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REVIEW

Calorie restriction and its impact on gut microbial composition and global metabolism

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Abstract **Calorie restriction (CR) is a dietary regimen that reduces calorie intake without incurring malnutrition or a reduction in essential nutrients. It has long been recognized as a natural strategy for promoting health, extending longevity, and prevents the development of metabolic and age-related diseases. In the present review, we focus on the general effect of CR on gut microbiota composition and global metabolism. We also propose mechanisms for its beneficial effect. Results showed that probiotic and butyrate-producing microbes increased their relative abundance, whereas proinflammatory strains exhibited suppressed relative abundance following CR. Analyses of the gut microbial and host metabolisms revealed that most host microbial co-metabolites were changed due to CR. Examples of dramatic CR-induced changes in host metabolism included a decrease in the rate of lipid biosynthesis and an increase in the rates of fatty acid catabolism, β-oxidation, glycogenolysis, and gluconeogenesis. The observed phenotypes and the further verification of the direct link between gut microbiota and metabolome may benefit patients that are at risk for developing metabolic disease. Thus, improved gut microbiota composition and metabolome are potential biomarkers for determining the effectiveness of dietary interventions for age-related and metabolic diseases.**

Keywords caloric restriction; gut microbiota; metabolome

Introduction

Calorie restriction (CR), also called energy restriction, is a dietary regimen that reduces calorie intake without incurring malnutrition or reduction in essential nutrients. It has been recognized as a health-promoting strategy for a long time and shown to oppose age-related physiological and pathological changes, thereby extending longevity [1–3]. The roles of CR at the cellular and molecular levels have been identified in many studies; for instance, CR enhances cellular protection, and energy metabolism along with reduction of both inflammation and oxidative damage [4–6]. Thus, CR reduces mortality in mammals due to causes that are age-related or pathological causes, including diabetes, cancer, cardiovascular disease, and brain atrophy [1,7]. CR with its health-beneficial and lifespan-extending effects has been applied and studied in such diverse species as fish, hamsters, mice, rats, and dogs [3,8–12]. Likewise, CR-induced beneficial effects have been observed in nonhuman primates and humans [13–16]. Recently, knowledge of gut microbiota and metabolic changes that result from CR has substantially increased.

Humans are considered superorganisms because diverse and dense microbiota populations colonize their gastrointestinal tracts [8,17,18]. The gut microbiota is considered a separate metabolic organ of the host because of its ability to modulate host nutrition, metabolism, and immunity [19]. The composition of gut microbiota is shaped mainly by diet [20]. Substantial evidence of dramatic diet-induced changes in microbiota composition resulting from fasting, use of laxatives, and low fat dietary intervention has been obtained; these changes counteract metabolic damages associated with obesity and high-fat diet [21]. Thus, these CR-induced alterations of the intestinal microbiota suggested that animals can establish a balanced gut microbiota composition via CR, providing health advantages to the host.

Identifying the biochemical alterations related to different diets provide valuable insights into the

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associations between metabolism and phenotypes [22–26]. Global metabolomics captures system end point responses to these biological perturbations by measuring the chemical compositions of biological samples, such as biofluids and tissues. Studies have provided comprehensive information on the metabolic pathways and networks altered through CR intervention. These studies were important for uncovering the molecular mechanism of CR. This review aims to highlight studies that address the relationships between the gut microbiota and the metabolic changes that occur with CR.

CR and gut microbiota

The mammalian gastrointestinal tract harbors a complex community of over 100 trillion microbial cells that can influence host physiology, nutrition, metabolism, and immune function. The dysbiosis of gut microbiota has been proven to be associated with several intestinal diseases, such as inflammatory bowel disease and colorectal cancer, as well as some systematic diseases such as diabetes and neurological diseases. Several studies found that the composition of the gut microbiota can be greatly changed by restricting dietary intake, without significant alterations in their α-diversity [21,27–29]. CR studies showed that the gut microbiota exerts more prominent effects than some diseases, such as viral infection [30], or physical exercise [3]. However, some reports refute these observations [31–33], stating that the effect of CR on the microbiota is minimal. Such discrepancies might have been due to the limitation of technology for gut microbiota analysis at the time of these studies, the short duration of the CR, or use of a small study population. Moreover, during a rapid growing phase such as that of a 7 – 16 weeks old rat, the gut microbiota composition could be mainly ascribed to normal physiological changes. Therefore, perturbation of the abundant genera and families might not have been detected [21]. The advent of improved sequencing technology and well-designed study protocols have allowed new findings related to the impact of CR on gut microbiota composition to emerge.

Studies of the CR effect on gut microbiota have been performed in mouse and rat, as well as, human models. The diet restriction regimens utilized were 10%–40% calorie restricted based on either a normal or high fat diet for animal studies, or 700–1500 kcal/day/person for human studies. Most of these reported studies focused on either normal or obese models [3,21,27], whereas some were conducted on participants with a pathological condition such as non-alcoholic fatty liver disease (NAFLD) [28] or influenza [30].

Factors that might have influenced the CR impact on gut microbiota were dietary composition and age of models. One murine study [3] showed that most of the microbes responding to 30% restricted high fat diet (60% fat, D12492, Research Diets) were not found in mice with 30% restricted normal diet (10% fat, D12450B, Research Diets). Furthermore, the CR-induced microbiota alterations were different in mice of mid-life and late-life ages. Another human study [29] showed that enormous differences in intestinal microbial composition existed between lean vs. obese subjects and such differences were absent after long-term CR. Thus, CR intervention adjusted the gut microbiota composition of obese subjects to that of the lean subjects which were dramatically different before intervention. Firmicutes, Bacteroidetes, and Proteobacteria are the main phyla in the gut microbiota; however, results regarding CR-induced alterations in the relative abundances of these bacteria varied [27,30,34,35]. Some studies reported that dietary intervention reduced the Bacteroidetes population in favor of Firmicutes [34], whereas long-term (45 days) CR in obese subjects enriched Bacteroidetes and greatly reduced the Firmicutes:Bacteroidetes ratio [35]. The inconsistent results might be due to the variable diversity of the microbes present under a specific phylum, and dietary intervention may have led to remarkably relative alterations in low-level taxa without affecting the relative abundance of a major phylum.

The alterations of microbes in different levels of taxa from different studies are summarized in Fig. 1. Generally, CR treatment showed a substantial effect on the relative abundances of microbes belonging to the Clostridiales order, that is, a decrease in families, Eubacteriaceae, Lachnospiraceae, Peptostreptococcaceae, and Erysipelotrichaceae. It also led to decreased abundance in the Bacteroidales order, whereas certain family levels belonging to this order showed an increasing trend, for example, for the families, Porphyromonadaceae, Rikenellaceae, and Bacteroidaceae [3,21,29]. Notably, in some studies, the operational taxonomic units (OTUs; several hits > 97% sequence identity) were annotated to species levels, such as increased abundances of *Clostridium saccharolyticum*, *Clostridium* cluster XIVa, *Eubacterium limosum*, *Eubacterium cellulosolvens*, *Anaerostipes hadrus*, *Blautia hydrogenotrophica*, and *Butyrivibrio fibrisolvens*, and decreased abundances of *Clostridium perfringens*, *Agathobacter rectalis*, *Ruminococcus gnavus*, and *Akkermansia muciniphila*. The widely reported microbes or altered functions with CR intervention are summarized in detail in the following sections.

Probiotic strains

Studies have shown an overall increased relative abundance of probiotic microbes, such as *Bifidobacterium* spp. and *Lactobacillus* spp. in CR-treated mammals [3,21,35]. Increased relative abundance of probiotic strains may explain some of the benefits of CR given the acknowledged role of these genera in promoting intestinal

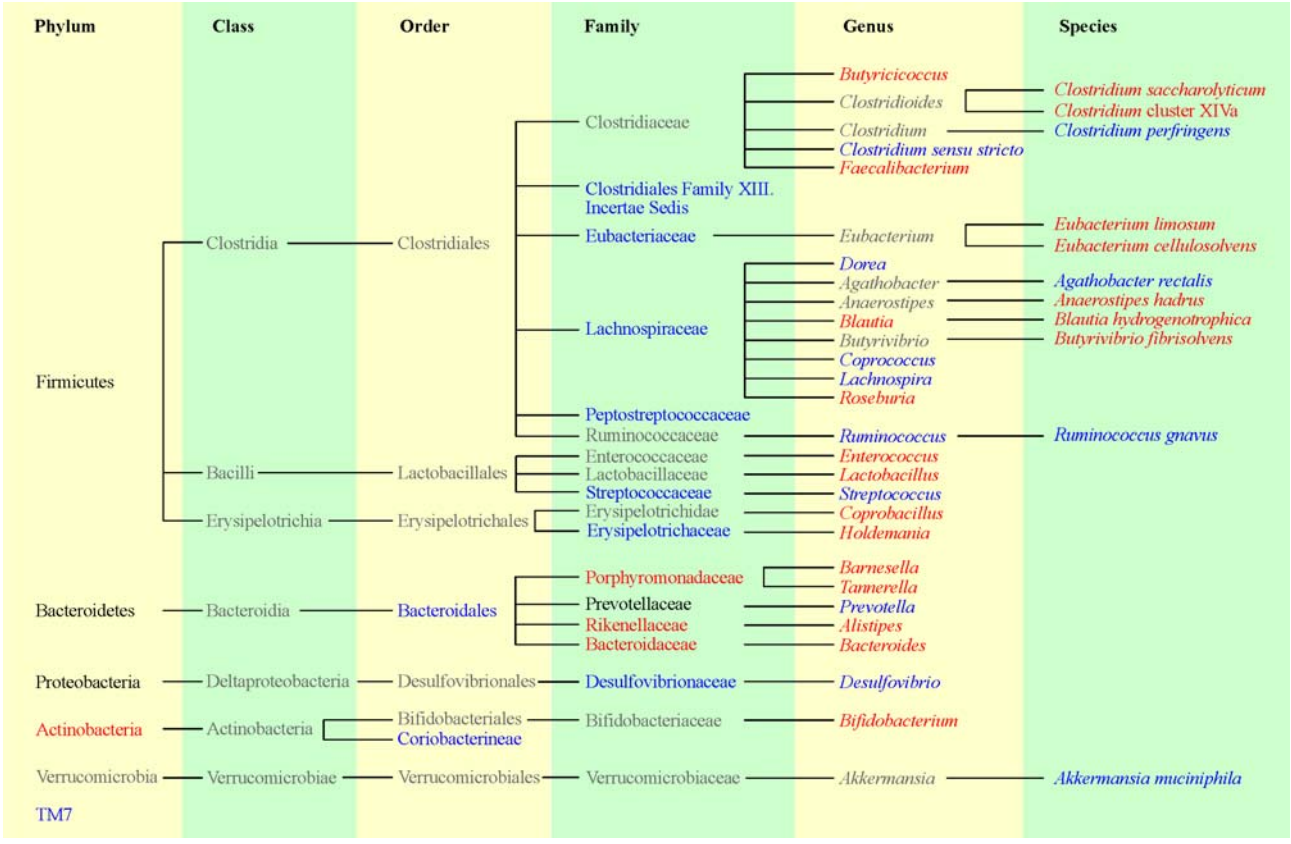

Fig. 1 Phylogenetic tree of all reported taxa. The abundance of taxa in red indicates increased abundance, blue indicates decreased abundance, and black indicates controversial alteration after CR intervention. The taxa in gray have not been reported in CR-related studies.

homeostasis by protecting against pathogen-induced gut barrier disruption, inhibiting pathogen adhesion to the intestinal wall, and reducing inflammatory cytokines [36,37]. The anti-inflammatory effect of *Lactobacillus* has been attributed to its surface antigens, which have structural or enzymatic functions. The increased abundances of *Lactobacillus* and *Bifidobacterium* in subjects under CR conditions correlated with decreases in body weight, total cholesterol, and triglycerides, and thus *Lactobacillus* spp. growth might be correlated to a diet-dependent effect on lipid metabolism [21,36,38].

Proinflammatory microbes

Some harmful microbes inducing inflammation were inhibited with CR treatment. Desulfovibrionaceae, Streptococcaceae, and TM7 induced mild inflammation, which is positively associated with obesity, diabetes, and inflammatory mucosal processes in inflammatory bowel diseases [39–41]. After CR intervention (45-day 25%-restricted diet for mice and 28-day 800 kcal/day diet for humans), the circulating lipopolysaccharide (LPS) binding protein (LBP) was reduced [3,27]. LBP is an important biomarker because it can bind to antigens produced by Gram-positive bacteria; thus, the levels of LBP can reflect the antigen load in the circulation and the inflammatory response of the host [42]. With CR intervention, the antigen translocation from the gut to the blood might be considerably reduced due to the decreased abundance of Gram-positive bacteria [39,43].

Butyrate-producing microbes

The growth of some butyrate-producing microbial strains, such as *Coprobacillus*, *Holdemania*, *Eubacterium cellulosolvens,* and *Clostridium saccharolyticum* was increased with CR [34]. Meanwhile, metagenomic data showed an increase in the metabolic capacity of Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs for butyrate fermentation after six months of very low carbohydrate diet in humans [34]. Butyrate is a short-chain fatty acid produced in the colon from fermented dietary fiber by gut microbiota. It is the main energy source of enterocytes and has been reported to have an anti-inflammatory effect by decreasing gut permeability [44]. Moreover, studies in murine models showed that the butyrate activation of intestinal glucagon-like peptide 1 (GLP-1) in enteroendocrine cells improved glycemic and insulin responses. In

animals, butyrate-producing bacteria were reported to prevent diet-induced obesity [45] and alleviate NAFLD [46]. Some studies did not reveal significant differences in fecal butyrate content after CR intervention, which was explained by low prebiotic substrate levels in the diet [34,47].

Microbes with amino acid degradation function

The metabolic functional changes in gut microbiota with CR intervention were also observed for bacteria associated with amino acid metabolism [28]. Increased lysine biosynthesis along with decreased phenylalanine and tryptophan synthesis and branched amino acid degradation were observed in the microbial metabolism of essential amino acids.

CR and the global metabolome

The global metabolome experiences a dramatic shift with CR intervention, as evidenced by metabolomics and transcript analyses in blood, urine, liver, and muscle (summarized in Table 1). The metabolism rapidly switched from lipid biosynthesis to fatty acid catabolism and stimulated downstream β-oxidation. In addition, CR induced increased glycogenolysis and gluconeogenesis [48,49]. Most importantly, due to the 25% restricted dietary CR impact on the composition of gut microbiota, gut microbial metabolites were subsequently changed [8]. Important published metabolites and metabolic pathways are summarized in the following sections.

The global metabolism is more affected by diet composition than by energy supply alone. High dietary protein-to-carbohydrate ratio (low P/C ratio (7:1): 10% protein, 20% fat, and 70% carbohydrate; high P/C ratio (1.3:1): 32% protein, 22% fat, and 46% carbohydrate) was positively associated with improvement in glycemic control [50]. In contrast, some studies suggested that increased protein intake may be ineffective and even detrimental to the maintenance of glucose homeostasis [51,52] because increased levels of a single type of amino acid, that is, branched-chain amino acids (BCAA), produced from dietary protein were positively associated with insulin resistance [53,54]. Thus, the investigations of the CR effect with different diet compositions were inconsistent. Moreover, to evaluate the CR impact on the metabolism, not only caloric intake but also dietary macronutrient composition should be considered. The effect of CR varies among the metabolomes of various biofluids and tissues in the body. For instance, the urinary metabolome showed more fluctuations than the metabolomes of other biofluids, such as blood and saliva, indicating that urine samples were more sensitive to differences in diet than other body fluids [55]. Gender difference in metabolism alteration was observed in several studies [56,57]. Some other significant factors that have been investigated in the studies of CR and gut microbiota, such as age and baseline body weight, should also be included in studies of CR-induced changes in the metabolome.

Host-microbial co-metabolites

Metabolites with aromatic chemical structures, including indoles and benzoic acids, are main metabolites derived from biochemical degradation reactions involving *Clostridium scatalogens, C. difficile,* or certain *Lactobacillus* strains [58,59]. Such host microbial co-metabolites, including hippurate, p-cresol, dimethylglycine, phenylacetylglycine, and 4-hydroxyphenylacetic acid [8,22,60,61], were associated with gut microbial homeostasis that was modulated by diet [62,63], although with observed differences in the alteration trend for diverse species and ages. Interestingly, increased levels of hippurate and dimethylgycine have been considered as age-induced biomarkers [64,65]. Their decreased levels in aged mammals resulting from a 40% restricted dietary CR treatment for 12 weeks may be the effect of modulated gut microbiota activity and improved nutrient digestibility, thereby providing supporting evidence for the beneficial effect of CR as anti-aging and causing increased longevity [66].

The CR intervention (20% and 40% restricted diet for five days) was associated with elevated plasma levels of trimethylamine-N-oxide (TMAO) and reduced levels of trimethylamine (TMA), choline, and glycerophosphocholine [67]. Consistently, changes in the levels of aliphatic amines, including TMA, dimethylamine (DMA), and TMAO occurred in the urine [22]. Choline and methylamines (TMA and DMA) are metabolites derived from host-microbial interactions in the large intestine; choline can be metabolized by gut microbiota to DMA and TMA, and liver flavin monooxygenases ultimately metabolizes TMA to TMAO [68]. Therefore, changes in the levels of these compounds may indicate the relative abundance of methylamine-producing bacterial resulting from CR-induced changes in gut microbial community and activity.

Lipids

Alterations in blood lipoproteins has been widely shown in the studies of CR in mouse, rat, dog, and monkey models [13,69–71], as well as in human clinical studies [14,72]. Changes in lipoproteins were manifested by the increase in blood and fecal high density lipoprotein (HDL) levels and reduction in low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels [13,15,22,48,49,67]. Lower levels of LDL and VLDL are associated with a lowered risk of cardiovascular disease. Increasing the

Table 1 CR related metabolites

Metabolite types	Increased after CR	Decreased after CR	Inconsistent in the reports
Host-microbial co-metabolites	Trimethylamine-N-oxide (TMAO)	Hippurate, dimethylgycine, trimethylamine (TMA)	p-Cresol, phenylacetylglycine, 4-hydroxyphenylacetic acid
Lipoprotein	High density lipoprotein (HDL)	Low density lipoprotein (LDL), very low-density lipoprotein (VLDL)	
Glyceryl derivatives of lipids and phospholipid choline		Choline, glycerophosphocholine, phos phatidylcholine (PC) (18:0/20:4), sphingomyelin (SM) (d18:0/16:1), lysoPCs (C16:1, C16:0, C17:0, C18:2, C18:1, C18:0, C20:4, C20:3, and C22:6), diacylglycerol lipids, triacyl glycerol lipids	
Free fatty acids		n-6 polyunsaturated fatty acids, palmitoleic acid (C16:1 n7), heptadecenoic acid (C17:1 n7), γ-linolenic acid (C18:3 n6), dihomo-γ-linolenic acid	n-3 polyunsaturated fatty acids, mono unsaturated fatty acids, saturated fatty acids
Ketone bodies	Acetoacetate, 3-hydroxybutyrate		
Bile acids	Taurocholic acid, taurodeoxycholic acid, deoxycholic acid, lithocholic acid, ω-muricholic acid, hyodeoxy cholic acid		
Amino acids	Glutamate, methionine, glutamine, alanine	Branched-chain amino acids, aromatic amino acids	
Others	Carnitine, gluconate	Pyruvate	

beneficial lipoprotein, HDL, has been closely related to attenuation of age-related disorders, metabolic dependent diseases, and cardiovascular diseases [73].

Glyceryl derivatives of lipids and phospholipid choline in liver [60] and serum [16,71] were significantly decreased after CR intervention, such as phosphatidylcholine (PC) (18:0/20:4), sphingomyelin (SM) (d18:0/16:1), and some lysoPCs (C16:1, C16:0, C17:0, C18:2, C18:1, C18:0, C20:4, C20:3, and C22:6). Many of these compounds are involved in fatty acid catabolism, where triacylglycerols and phospholipids become hydrolyzed into non-esterified fatty acids, glycerol, and phosphocholine [67]. Such metabolic phenotypes were observed in some pathological models. In db/db mice, reduction in the levels of most glycerolipids [diacylglycerol (DG) and TG] and related hepatic enzymes were observed [4]. In NAFLD, the CR-induced reduction of liver triglyceride (TG) may have contributed to the prevention of further hepatic alterations associated with insulin resistance (IR) [66]. Notably, the decreased level of SM (d18:0/16:1) was observed in overweight and obese women after eight weeks of CR intervention [74], whereas the proportion of plasma SM increased with age and was elevated in obese models [75,76].

Free fatty acids

CR induced lipolysis in liver and adipose tissues and increased free fatty acid (FFA) release into circulation [4]. Enhanced autophagy induced by CR has been reported to increase lipolysis [77]. A controversial study showed that increased circulating fatty acids were positively associated with the development of IR. This result was explained by the fact that IR can be induced not only by FFA elevation but increased TG concentration [78]. Thus, the beneficial effect of CR in lipid metabolism may be due to decreased lipid storage and increased FFA in the circulation.

The CR-induced alteration in the levels of unsaturated fatty acids (UFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SFA) was inconsistent in several studies. In one murine study, CR increased MUFA levels in the liver, whereas PUFA levels decreased and no changes were observed in SFA levels [60]. In contrast, a clinical study [79] with obese subjects participating in a very low carbohydrate diet intervention presented with overall UFA levels, MUFA, and n-6 PUFAs that were decreased after dietary intervention, whereas SFAs and n-3 PUFA increased remarkably. Among these FFAs, four UFA levels were associated with improved metabolic markers, including palmitoleic acid (PA) (C16:1 n7), heptadecenoic acid (HA) (C17:1 n7), gamma-linolenic acid (GLA) (C18:3 n6) and dihomo-gamma-linolenic acid (DGLA). The consistent findings within these studies were lower PUFA levels, especially n-6 PUFA levels after CR intervention. The lower levels of n-6 PUFA were believed to be due to decreased inflammation and susceptibility to oxidation of the cellular membranes [60].

FFA related metabolites were also altered after CR intervention. FFA oxidation is the major fuel for ketone

bodies and increased levels of the circulating ketone bodies, acetoacetate and 3-hydroxybutyrate were observed to be simultaneously elevated after CR and were high in the subjects with higher percentage of weight loss [15,48,67,71,80]. The serum level of carnitine was also increased with CR intervention (40% dietary reduction for 12 weeks), greatly influencing fatty acid oxidation *in vivo* [70].

Bile acids

Bile acids (BAs) are downstream metabolites of cholesterol that are synthesized in the liver and secreted from the gall bladder into the intestine to aid lipid uptake by the intestine. Hepatic cholesterol accumulation can be alleviated through the production of bile acids [81]. Bile-acid-activated signaling pathways have become attractive therapeutic targets for metabolic disorders, such as obesity, type 2 diabetes, hypertriglyceridemia, and atherosclerosis, as well as other associated chronic diseases, such as non-alcoholic steatohepatitis [82,83]. The results have shown that a 40% CR increased the BA pool size (162%) and total BAs in gallbladder, small intestinal contents, and serum, with contributions mainly from taurocholic acid (TCA) and some secondary BAs, such as taurodeoxycholic acid, deoxycholic acid, lithocholic acid (LCA), ω-muricholic acid, and hyodeoxycholic acid [84–86]. Increases in these CR-induced BAs might be due to an increase in the rate of BA synthesis, conjugation in liver and intracellular transport in the ileum. This interpretation has been supported by the observation of increased expression of BA-synthetic (cytochrome P450 7a1 (Cyp7a1)), conjugating enzyme bile acid-CoA ligase (BAL) and the ileal BA binding protein (Ibabp) levels [84,87]. Hepatic bile acids can spill into the circulation and induce energy expenditure and glucose homeostasis [87,88]. Recent studies showed that bile acids play an important role in CR-induced longevity. One bile acid, LCA, was found to be an anti-aging compound in yeast [89], where it influenced various longevity-related processes.

Pyruvate and TCA cycle

The levels of pyruvate in blood were significantly decreased after CR and fasting intervention [67,90]. Pyruvate kinase enzyme levels decreased in liver samples of mice with 30% restricted CR undergoing a step-down feeding regime [60]. Pyruvate is a key metabolic intermediate at the intersection between glycolysis and the TCA metabolic cycles [67]. CR promotes the TCA cycle and reduces glucose levels in contrast to metabolic phenotypes with high calorie intake; these phenotypes are characterized by elevated glucose levels and depleted levels of the TCA cycle intermediates, citrate, succinate, 2-ketoglutarate, and cis-aconitate. Therefore, such metabolic characteristics of CR can potentially be used to prevent the development of insulin resistance, diabetes, and hyperglycemia [22].

Pentose phosphate pathway (PPP)

Increased levels of gluconate [13], which is a key metabolite of the PPP, and increased flux through the PPP, were observed under CR. This increase may be attributed to the CR-induced upregulation of peroxisome proliferator-activated receptor α (PPARα) expression [91]. Moreover, aging was associated with decline in the expression levels of PPP genes, although this association was counteracted by CR [91, 92]. The PPP is involved in the biosynthesis of nicotinamide adenine dinucleotide phosphate which is essential for various reductive biosynthetic processes, such as lipogenesis, cholesterol synthesis, and nucleotide production. The PPP also plays a substantial role in the regulation of β-oxidation in muscles, hepatic glucose output and systemic insulin sensitivity [93]. Thus, CR may upregulate PPARα expression in skeletal muscles [91] and may increase insulin sensitivity in the liver and peripheral tissues by increasing glucose flux through the PPP and enhancing fatty acid synthesis [94].

Amino acids

The levels of some amino acids, such as phenylalanine and tyrosine, were considerably reduced after CR [67,80,95,96], confirming that decreased levels of branched-chain and aromatic amino acids are significantly associated with weight loss and decreased homeostasis model assessment of insulin resistance (HOMA-IR) scores in overweight or obese subjects [97]. Studies of BCAA supplementation in humans [51] and animals [98] demonstrated that circulating branched chain and aromatic amino acids directly promoted IR through the obstruction of insulin signaling in skeletal muscles.

High levels of some amino acids were observed in CR animals, such as serine and glutamate, implying the maintenance of protein turnover rate after CR intervention [99]. These amino acids are known to be important for neurotransmitter biosynthesis and preservation of neurological function [100]. Plasma levels of the glucogenic amino acids, methionine, glutamine, alanine, and valine were also increased, promoting a CR-induced switch in energy metabolism toward energy conservation and gluconeogenesis [48].

Prospective

Early microbial biomarkers predictive of the clinical benefits associated with CR treatment might serve as

diagnostic tools for identifying overfeeding-related dysbiosis. A recent study showed that some strains have the capability to predict the personal effects of CR treatment [101]. Individuals with a high baseline abundance of *A. muciniphila* before CR showed improved clinical outcomes after CR, such as improvement in glucose homeostasis, blood lipids, and body composition. The abundance of *A. muciniphila* in these participants was reduced during CR, however, it still remained considerably higher than in those with lower abundance pre-CR [101]. Thus, the levels of *A. muciniphila* could potentially be used for predicting the effect of CR. Another study [21] suggested that *Lactobacillus* served as a marker of dietary intervention in experimental animal models because the increased relative abundance of *Lactobacillus* correlated with the lowering of cholesterol and triglycerides shortly after dietary restriction. Additionally, its high abundance persisted after long-term CR treatment.

The calorie restricted diet could be supplemented with some beneficial microbes; thus, the CR impact could be more effective. Some studies [21] have applied a calorie restriction diet with *L. fermentum* CRL1446 and found that such a diet accelerated the microbiota replacement and promoted the presence of the probiotics, *Bifidobacterium* and *Lactobacillus*, in the intestine.

Conclusions

Calorie restriction is a strong environmental force that alters the gut microbiota and global metabolome. Further verification of the direct link between gut microbiota and metabolome as well as, the observation of host phenotypes that correlated with a particular gut microbe composition, metabolite concentrations and physiological parameters would benefit patients at risk for developing metabolic disease. These findings would provide essential metabolic information associated with gut microbial composition under CR conditions for the consideration of tailored therapeutic treatments to a specific metabolic condition [102–106]. More importantly, the causality between gut microbiota, global metabolism, and CR-induced phenotypes should be evaluated. Strategies have been proposed for identifying causative factors through functional microbiome-wide association studies and mechanistic investigations. Given the potential key role of these factors in mediating the health-promoting actions of CR, improved gut microbiota composition and its resulting metabolome may become a novel biomarker for identifying effective dietary interventions for age-related and metabolic diseases.

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Compliance with ethics guidelines

Xiaojiao Zheng, Shouli Wang, and Wei Jia declare that they have no conflicts of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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