoshimatsu, H. (2010). Isoleucine Prevents the Accumulation of Tissue Triglycerides and Upregulates the Expression of PPARα and Uncoupling Protein in Diet-Induced Obese Mice. J. Nutr. *140*, 496–500. https://doi.org/10.3945/jn.109.108977.
106. Honda, T., Ishigami, M., Luo, F., Lingyun, M., Ishizu, Y., Kuzuya, T., Hayashi, K., Nakano, I., Ishikawa, T., Feng, G.-G., et al. (2017). Branched-chain amino acids alleviate hepatic steatosis and liver injury in choline-deficient high-fat diet induced NASH mice. Metabolism *69*, 177–187. https://doi.org/10.1016/j.metabol.2016.12.013.
107. Lee, J., Vijayakumar, A., White, P.J., Xu, Y., Ilkayeva, O., Lynch, C.J., Newgard, C.B., and Kahn, B.B. (2021). BCAA Supplementation in Mice with Diet-induced Obesity Alters the Metabolome Without Impairing Glucose Homeostasis. Endocrinology *162*, bqab062. https://doi.org/10.1210/endocr/bqab062.
108. Hinkle, J.S., Rivera, C.N., and Vaughan, R.A. (2022). AICAR stimulates mitochondrial biogenesis and BCAA catabolic enzyme expression in C2C12 myotubes. Biochimie *195*, 77–85. https://doi.org/10.1016/j.biochi.2021.11.004.
109. Vallejo, F.A., Vanni, S., and Graham, R.M. (2021). UCP2 as a Potential Biomarker for Adjunctive Metabolic Therapies in Tumor Management. Front. Oncol. *11*, 640720. https://doi.org/10.3389/fonc.2021.640720.
110. Li, H., Xu, M., Lee, J., He, C., and Xie, Z. (2012). Leucine supplementation increases SIRT1 expression and prevents mitochondrial dysfunction and metabolic disorders in high-fat diet-induced obese mice. Am. J. Physiol. Endocrinol. Metab. *303*, E1234–E1244. https://doi.org/10.1152/ajpendo.00198.2012.
111. Marinho, R., Mekary, R.A., Muñoz, V.R., Gomes, R.J., Pauli, J.R., and de Moura, L.P. (2015). Regulation of hepatic TRB3/Akt interaction induced by physical exercise and its effect on the hepatic glucose production in an insulin resistance state. Diabetol. Metab. Syndrome *7*, 67. https://doi.org/10.1186/s13098-015-0064-x.
112. Zhao, H., Zhang, F., Sun, D., Wang, X., Zhang, X., Zhang, J., Yan, F., Huang, C., Xie, H., Lin, C., et al. (2020). Branched-Chain Amino Acids Exacerbate Obesity-Related Hepatic Glucose and Lipid Metabolic Disorders via Attenuating Akt2 Signaling. Diabetes *69*, 1164–1177. https://doi.org/10.2337/db19-0920.
113. Li, Y., Xu, S., Mihaylova, M.M., Zheng, B., Hou, X., Jiang, B., Park, O., Luo, Z., Lefai, E., Shyy, J.Y.J., et al. (2011). AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. Cell Metabol. *13*, 376–388. https://doi.org/10.1016/j.cmet.2011.03.009.
114. Iwao, M., Gotoh, K., Arakawa, M., Endo, M., Honda, K., Seike, M., Murakami, K., and Shibata, H. (2020). Supplementation of branched-chain amino acids decreases fat accumulation in the liver through intestinal microbiota-mediated production of acetic acid. Sci. Rep. *10*, 18768. https://doi.org/10.1038/s41598-020-75542-3.
115. Nishitani, S., Takehana, K., Fujitani, S., and Sonaka, I. (2005). Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. Am. J. Physiol. Gastrointest. Liver Physiol. *288*, G1292–

G1300. https://doi.org/10.1152/ajpgi.00510.2003.

116. Konstantis, G., Pourzitaki, C., Chourdakis, M., Kitsikidou, E., and Germanidis, G. (2022). Efficacy of branched chain amino acids supplementation in liver cirrhosis: A systematic review and meta-analysis. Clin. Nutr. *41*, 1171–1190. https://doi.org/10.1016/j.clnu.2022.03.027.

117. Kwan, S.Y., Jiao, J., Joon, A., Wei, P., Petty, L.E., Below, J.E., Daniel, C.R., Wu, X., Zhang, J., Jenq, R.R., et al. (2022). Gut microbiome features associated with liver fibrosis in Hispanics, a population at high risk for fatty liver disease. Hepatology *75*, 955–967. https://doi.org/10.1002/hep.32197.

118. Bluemel, S., Wang, L., Kuelbs, C., Moncera, K., Torralba, M., Singh, H., Fouts, D.E., and Schnabl, B. (2020). Intestinal and hepatic microbiota changes associated with chronic ethanol administration in mice. Gut Microb. *11*, 265–275. https://doi.org/10.1080/19490976.2019.1595300.

119. Serena, C., Ceperuelo-Mallafré, V., Keiran, N., Queipo-Ortuño, M.I., Bernal, R., Gomez-Huelgas, R., Urpi-Sarda, M., Sabater, M., Pérez-Brocal, V., Andrés-Lacueva, C., et al. (2018). Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota. ISME J. *12*, 1642–1657. https://doi.org/10.1038/s41396-018-0068-2.

120. Wan, Y., Yuan, J., Li, J., Li, H., Yin, K., Wang, F., and Li, D. (2020). Overweight and underweight status are linked to specific gut microbiota and intestinal tricarboxylic acid cycle intermediates. Clin. Nutr. *39*, 3189–3198. https://doi.org/10.1016/j.clnu.2020.02.014.

121. Haas, E.A., Saad, M.J.A., Santos, A., Vitulo, N., Lemos, W.J.F., Martins, A.M.A., Favarato, D., Favarato, D., Magro, D.O., Magro, D.O., et al. (2022). A red wine intervention does not modify plasma trimethylamine N-oxide but is associated with broad shifts in the plasma metabolome and gut microbiota composition. Am. J. Clin. Nutr. *116*, 1515–1529. https://doi.org/10.1093/ajcn/nqac286/6751899.

122. Galarregui, C., Cantero, I., Marin-Alejandre, B.A., Monreal, J.I., Elorz, M., Benito-Boillos, A., Herrero, J.I., de la O, V., Ruiz-Canela, M., Hermsdorff, H.H.M., et al. (2021). Dietary intake of specific amino acids and liver status in subjects with nonalcoholic fatty liver disease: fatty liver in obesity (FLiO) study. Eur. J. Nutr. *60*, 1769–1780. https://doi.org/10.1007/s00394-020-02370-6.

123. van den Berg, E.H., Flores-Guerrero, J.L., Gruppen, E.G., de Borst, M.H., Wolak-Dinsmore, J., Connelly, M.A., Bakker, S.J.L., and Dullaart, R.P.F. (2019). Non-Alcoholic Fatty Liver Disease and Risk of Incident Type 2 Diabetes: Role of Circulating Branched-Chain Amino Acids. Nutrients *11*, 705. https://doi.org/10.3390/nu11030705.

124. Grzych, G., Vonghia, L., Bout, M.A., Weyler, J., Verrijken, A., Dirinck, E., Chevalier Curt, M.J., Van Gaal, L., Paumelle, R., Francque, S., et al. (2020). Plasma BCAA Changes in Patients With NAFLD Are Sex Dependent. J. Clin. Endocrinol. Metab. *105*, dgaa175. https://doi.org/10.1210/clinem/dgaa175.

125. Yu, D., Richardson, N.E., Green, C.L., Spicer, A.B., Murphy, M.E., Flores, V., Jang, C., Kasza, I., Nikodemova, M., Wakai, M.H., et al. (2021). The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine. Cell Metabol. *33*, 905–922.e6. https://doi.org/10.1016/j.cmet.2021.03.025.

126. Wang, Z., Lu, Z., Lin, S., Xia, J., Zhong, Z., Xie, Z., Xing, Y., Qie, J., Jiao, M., Li, Y., et al. (2022). Leucine-tRNA-synthetase-2-expressing B cells contribute to colorectal cancer immunoevasion. Immunity *55*, 1067–1081.e8. https://doi.org/10.1016/j.immuni.2022.04.017.