



## Methionine restriction - Association with redox homeostasis and implications on aging and diseases

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### ABSTRACT

Methionine is an essential amino acid, involved in the promotion of growth, immunity, and regulation of energy metabolism. Over the decades, research has long focused on the beneficial effects of methionine supplementation, while data on positive effects of methionine restriction (MR) were first published in 1993. MR is a low-methionine dietary intervention that has been reported to ameliorate aging and aging-related health concomitants and diseases, such as obesity, type 2 diabetes, and cognitive disorders. In addition, MR seems to be an approach to prolong lifespan which has been validated extensively in various animal models, such as *Caenorhabditis elegans*, *Drosophila*, yeast, and murine models. MR appears to be associated with a reduction in oxidative stress via so far mainly undiscovered mechanisms, and these changes in redox status appear to be one of the underlying mechanisms for lifespan extension and beneficial health effects. In the present review, the association of methionine metabolism pathways with redox homeostasis is described. In addition, the effects of MR on lifespan, age-related implications, comorbidities, and diseases are discussed.

### 1. Introduction

Disturbed redox homeostasis is closely related to aging and metabolic dysfunction and is influenced by diet and energy intake, with the organism adapting to changes in nutrients available in the environment. A network of nutrients and nutrient-sensing pathways regulates metabolism, growth, and aging. As a dietary intervention, caloric restriction (CR), without causing malnutrition, is recognized as an experimental method capable of prolonging lifespan and positively affecting metabolic health and various diseases of the organism, such as obesity, type 2 diabetes, and cardiovascular disease [1], partly by reducing cellular oxidative stress [2–5]. However, it is difficult for individuals to stick to CR for decades. With increasing evidence that protein restriction (PR)

may extend lifespan and reduce the risk of age-related diseases, numerous studies have focused on investigating the role of amino acids in the diet [6–8]. Studies have shown that restriction of a specific amino acid, methionine restriction (MR), has similar physiological effects as CR and is related to longevity, metabolic health but also cognitive disorders.

The restriction of methionine seems to be related to a reduction of oxidative stress, through mechanisms not yet discovered, but these changes of the redox status seem to be one of the underlying mechanisms of life-span prolongation [9] and beneficial health effects [10,11]. Proposed mechanisms for reducing a pro-oxidative environment by methionine restriction are multifold and include (i) induction of autophagy in particular mitophagy and, therefore, the enhanced removal of reactive oxygen species (ROS)-producing, non-functional mitochondria [12], (ii) a reduction of ROS production within the mitochondria [13]

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ABBREVIATIONS	
Acox1	acyl-coenzyme A oxidase 1
AD	Alzheimer's disease
ADF	alternate day fasting
AGE	advanced glycation endproduct
ATF4	activating transcription factor 4
A $\beta$	beta-amyloid
B2M	$\beta$ 2-microglobulin
BAT	brown adipose tissue
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
BHMT	betaine homocysteine methyltransferase
<i>C. elegans</i>	<i>Caenorhabditis elegans</i>
CBS	cystathionine- $\beta$ -synthase
CGL	cystathionine- $\gamma$ -lyase
CNS	central nervous system
CO	carbon monoxide
Cpt1a	carnitine palmitoyltransferase
CVD	cardiovascular disease
DACD	diabetes-associated cognitive decline
dcSAM	decarboxylated SAM
<i>E. coli</i>	<i>Escherichia coli</i>
EOD	every-other-day
ER	endoplasmic reticulum
FGF	fibroblast growth factor
FGFRs	fibroblast growth factor receptors
GPx	glutathione peroxidases
GSH	glutathione
GSTP	glutathione S-transferase
H <sub>2</sub> S	hydrogen sulfide
HDL-C	high-density lipoprotein cholesterol
HFD	high-fat diet
HO-1	hemoxygenase-1
HPA	hypothalamic-pituitary-adrenal
IBD	inflammatory bowel disease
IER	intermittent energy restriction
IR	insulin resistance
IUGR	intrauterine growth restriction
Keap1	Kelch-like ECH-associated protein 1
LDL-C	low-density lipoprotein cholesterol
LOVs	lacto-ovo vegetarians
LPD	low-protein diet
MAPK	mitogen-activated protein kinase
MAT	methionine andenosyltransferase
MCI	mild cognitive impairment
MCM	methionine cycle metabolites
MD	methionine deprivation
MDA	malondialdehyde
Met	methionine
MPO	myeloperoxidase
MR	methionine restriction
MTA	5'-methylthioadenosine
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
mtROS	mitochondrial ROS
Nfe2l2	hepatic Nrf2
Nfe2l2 <sup>fl/(Alb)</sup>	liver-specific deletion of Nfe2l2
NF $\kappa$ B	nuclear = factor 'kappa-light-chain-enhancer' of activated B-cells
NO	nitric oxide
Nrf2	nuclear factor erythroid 2-related factor 2
3-NT	3-Nitrotyrosine
ob/ob	leptin-deficient obese
OCM	one-carbon metabolism
PEPCK	phosphoenolpyruvate carboxykinase
Ppargc1a	peroxisome proliferator-activated receptor $\gamma$ coactivator 1- $\alpha$
PVN	paraventricular nucleus
ROS	reactive oxygen species
SAH	S-adenosylhomocystein
SAHH/AHCY	S-adenosylhomocystein hydrolase
SAM	S-adenosylmethionine
SCN	suprachiasmatic nucleus
SNA	sympathetic nerve activity
SOD	superoxide dismutase
SRB	sulfate-reducing bacteria
STZ	streptozotocin
sWAT	subcutaneous white adipose tissue
T2D	type 2 diabetes
TASIR1/TASIR3	taste 1 receptor member 1 and 3
TC	total cholesterol
TG	triglyceride
TMAO	trimethylamine-N-oxide
TRF	time-restricted fasting
TSAA	total sulfur amino acid
WAT	white adipose tissue
WD	western diet

and effects on the transsulfuration and glutathione (GSH) pathways [14, 15].

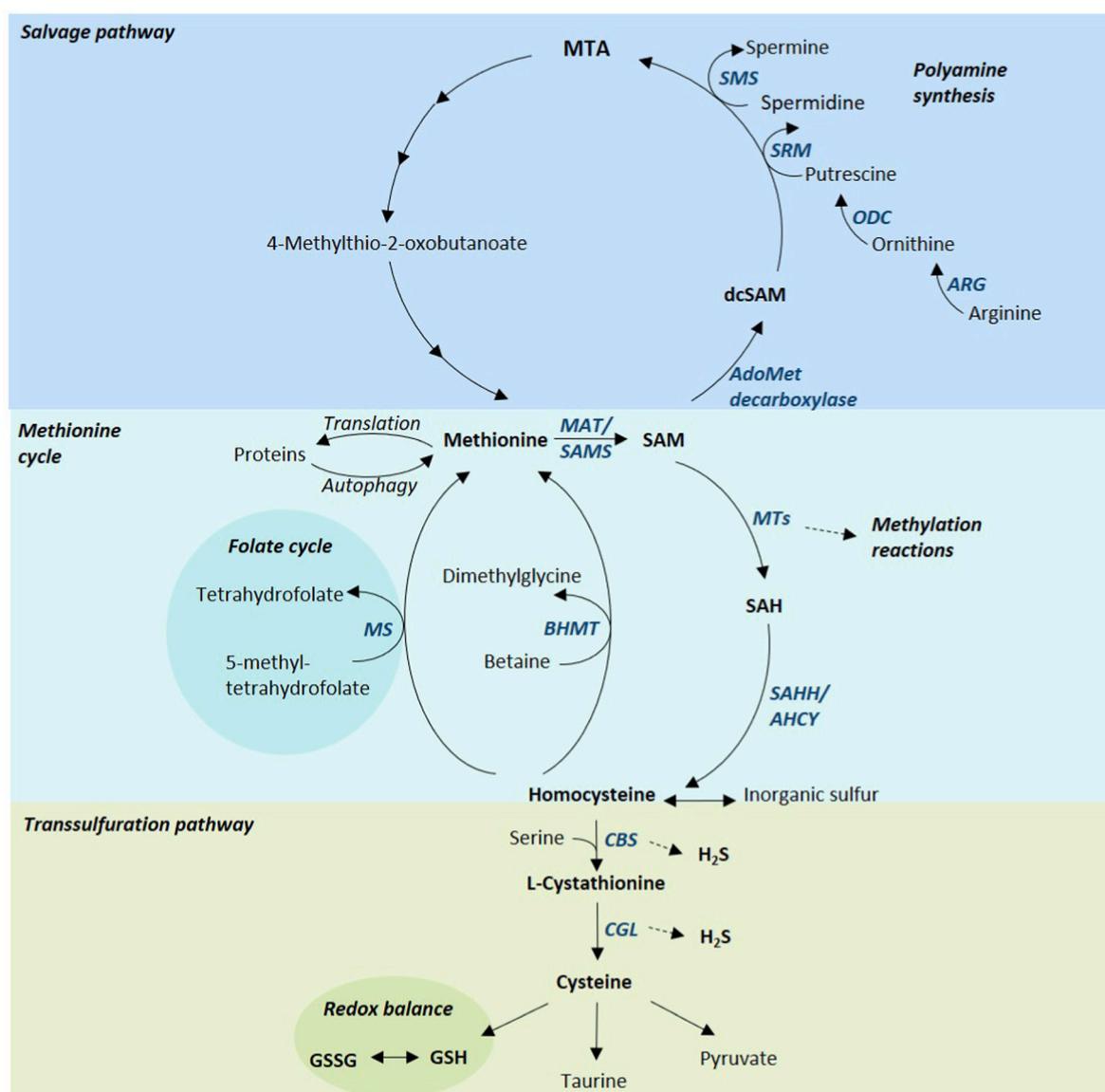
This review will give an overview on the possible interaction of the methionine pathways with the redox status of cells, and in particular the effects of methionine restriction.

## 2. Methionine metabolism

Methionine is an amino acid that occurs in two chiral forms. While D-methionine hardly occurs in nature, L-methionine is a component of most proteins. Besides cysteine, methionine is the only proteinogenic amino acid containing sulfur [16]. Due to the thioether group, Methionine is less reactive than cysteine, whose sulfur atom is located in a sulfhydryl group. As an essential amino acid, methionine cannot be synthesized endogenously and must be provided by the diet [17]. In metabolism, methionine is a supplier of methyl groups (-CH<sub>3</sub>) and necessary for cell growth and normal cell function [18]. Furthermore, methionine is involved in the metabolism of polyamines, and GSH plays

an important role in oxidative stress resistance [17,19]. Methionine metabolism can be divided into three connected pathways: the methionine cycle, the transsulfuration pathway and the salvage pathway [20] (Fig. 1).

In the methionine cycle, methionine is converted to the universal methyl-donor S-adenosylmethionine (SAM) by the enzyme methionine adenosyltransferase (MAT) [21]. In this reaction, all three phosphates are removed from ATP, indicating the "high-energy" nature of this sulfonium ion. As a principal methyl donor, SAM is involved in different methylation processes of DNA, RNA, and proteins [22]. After donating a methyl group, S-adenosylhomocysteine (SAH) is generated, which is a product inhibitor of SAM-dependent methylation reactions. SAH hydrolase (SAHH/AHCY) catalyzes the reversible hydrolysis of SAH to adenosine and L-homocysteine. The methionine cycle is closed by the followed remethylation of homocysteine to methionine. This process can be conducted via the folate cycle with 5-methyltetrahydrofolate as a methyl donor or by the betaine homocysteine methyltransferase (BHMT) requiring betaine as a methyl donor [23].

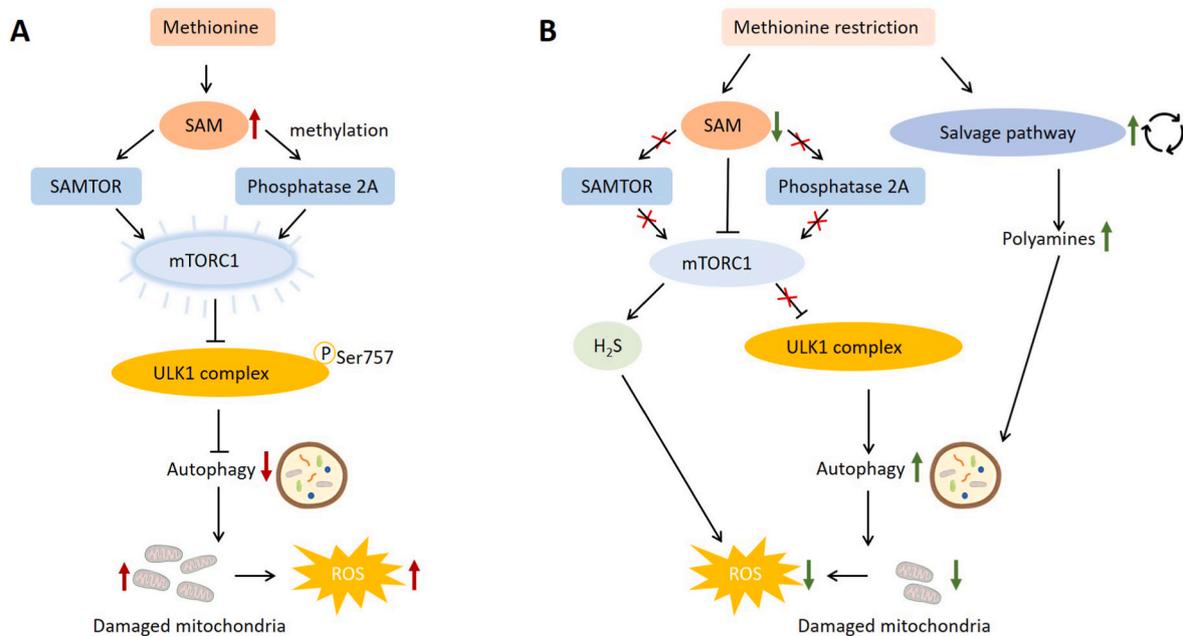


**Fig. 1. Schematic methionine metabolism** (modified according to Parkhitko et al. [15]). The methionine metabolism can be divided into three main parts. In the methionine cycle the essential amino acid methionine is converted by methionine adenosyltransferase to S-adenosylmethionine (SAM), a principal methyl donor. SAM can be demethylated to S-adenosylhomocysteine and hydrolyzed to homocysteine by the S-adenosylhomocysteine hydrolase. Homocysteine can be either remethylated back to methionine through the folate cycle or by betaine homocysteine methyltransferase. Another pathway is the transsulfuration of homocysteine. In this route homocysteine is needed for the synthesis of L-cystathionine by cystathionine- $\beta$ -synthase. L-cystathionine can be hydrolyzed by the cystathionine- $\gamma$ -lyase to cysteine, a precursor for taurine, pyruvate and glutathione, which is important for the redox balance. As side product of both enzymes, hydrogen sulfide is built. Another possibility to regenerate methionine is through the salvage pathway. Thereby SAM is decarboxylated and serves as aminopropylgroup donor for the synthesis of polyamines like spermidine and spermine. For this synthesis putrescine is formed in parallel through arginase and ornithine decarboxylase activity and then synthesized to spermine and spermidine. The needed decarboxylated SAM here is thereby converted to 5'-methylthioadenosine and synthesized back to methionine. AdoMet = S-adenosylmethionine, ARG = arginase, BHMT = betaine homocysteine methyltransferase, CBS = cystathionine- $\beta$ -synthase, CGL = cystathionine- $\gamma$ -lyase, dcSAM = decarboxylated SAM, GSH = glutathione, GSSG = glutathione disulfide, H<sub>2</sub>S = hydrogen sulfide, MAT = methionine adenosyltransferase, MS = methionine synthase, MT = methyltransferase, MTA = 5'-methylthioadenosine, ODC = ornithine decarboxylase, SAH = S-adenosylhomocysteine, SAHH/AHCY = S-adenosylhomocysteine hydrolase, SAM = S-adenosylmethionine, SMS = spermine synthase, SRM = spermidine synthase.

Besides the remethylation pathway homocysteine can be utilized in the transsulfuration pathway [23]. In this metabolic pathway the transfer of sulfur from homocysteine to cysteine occurs and is the only route for biosynthesis of cysteine. The rate-limiting cystathionine- $\beta$ -synthase (CBS) synthesizes cystathionine through the condensation of homocysteine and serine. Cystathionine can be hydrolyzed by the cystathionine- $\gamma$ -lyase (CGL) to produce cysteine, which is involved in the synthesis of proteins, GSH, and taurine [20]. GSH, a tripeptide of cysteine, glutamic acid and glycine, is one of the most important thiol redox buffers and can scavenge ROS. After scavenging

ROS, GSH is reversibly oxidized to GSSG [20,24] or S-nitrosoglutathione, the reaction product of \*NO and GSH that can be restored by the enzyme S-nitrosoglutathione reductase (GSNOR) in a NADH-consuming manner [25].

GSH is found free or protein bound in eukaryotic cells. Since the GSH reductase is constitutively active and inducible during oxidative stress, free GSH is almost only present in its reduced form. Therefore, the GSH:GSSG ratio is a key redox sensor and can be used as a marker of oxidative stress. Under normal conditions in mammalian cells the molar GSH:GSSG ratio exceeds 100:1 whereas in various oxidative stress models



**Fig. 2.** The possible mechanism of methionine on mTORC1 activity and autophagy. **A:** Methionine can lead to higher circulating SAM concentrations. This is sensed by SAMTOR leading to an activation of mTORC1. Also intracellular SAM can methylate phosphatase 2A which activates mTORC1. Activated mTORC1 suppresses the activity of the ULK1 complex through a specific phosphorylation on SER757 resulting in autophagy inhibition. This can lead to higher levels of damaged cell organelles such as mitochondria producing more ROS. **B:** Under methionine restriction SAM levels are altered leading to a suppressed mTORC1 activity. The ULK1 complex is active and promotes autophagy. Also the suppressed mTORC1 can lead to higher H<sub>2</sub>S production, a radical scavenger leading to lower ROS levels. Besides, the possible upregulation of the salvage pathway in the methionine cycle by methionine restriction leads to higher levels of polyamines, able to stimulate cytoprotective autophagy. mTORC1 = mammalian target of rapamycin complex 1, SAM = S-adenosylmethionine, ROS = radical oxygen species.

decreased ratios of 10:1 or even 1:1 have been observed [26,27]. The extracellular GSH/GSSG ratio and Cys/cystine ratio in plasma can be used to quantify oxidative stress associated with a number of unhealthy risk factors [28]; in addition, the GSH/GSSG ratio and the Cys/cystine ratio can be influenced by the SAA content of meals [29]. Furthermore, in an SAA-deficient diet, additional intake of drugs, such as therapeutic doses of acetaminophen, may alter SAA metabolism to maintain plasma cysteine/cystine redox potential (E(h)CySS) [30].

It has been shown that an activation of the transsulfuration pathway also promotes the production of the signaling molecule hydrogen sulfide (H<sub>2</sub>S) [31]. As a side product of cystathionine-β-synthase and cystathionine-γ-lyase H<sub>2</sub>S is generated and has been recognized as the third gaseous signaling molecule together with nitric oxide (NO) and carbon monoxide (CO) [32]. Although it is toxic in high concentrations, under physiological conditions, low concentrations of endogenous H<sub>2</sub>S have a protective effect. It protects cells from oxidative stress by modulating neuronal transmission, smooth muscle relaxation, release of insulin, and the inflammatory response [33,34]. Since transsulfuration modulates several physiological processes and plays a central role in maintaining redox balance, dysregulation of this pathway can lead to deleterious effects. Cysteine and H<sub>2</sub>S participate in a multitude of signaling processes and need to be highly regulated for normal cellular processes with multi-level controls (details on regulators of transsulfuration can be found in Ref. [31]).

To regenerate methionine, the transsulfuration pathway intersects with the transmethylation pathway, where homocysteine can be remethylated back to methionine as already mentioned above. However, impaired homocysteine remethylation and aberrancy in methyltransferase reactions can lead to methionine deficiency and homocysteine elevation, a process that seems to be associated to NAFLD, metabolic syndrome and cardiovascular risk as well as inflammation, oxidative stress, unfolded protein response, and cell death [35–41].

Another way to regenerate methionine is through the salvage pathway, also known as 5'-methylthioadenosine (MTA) cycle, which

regenerates methionine through SAM and is involved in the production of polyamines [42]. Briefly, SAM is decarboxylated by adenosylmethionine decarboxylase to dcSAM (decarboxylated SAM) and can serve as an aminopropyl group donor. In parallel, arginine is converted to ornithine by arginase and then decarboxylated by ornithine decarboxylase to putrescine. Putrescine is involved in the production of spermidine and spermin through spermidine synthase and spermin synthase, which use dcSAM as aminopropyl donor. Meanwhile, dcSAM is converted to MTA and through multiple enzymatic steps synthesized back to methionine [20]. Polyamines produced by this route are thought to play a dual role in maintaining redox balance. Polyamines may act protectively as free radical scavenger of hydroxyl radicals formed by fenton-like reactions, but not against superoxide radicals [43].

In addition, they may influence autophagy and interact with signaling pathways that modulate cellular responses [19]. While low polyamine levels promote growth cessation, high concentrations are associated with rapid proliferation or cancer. Dysregulated polyamine metabolism could lead to an imbalanced metabolic redox state. Therefore, maintaining intracellular polyamine homeostasis seems to be very important [44].

In addition to these three major pathways, several paralogous pathways contribute to methionine metabolism, as described by Sekowska et al. [45]. Studies show that chronic high exposure to methionine can lead to increased oxidative stress and contribute to multiple diseases and methionine metabolism dysregulation [17]. Dietary interventions such as methionine restriction could therefore contribute to the pathogenesis of multiple diseases as well as life span extension [17,23,46–49].

### 3. Methionine restriction (MR), aging, and diseases

#### 3.1. Aging/lifespan

Life expectancy has been rising in most countries over the past

**Table 1**  
Antioxidant effects of MR.

Studied condition	Model/Species/Strains	Age	Diet composition/MR content	Intervention duration	Effects	Ref.
Obesity	(M) C57BL/6J mice	14 weeks old	HFD: 24% Fat; SD: 0.86% Met; MR: 0.17% Met	22 weeks	Body weight ↓; fat mass ↓; lean mass per BW ↑; tissue mass per BW ↑; serum/liver ROS, GSH/GSSG ↑; serum/liver MDA ↓; liver GSH ↓; hepatic Nrf2, HO-1 and NQO-1 genes ↑	[83]
Obesity	(M) C57BL/6J mice	29 weeks old	HFD: 24% Fat; SD: 0.86% Met; MR: 0.17% Met	15 weeks	Plasma SOD ↑; plasma MDA ↓; heart Nrf2, HO-1 and NQO-1 genes ↑	[86]
Healthy	(M) Wistar rats	7 weeks old	SD: 0.86% Met; MR: 0.34% Met	7 weeks	Kidney 8-oxodG -; brain 8-oxodG ↓; kidney/brain GSA, AASA, CEL, CML, MDAL ↓	[92]
Obesity	(M) ob/ob mice	10 weeks old	SD: 0.86% Met; MR: 0.12% Met	14 weeks	Body weight ↓; adiposity ↓; lean body weight ↓; plasma total cholesterol and LDL ↓; hepatic TG ↓; VLDL ↑; serum ALT and AST ↓; hepatic Scd1 gene ↓; hepatic FAO ↑	[113]
Obesity	(M and F) C57BL/6J mice	18 weeks old	WD: 42% high-fat, high-sucrose SD: 8.2 g/kg Met; MD: 0% Met	5 weeks	Body weight ↓; WAT UCP-1 gene in males ↑; Acc1 and Fasn in females ↑; skeletal muscle mTORC1 ↓	[116]
Healthy	(M) F-344 rats	6–7 weeks old	SD: 0.86% Met; MR: 0.17% Met	Short-term study 2 weeks and 4 weeks; long-term study: 1–6 months	Plasma 8-OHdG, 8-isoprostane, protein-bound GSH in long-term study ↓; free GSH in long-term study ↑; liver and kidney GSH in short-term study ↓; brain GSH in short-term study -; brain GSSG reductase in both short-term study and long-term study; liver GSH peroxidase in both short-term study and long-term study; kidney GSH peroxidase in both short-term study and long-term study ↑; brain GSH peroxidase in both short-term study and long-term study; liver total SOD activity, Mn-SOD in long-term study	[123]
Obesity	(M) C57BL/6J mice	5 weeks old	HFD: 20% Fat; SD: 0.86% Met; MR: 0.17% Met	22 weeks	Body weight ↓; body fat rate ↓; plasma lipid levels ↓; colon MDA ↓; colon/ileum GSH-Px ↑; colon/ileum GSH/GSSG ↑	[119]
Healthy	(M) C57BL/6J mice	6 weeks old	SD: 0.86% Met; MR: 0.17% Met	8 weeks	Insulin sensitivity ↑; hepatic glucose production ↓; hepatic FGF21 ↑; HepG2 cells GSH, GSSG ↓	[139]
Healthy	(M) Wild-type, Gcn2 <sup>-/-</sup>	5 weeks old (Exp. 1) 7 weeks old (Exp. 5&6)	SD: 0.86% Met; MR: 0.17% Met	14 weeks (Exp. 1) 8 weeks (Exp. 5&6)	Insulin, glucose ↓ (Exp. 1); energy expenditure ↑ (Exp. 1); GSH (Exp. 5&6) ↓; hepatic NQO-1 gene ↑ (Exp. 5&6); targets of Nrf2 (Exp. 5&6) ↑	[150]
Healthy	(M) Wild Type, Nfe2l2 <sup>fl/Alb</sup>	8 weeks old	SD: 0.86% Met; MR: 0.17% Met	8 weeks	Energy expenditure ↑; hepatic Nfe2l2 gene -; serum FGF21 ↑; Nfe2l2 target genes -	[152]
Healthy	(M) Wistar rats	10 weeks old	SD: 0.86% Met; MR: 0.17% Met	7 weeks	Brain 8-oxodG, GSA, AASA, CEL, CML ↓	[173]
Aging	(M) Wistar rats	8 months and 26 months	SD: 0.86% Met; MR: 0.17% Met	8 weeks	Peroxisomal β-oxidation, GSA, 2-SC ↓	[174]
Glioma	The human glioma cell lines U87 and U251	N/A, cell culture	Met-Cys double deprivation	96 h	GSH ↓; ROS ↑; LC3-II ↑	[175]
Aging	(M) C57BL/6J mice	2, 12, and 15 months old	SD: 0.86% Met; MR: 0.34% Met	3 months	Brain NQO-1, HO-1 genes ↑; brain MDA ↓; serum/liver/brain FGF21 ↑	[263]
IBD	(M) ICR mice	Not mentioned	SD: 0.8% Met; MR: 0.14% Met	7 days	Colon MPO ↓; colon SOD, CAT, GPx ↑; colonic nuclear Nrf2 ↑	[251]

a Symbol: ↑ means increase; ↓ means decrease; - means no effects.

b M means male; F means female.

c HFD means high fat diet; SD means standard diets; MR means methionine restriction; MD means methionine deprivation; WD means western diet.

decades, as well as the prevalence of aging-associated pathological conditions [50]. It is suggested that a long-term, low-fat, whole-food vegan diet may increase life expectancy in humans by down-regulating IGF-I activity [51]. The influence of dietary interventions and restrictions, including CR and PR, on (metabolic) health and aging, has been investigated for more than 60 years [52,53]. Recent evidence shows that especially the quantity, source, and amino acid composition are strongly associated with the positive effects on lifespan extension and metabolic health [54].

Dietary methionine restriction prolongs mammalian lifespan [55], although it should be noted that Western diets contain methionine at levels many times higher than dietary requirements [56]. The adverse effects of this amino acid on lifespan have been strongly related to the disadvantageous ability of methionine to promote oxidative stress by several mechanisms, which might promote the aging process.

Methionine restriction (MR) was first reported by Dr. Norman Orentreich in 1993 [55]. They restricted the essential amino acid

L-methionine from 0.86 to 0.17% of the diet, resulting in a 30% longer lifespan of male Fischer 344 rats. Similar results have been demonstrated in other models of yeast [57], *Drosophila* [58], *Caenorhabditis elegans* (*C. elegans*) [59], mice [60,61] and rats [55,62,63]. Richie et al. were able to show that 80% MR in Fisher 344 rats resulted in a 44% increase of lifespan compared to controls [63]. In a mouse model of BALB/cJ × C57BL/6J F1 mice, a diet with 0.15% methionine compared to 0.43% methionine led to lifespan extension [60]. The positive effects on lifespan extension are associated with favorable metabolic responses on low-methionine diet in rodents [64,65]. Studies in other species under specific growth conditions support these results. For example, Carbreiro et al. demonstrated that metformin-induced altered methionine metabolism in *Escherichia coli* (*E. coli*) led to MR in *E. coli*, resulting in a prolongation of lifespan in their host *C. elegans* [59]. *Drosophila* fed with MR also had longer lifespans, but only under conditions of a low amino acid status, while a high amino acid status prevented the effect [58]. In cell culture experiments in human diploid fibroblast by Koziel

et al., it was observed that under MR conditions, the replicative lifespan was extended while cellular senescence was postponed [66].

The underlying mechanisms of the beneficial effects of MR on aging are very complex and not yet fully understood [18]. One possibility could be through influencing insulin/IGF-1 and mTORC1 (mammalian target of rapamycin complex 1) signaling, which regulated longevity across species in other dietary restriction and protein restriction models [67–69]. Like growth factors, insulin, and amino acids, methionine can contribute to mTORC1 activation, a complex with several subunits and central regulator of cell functions [70]. Activated mTORC1 is suppressing autophagy by inactivation of Ulk1 (Ser757) through phosphorylation [71] (Fig. 2A). However, autophagy plays an important role in the removal of damaged organelles such as mitochondria. Interestingly, recent data from Plummer et al. suggest that the autophagic activity underlying the lifespan extension by MR could be specifically that of mitophagy (the autophagy-dependent degradation of mitochondria), but not non-specific bulk macroautophagy or any other known form of selective autophagy [12]. Damaged mitochondria produce more ROS [72], therefore mitophagy is important to maintain mitochondrial quality.

Suppression of mTORC1 signaling pathways by MR could therefore extend chronological and replicative lifespan by reducing oxidative stress caused of damaged organelles [49] (Fig. 2B). Studies show that methionine can influence the mTORC1 activity via multiple pathways. On the one hand, SAM, a metabolite of methionine, is sensed by SAMTOR, resulting in mTORC-1 activation and autophagy suppression. On the other hand, intracellular SAM can methylate protein phosphatase 2A, which also activates mTORC1 [18,70]. MR is thought to alter SAM availability and thereby contribute to lifespan extension by suppressing mTORC1 activity [20]. Also, extracellular methionine is sensed by the taste 1 receptor member 1 and 3 (TAS1R1/TAS1R3) resulting in mTORC1 activation through phospholipase C, increase in intracellular calcium, and mitogen-activated protein kinase (MAPK) activation [18,73]. Lower extracellular methionine levels could therefore suppress this activation. Further, the induction of autophagy is closely related to the salvage cycle described in chapter 2 [19].

In addition, methionine is indirectly involved in the synthesis of polyamines, as the dcSAM formed in the methionine cycle serves as an aminopropyl donor for polyamine synthesis [20]. Polyamines like Spermidine stimulate cytoprotective autophagy, and it could be shown that supplementation with spermidine could extend lifespan across species [74–77]. Controversially, although methionine is required for the synthesis of spermidine, MR actually resulted in a 10-fold increase in spermidine in studies by Barcena et al. in progeroid mice, leading to an increase in lifespan [61]. The upregulation of the salvage pathway in MR could be a possible target for the positive effect [18]. Another mechanism on lifespan extension could be through the positive effect of MR on aging-associated metabolic diseases. In adult mice fed MR, the negative effects of aging on body mass, obesity, and insulin resistance were reversed by induction of fibroblast growth factor (FGF) 21 in the liver [78].

The positive effects of MR on lifespan extension are partially mediated through reducing oxidative stress, which is closely related to aging and aging-associated diseases. Induction of autophagy, H<sub>2</sub>S production, and reduction in free radical leakage from mitochondria seem to contribute there [18]. An increased flux via the transsulfuration pathway has been described in different MR models and is postulated as contributing factor for lifespan extension [79,80]. It could be caused by an enhanced cystathionine  $\gamma$ -lyase expression when sulfur-containing amino acids are restricted [81]. Human studies in centenarians also revealed a specific plasma profile associated with an enhanced transsulfuration pathway and highly regulated methionine metabolism [80]. Contributing to the beneficial aging effect appears to be enhanced H<sub>2</sub>S synthesis as a byproduct of this pathway. H<sub>2</sub>S can act as a ROS scavenger and upregulate antioxidant defense mechanisms [18].

Suppression of the mTOR pathway by MR can also lead to increased

H<sub>2</sub>S production, as mentioned above [71]. *In vivo* and *in vitro* studies by Wang et al. demonstrated that MR can effectively delay senescence through higher H<sub>2</sub>S production and mTOR suppression by AMPK in renal aging [82]. This is supported by various studies across species observing an enhanced H<sub>2</sub>S production by MR [83–86]. In addition, the transsulfuration pathway is required for the synthesis of GSH, an important regulator of redox balance [24]. The antioxidant effects of MR are summarized in Table 1. Studies have shown that an 80% MR increases the GSH content in erythrocytes of rats, which correlates with a reduction in age-related diseases and life expectancy [63,87]. However, it was also observed that GSH was reduced by MR in the liver and several tissues, although oxidative stress was not enhanced [63,65,87–89]. The low levels of hepatic GSH could be compensated by increased oxidative capacity [89]. GSH decrease and GSSG:GSH ratio increase are possible to contribute to extend lifespan by MR [62]. However, the influences and mechanisms of MR on GSH and GSH:GSSG ratio are still unclear, and further studies are needed.

In addition, methionine seems to stimulate mitochondrial ROS production. Mitigation of mitochondrial ROS (mtROS) by MR is another mechanism that contributes to the maintenance of a redox balance for healthy aging [18]. Sanz et al. demonstrated that 80% MR decreased mtROS production of complexes I and III in the liver and heart, similar to the results of Caro et al. [90–92]. Lifespan extension could be partially mediated by attenuating mtROS overproduction. The underlying mechanism appears to be a direct and rapid effect of methionine or methionine metabolites on mitochondrial complexes [13]. It has been reported that the reaction of methionine with hydroxyl radicals produces methionine radicals as intermediates and methanethiol as the final gaseous product [93]. Intermediate radicals or methanethiol itself may react with complex I or III in mitochondria, leading to overproduction of mtROS.

In summary, reduction of oxidative stress, induction of autophagy (mitophagy), and activation of the transsulfuration pathway seems to contribute most to lifespan extension by MR, but the mechanisms are very complex and not yet fully understood.

### 3.2. Cardiovascular disease and associated risk factors

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the world and aging is the dominant risk factor for CVD. Aging has remarkable effects on the heart and arterial system, leading to an increase in CVD including atherosclerosis, hypertension, myocardial infarction, and stroke [94]. Inappropriate diet contributes to “unsuccessful” aging and aging-related diseases [95] and is also a major risk factor for CVD, which can thus also be seen as a diet-associated disease. Diets rich in red meat, such as western diet (WD) correlate with increased CVD risk. One reason for the increased CVD risk observed in high red meat diets is thought to be linked to gut microbiota-dependent generation of trimethylamine-N-oxide (TMAO) from L-carnitine, a nutrient abundant in red meat [96]. TMAO is pro-inflammatory, able to impair vascular function and structure, and may up-regulate scavenger receptors and inhibit reverse cholesterol transport.

In general, a westernized diet, such as high fat diet (HFD) is characterized by a high proportion of saturated fat. Increased intake of fat, especially saturated fat, is associated with the increase in cardiometabolic diseases and obesity [97]. In fact, obesity is another independent risk factor for CVD [98]. Obesity is a multifactorial disease caused by the interaction of multiple factors such as genetics, environment, and biology, leading to the expansion of adipose tissue. Both sexes and all ethnic groups are affected by obesity at all ages [99] and obesity is associated with lower life expectancy because of a dramatic increase in the risk of comorbidities such as diabetes and CVD, including hyperglycemia, hypertension, and dyslipidemia. Furthermore, obesity-related diseases appear to accelerate cellular processes also observed in normal aging [100]. MR may delay the occurrence of CVD and associated risk factors. A diet high in animal protein/methionine

increases total homocysteine and methionine concentrations and thus the risk of CVD [101,102], whereas a low-fat vegetarian and vegan diet is associated with a reduction in cardiovascular risk factors [103]. Notably, MR can induce hyperhomocysteinemia in rats, which is a risk factor for the development of CVD [104]. However, Ables et al. [105] consider that MR may improve cardiac adaptability despite hyperhomocysteinemia.

It has been reported that total fat mass increases with age and its distribution changes, especially in the abdominal region [106]. In particular, abdominal obesity is a risk factor for CVD worldwide [107]. MR to alleviate obesity, such as reducing body weight and abdominal fat deposition and increasing energy expenditure, has been extensively studied.

MR was able to prevent weight gain and fat accumulation in both mouse and human studies [108,109]. Restricting methionine content from 0.86% to 0.17% can effectively increase total energy expenditure and core temperature and regulate metabolic flexibility, as shown in F344 rats. Dietary MR produced a persistent increase in uncoupling protein 1 expression in brown and white adipose tissue in combination with decreased leptin and increased adiponectin serum levels [110]. Compared with HFD-induced obese mice, 22-week MR (0.17% Met) significantly increased average heat production during the light and dark cycle [111]. Interestingly, MR also seems to be able to restore the circadian misalignment induced by a HFD. MR (0.17% Met; control diet 0.86% Met) improved the HFD-disrupted cyclical fluctuations of lipidolysis genes and the circulating lipid profile in C57BL/6 J mice. Also, MR improved the expression of clock-controlled genes in the liver and the brown adipose tissue [112].

In addition, obesity may affect heart function through risk factors such as dyslipidemia. MR has been evidenced to improve lipid metabolism. The plasma levels of triglyceride, total cholesterol, and low-density lipoprotein cholesterol were decreased, while high-density lipoprotein cholesterol was increased significantly in HFD-fed mice after MR [84,111]. Similar effects were shown in leptin-deficient obese (ob/ob) mice after 14 weeks of 0.12% MR treatment [113], suggesting that MR can effectively improve the dyslipidemia of obese mice.

Lipid metabolism is a driving force for the pathological changes in CVD, and remodeling lipid metabolism by MR and reducing fat mass could therefore reduce a risk factor for CVD.

A 2011 study showed in 26 obese adults randomized to MR (2 mg Met/kg body weight/day) or control diet (35 mg Met/kg body weight/day) that MR intervention increased fat oxidation and decreased carbohydrate oxidation and resulted in a decrease in intrahepatic lipid content. Comparable weight loss was observed in both groups [114].

In F344 rats it was shown that MR induced a coordinated down-regulation of lipogenic genes in the liver, resulting in a corresponding reduction in the capacity of the liver to synthesize and export lipids [115]. In this study, MR also remodeled the morphology of adipocytes in all three depots (epididymal, visceral and subcutaneous WAT), increased mitochondrial density in two depots (visceral and subcutaneous), increased TCA flux, and increased the capacity of subcutaneous WAT to oxidize palmitate. Changes in gene expression within WAT and liver reveals that dietary MR produced fundamentally different responses between the tissues with respect to lipid metabolism, however the coordinated remodeling of lipogenic gene expression between liver and WAT induced by dietary MR resulted in a significant decrease in circulating and hepatic lipid levels, beneficial to the overall metabolic profile of the animal.

In addition, MR also upregulated genes related to mitochondrial  $\beta$ -oxidation, further reversing hepatic steatosis in ob/ob mice [113]. In a short-term methionine deprivation (MD), genes involved in hepatic fatty acid  $\beta$ -oxidation, including peroxisome proliferator-activated receptor  $\gamma$  coactivator 1- $\alpha$  (*Ppargc1a*), carnitine palmitoyltransferase (*Cpt1a*), and acyl-coenzyme A oxidase 1 (*Acox1*), were significantly upregulated in female C57BL/6J mice, but not in male ones [116]. Consistent with that, greater changes in lipogenic gene expression in the WAT of female mice

compared to male mice were detected in that study. An important note from the authors is that they studied only young mice, and the question arises whether the sex-specific effects of MR persist in older animals after the levels of sex hormones, that drive many sexually dimorphic phenotypes, have declined.

In addition to fat deposition and impaired lipid metabolism, systemic oxidative stress appears to be an important link between CVD, associated risk factors, and aging. Chronic or long-lasting oxidative stress may cause cell damage by oxidizing cellular components such as proteins, lipids, and DNA [117], and is closely related to antioxidant enzymes like GSH, glutathione peroxidases (GPx), and superoxide dismutase (SOD) [118]. MR is able to reduce oxidative stress and oxidation-derived damage. In HFD-fed mice it was shown that MR is able to improve intestinal barrier function, inflammatory response, and oxidative stress by regulating the intestinal microbiota and its metabolites [119]. Improved gut homeostasis may be also associated with decreased body weight, body fat rate, blood glucose and plasma lipid levels by MR.

Furthermore, the nuclear factor erythroid 2-related factor 2/Kelch-like ECH-associated protein 1 (Nrf2/Keap1) pathway plays a role in stress response [120]. Nrf2 is a master regulator of multiple antioxidant enzymes, modulates cellular redox balance and senses the status of cellular oxidative stress. This is done by stimulating the activity of components of antioxidant defense, such as SOD, GPx, heme oxygenase-1 (HO-1), glutathione reductase, thioredoxin reductase, ferritin, and NAD(P)H:quinone oxidoreductase (NQO1). Inducing HO-1 in obesity provides an antioxidant environment that can decrease the formation of adipocytes by reducing visceral adipose precursor proliferation, contributing to hyperplastic adipose tissue expansion [121, 122]. A diet restricting methionine to 80% (0.17% Met) significantly increases plasma SOD and decreases MDA levels while increasing mRNA expression of Nrf2, HO-1, and NQO-1 in the heart of HFD-fed mice with cardiovascular impairment [86].

In F344 rats MR was associated with a reduction in oxidative stress biomarkers, including plasma 8-hydroxydeoxyguanosine (8-OHdG), 8-isoprostane and erythrocyte protein-bound GSH after one month with levels remaining low for at least six months. However, no changes in the activities of GSH reductase in liver and kidney and SOD in liver were observed as a result of MR feeding, indicating that oxidative stress is reduced by MR feeding in rats, but this effect cannot be explained by changes in the activity of antioxidant enzymes [123].

The mechanism by which MR ameliorates oxidative stress during CVD may be the activation of autophagy and hepatic H<sub>2</sub>S generation [85,124]. Activating transcription factor 4 (ATF4) is considered a master regulator of metabolism and is essential for the autophagy gene transcription program [125].

Although ATF4 is not required for many responses to MR, including body weight reduction and body composition shift (towards leanness), it is required for maintenance of redox homeostasis through the trans-sulfuration pathway leading to production of endogenous H<sub>2</sub>S [126]. In *C. elegans*, the ATF4/CTH2/H<sub>2</sub>S pathway also increases stress resistance by suppressing mTORC1 [127]. Furthermore plasma H<sub>2</sub>S levels are negatively correlated with adiposity [128] and H<sub>2</sub>S modulates Sirt1, which in turn is able to interact with mTOR to suppress oxidative stress [129]. In addition, oxidative stress triggers disruption of signaling pathways associated with metabolism and epigenetics, including microRNAs [130]. Similarly, MR regulates miR-328-3p, a type of microRNA that directly targets CGL and modulates endogenous H<sub>2</sub>S levels, thereby relieving oxidative stress and ER stress and improving homeostasis and metabolic efficiency in HFD-fed mice [83]. In conclusion, MR can reduce body weight, increase energy expenditure, and balance redox status. However, on the one hand, MR may induce hyperhomocysteinemia, but on the other hand, it may improve cardiac adaptability. Since a sex-specific response to MR has been demonstrated in young C57BL/6J [116], it is important from a translational perspective to also conduct studies at older ages, especially if we want to investigate and better understand the effects of MR on age-related

diseases such as CVD and associated risk factors.

### 3.3. Type 2 diabetes/insulin resistance

Insulin resistance (IR) is a set of clinical manifestations resulting from a decrease in the sensitivity of target tissue cells in liver, muscle, and adipose tissue to insulin, leading to a decrease in the efficiency of glucose uptake and utilization. IR, in addition to progressive pancreatic islet beta-cell loss, is part of the pathogenesis of type 2 diabetes (T2D). T2D is generally manifested after the age of 40 and is thereby regarded as typical age-related disease [131]. Indeed, recent data from Fazeli et al. [132] suggest as already other data before, that aging is an independent risk factor for T2D and this disorder is closely related to the aging process.

Vegan and vegetarian diets contain lower concentrations of methionine compared to omnivorous diets and this could explain the reduced incidence of diabetes in humans following the former diets [48]. Castaño-Martínez et al. [48] found that vegans had increased insulin sensitivity.

An elevated concentration of circulating fibroblast growth factor 21 (FGF21) has been implicated as a potential underlying mechanism. Although plant based and animal based foods have different amino acid compositions and the beneficial effects of a vegetarian diet have been reported, a study in mice shows that total protein, not amino acid composition, has healthy metabolic effects [133]. Nevertheless, literature data concerning effects of animal and plant protein are contradictory. Pivovarova-Ramich et al. showed in a randomized clinical trial in individuals with T2D, that both plant and animal protein based diets (30% of energy coming from protein in both groups) similarly reduce oxidative stress markers malondialdehyde (MDA) and protein carbonyls, but led to an increase in 3-nitrotyrosine (3-NT) in plasma, related to changes in fasting insulin and insulin resistance [134]. In contrast, it was shown that replacement of red meat with soy protein reduced plasma MDA and increased plasma total antioxidant capacity [135], a beneficial effect that couldn't be confirmed by another study [136]. In accordance with epidemiological studies on red meat intake, a high-methionine diet in rats showed an increased level of MDA and 3-NT in the liver [137]. However, the animal protein based diet in the study from Pivovarova-Ramich et al. was rich in white meat and dairy food and the methionine content of the diet is unknown.

In various animal models, numerous studies have shown that MR improves systemic glucose homeostasis and insulin signaling in peripheral tissues [46,60,138]. MR has beneficial effects on glucose homeostasis in some tissues and organs of the body. In the liver, MR (0.17%) enhanced the inhibitory effect of insulin on glucose production in C57BL/6J mice during the 8-week intervention, which was related to the increase in Akt phosphorylation [139]. Methionine deprivation also rapidly restored normal glucose tolerance and improved insulin-stimulated glucose uptake and suppression of hepatic gluconeogenesis in both female and male mice fed a continuous high-fat, high-sugar diet [116].

In addition, NZO mice treated with MR showed a decreased hepatic glycogen content and increased hepatic phosphoenolpyruvate carboxykinase (PEPCK) protein expression, indicating increased gluconeogenesis [48]. MR resulted in a significant decrease in circulating and hepatic lipid levels through the coordinated transcriptional restructuring of fat metabolism between the liver and WAT, which may also improve insulin sensitivity, shown in F344 rats [115].

In skeletal muscle, MR improved expression and transport of GLUT4 and glycogen levels and increased the expression of glycolysis-related genes (*HK2*, *PFK*, *PKM*) in HFD-fed mice [140]. This suggests that MR alleviates insulin resistance and improves glucose utilization by promoting glucose uptake and glycogen synthesis, glycolysis, and aerobic oxidation in skeletal muscle. MR can also increase insulin sensitivity by enhancing mitochondrial biogenesis with increased mtDNA copy number, *TFAM*, and *PGC1-α* mRNA level in HFD-fed mice [140]. In the renal

cortex and HK-2 cells (a type of proximal tubule cells of the human kidney) of *Gnmt*-deficient mice, a low-protein diet (LPD) also had beneficial effects on diabetic kidney disease [141]. LPD protected the kidney by inhibiting mTORC1, which was related to the lower SAM levels caused by low methionine intake in the diabetic mice.

Because MR can maintain systemic glucose homeostasis, it is thought to be a preventive or complementary feeding pattern. Intrauterine growth restriction (IUGR) is prone to the development of T2D. An MR diet reduced hyperglycemia in pigs with IUGR by promoting hepatic protein kinase B signaling and glycogen synthesis [142], suggesting that MR may be a potential dietary strategy to prevent T2D in humans with IUGR.

Chronic inflammation and oxidative stress triggered by obesity and often as a result of inactivity/sedentary lifestyle over years, lead to IR and eventually to T2D [143], which also accelerates aging. Signaling molecules, including hydrogen peroxide, are involved in the regulation of cellular functions. Reactive molecules can lead to abnormal changes in intracellular signaling and cause chronic inflammation and IR [144]. MR improves insulin sensitivity possibly by activating FGF21-mediated antioxidant signaling pathways. FGF21 is a novel target involved in metabolic regulation and has significant effects on enhancing insulin sensitivity. FGF21 and its receptors (FGFRs) are widely distributed in liver, adipose tissue, and pancreas, and FGF21 is a key target and endocrine mediator of the metabolic phenotype induced by dietary MR. MR can increase the expression of hepatic FGF21 by activating GCN2/ATF4/PPAR $\alpha$  signaling in liver cells, thereby improving insulin sensitivity, accelerating energy expenditure, and promoting fat oxidation and glucose metabolism [145].

MR also enhances insulin-stimulated phosphorylation of PKB/Akt and S6 in kidneys of 10-month-old mice to lower blood glucose levels [146]. Moreover, MR was able to decrease GSH in HepG2 cells, thereby regulating the activation state of protein tyrosine phosphatases such as PTEN. A lowered presence of GSH limits the GSH-responsive degradation of PIP3 by PTEN, thereby enhancing the PIP3-dependent activation of Akt [139]. Consequently, a decrease of GSH by MR also triggers upregulation of glutathione S-transferase (GSTP), which appears to be initiated by the ERK-AP-1 pathway [147].

It has been reported that upregulation of renal FGF21 expression in a T1D mouse model resulted in renal protection, possibly because of the activation of the Nrf2 antioxidative pathway mediated by PI3K/Akt/GSK3 $\beta$ /Fyn [148]. In FGF21<sup>-/-</sup> mice, MR failed to increase energy expenditure and reduce serum triglycerides, suggesting that FGF21 is essential in energy metabolism [138,149]. In GCN2<sup>-/-</sup> mice, MR similarly improved insulin sensitivity and activated hepatic PERK via the GSH-dependent PERK-eIF2-ATF4-Nrf2 pathway [150], implying that FGF21 rather than GCN2 may be essential for the antioxidative effects of MR.

However, MR-activated hepatic Nrf2 (Nfe2l2) possibly also cooperates with hepatic ATF4 to activate various antioxidant stress response reactions [151].

Interestingly, Nfe2l2 does not appear to be essential for mediating the metabolic effects of dietary MR. It was shown that mice with liver-specific deletion of Nfe2l2 (Nfe2l2<sup>fl</sup>/Alb) treated with MR had no effect on the ability of the MR diet to increase FGF21, reduce body weight and adiposity, and increase energy expenditure [152]. Moreover, although FGF21 has been reported to induce adiponectin expression and secretion in WAT [153,154], the beneficial effects on glucose metabolism induced by MR in HFD-fed mice may also be independent of adiponectin and FGF21 [155]. These results could be due to different animal models, different tissues and organs, sex differences, and duration of MR intervention.

Overall, MR could be a preventive nutritional strategy to treat metabolic diseases by improving glucose homeostasis and insulin sensitivity and accelerating energy expenditure. The exact mechanisms by which MR improves these factors need further investigation. MR appears to partially reduce prooxidant factors through FGF21 and

thereby regulate insulin signaling. However, this hypothesis should be further explored in various animal models, taking into account many factors, including sex differences. Whether other signaling pathways synergize with FGF21 to improve insulin resistance after MR remains to be elucidated.

### 3.4. Brain aging and cognitive disorders

Aging seems to be the greatest risk factor for most neurodegenerative diseases due to the ever-increasing life expectancy and aging of populations. Aging leads to lipid alteration [156], insulin resistance [157], and complex vascular phenotypic changes [158] that render the brain prone to diseases.

The most common neurodegenerative diseases accompanied with cognitive disorders, Alzheimer's disease (AD) and Parkinson's disease (PD), are predominantly observed in elderly individuals, and the risk of these diseases increases with age [159]. A low-protein and low-methionine plant based diet in vegans and vegetarians has been associated with lower risk of ischemic stroke and neurotransmitter metabolism in addition to the cardiovascular and metabolic disease benefits already described [160–165].

A study in Chinese older adults ( $\geq 60$  years) showed that methionine cycle metabolites (MCMs) elevated by omnivorous diets can lead to mild cognitive impairment (MCI) [166], suggesting a benefit of avoidance of red meat, especially processed meat.

On the one hand, a restricted diet prolongs lifespan in several species; on the other hand, studies have shown that dietary restrictions, such as CR and IF, and improved brain function during aging are associated [167–169].

MR has the potential to affect brain physiology. Brain-derived neurotrophic factor (BDNF) is widely and highly expressed in the brain and plays a critical role in brain and neuron function. In *in vitro* experiments, MR promoted the level of BDNF in C2C12 cells (a kind of mouse myoblast cells) partly by enhancing glycolysis and lactic acid production [170]. Similarly, it is possible that MR stimulates the production of BDNF in the brain and protects neurons from damage. In addition, direct evidence shows that MR can regulate the physiological functions of the brain.

As mentioned above, MR may lead to weight reduction, but at the same time, individuals will have a stronger appetite simultaneously. The central mechanisms are unclear but involve sympathetic nervous signaling [171].

The level of methionine as a methyl donor nutrient affects DNA methylation in one-carbon metabolism. In C57BL/6J mice, it was shown that a diet with reduced methionine content during the developmental phase led to a direct downregulation of genes in the brain that are related to one-carbon metabolism (DNA methyltransferases), reducing anxiety-like behaviors that persist into adulthood [172].

Moreover, MR appears to be an antioxidant strategy for brain redox homeostasis. MR enhances mitochondrial activity and attenuates endogenous oxidative damage in the rat brain (frontal cortex), including fatty acids peroxidizability index, protein oxidation (GSA, CML, CEL, MDAL, AASA, and 2-SC), and mitochondrial DNA oxidation [173,174]. Mitochondrial ROS production, mitochondrial protein oxidation, and glyoxidation were also decreased in brain, whereas mitochondrial oxidative phosphorylation capacity was increased [92].

Double deprivation of methionine and cystine both *in vitro* and *in vivo* resulted in a decrease in GSH content, an increase in ROS levels, and an induction of autophagy in glioma cells [175], suggesting that MR has effects on the oxidative balance of neurons.

The above studies have shown that MR can alter physiological functions of the brain. How MR might mediate the improvement in cognitive functions via FGF21 and H<sub>2</sub>S will be discussed in the next two subsections.

#### 3.4.1. FGF21 and brain function

The initial descriptions of FGF21 provided evidence that it is a powerful metabolic regulator in the context of glucose homeostasis, lipid metabolism, and energy balance as already described, but it remains controversial how FGF21 signaling is anatomically organized to produce its diverse physiological effects. FGF21 can act on the different brain regions. Hepatic FGF21 crosses the blood brain barrier (BBB) and acts on the hypothalamus, and the activation of the hypothalamic-pituitary-adrenal (HPA) axis triggers gluconeogenesis [145]. In that process, FGF21 may play a role in the paraventricular nucleus (PVN) or suprachiasmatic nucleus (SCN) of the hypothalamus through ERK1/2 signaling [176]. Moreover, FGF21 enhanced sympathetic nerve activity (SNA) on BAT to induce UCP-1 expression and lipolysis through the central nervous system (CNS) [177]. Interestingly, by using genetic tools to delete FGF21 signaling in the CNS (Klbf1/(CamK2a)mice), little evidence was found that FGF21 signaling plays a significant role in increasing energy expenditure [178]. In contrast, the same study shows that deletion of FGF21 signaling in the brain fully blocked the ability of MR to increase food intake and energy expenditure. However, the authors point out that it is not clear where exactly FGF21 signaling is deleted in the brain of Klbf1/(CamK2a) mice and that it is likely that FGF21 may signal in multiple brain areas through redundant systems to coordinate its response.

A neuroprotective role of FGF21, was demonstrated in obese and insulin-resistant rats. HFD-fed male Wistar rats developed obesity-related insulin resistance and cognitive decline with impaired hippocampal synaptic plasticity, decreased dendritic spine density, brain mitochondrial dysfunction, and increased brain cell apoptosis [179]. These obese and insulin-resistant rats were found to have impaired FGF21 signaling in the brain. Treatment with recombinant human FGF21 was observed to improve peripheral insulin sensitivity, increase synaptic plasticity in the hippocampus, increase dendritic spine density, restore mitochondrial function in the brain, decrease brain cell apoptosis, and increase FGF21 signaling in the brain, resulting in prevention of cognitive decline.

Furthermore, *in vitro* and *in vivo* AD models demonstrated that FGF21 treatment attenuated neuronal apoptosis in the hippocampus and reduced ROS and 8-OHdG levels [180]. Sirt1, an NAD<sup>+</sup>-dependent protein deacetylase, can be upregulated by MR and alters the methionine metabolic pathway in kidney and liver [47,146]. Sirt1 may also promote systemic FGF21 signaling by increasing its supply from the liver and increasing the expression of  $\beta$ -klotho in target organs [181]. Furthermore, FGF21 can activate Nrf2 signaling via the FGFR/ $\beta$ -klotho receptor [182].

The above demonstrates that FGF21 and its co-receptors, which are upregulated by MR, are closely related to improved brain function and reduced levels of oxidative stress in the brain. The antioxidant mechanism of FGF21 may regulate NF $\kappa$ B (nuclear factor 'kappa-light-chain-enhancer' of activated B-cells) and AMPK $\alpha$ /Akt signaling pathways to increase antioxidant enzyme activity and also decrease the production of advanced glycation endproducts (AGEs) [183,184]. It is speculated that inhibition of the NF $\kappa$ B pathway could be a potential target for the treatment of AD [185].

The complex interplay of Nrf2 and NF $\kappa$ B signaling pathways can alter the balance of antioxidative or inflammatory responses [186]. Activation of Nrf2 increases *NQO-1* and *HO-1* expression, which efficiently neutralize ROS and detoxify toxic chemicals, thereby inhibiting ROS-mediated NF $\kappa$ B activation [187]. NF $\kappa$ B binds with cAMP-response-element-binding protein (CBP) in a competitive manner and inhibits the binding of CBP with Nrf2, resulting in inhibition of Nrf2 transactivation [188]. MR regulates NF $\kappa$ B [189], suggesting that MR may alter Nrf2 and NF $\kappa$ B signaling through activation of FGF21 to target oxidative stress and neuroinflammation in cognitive disorders.

On the other hand, brain alterations and mood disorders such as AD and depression are often accompanied by insulin resistance in the brain [190,191]. The increase of oxidative stress markers is correlated with

insulin receptor activation [192]. Elevated insulin levels can cause oxidative stress, which in turn further aggravates insulin resistance [193]. Akt/GSK-3 $\beta$  has previously been reported to play a key role in regulating insulin/glucose homeostasis, both in peripheral tissues and in the brain [194–197].

FGF21 may also be a potential regulator for the treatment of insulin resistance in the brain. Recombinant human FGF21 promoted Akt/GSK-3 $\beta$  signaling in the hippocampus of HFD-fed mice to sustain neurogenesis [198]. Moreover, Akt/GSK-3 $\beta$ /Fyn signaling increases Nrf2 activity to resist the beta-amyloid (A $\beta$ )-evoked oxidative stress [199]. As mentioned above, MR acts on Akt to regulate insulin homeostasis, but whether MR can further regulate downstream signaling of Akt or other insulin/glucose-related signaling in the brain is unclear.

The above studies suggest that MR improves brain insulin resistance and oxidative stress associated with cognitive disorders. On the one hand, FGF21 acts directly on the brain as an endocrine hormone via the BBB to balance the disturbed redox homeostasis; on the other hand, FGF21 indirectly reduces systemic oxidative stress and thus exerts neuroprotective effects. However, much additional work will be needed to understand how FGF21 mediates these important neuroprotective responses.

### 3.4.2. H<sub>2</sub>S and brain function

As mentioned previously, H<sub>2</sub>S is formed in the transsulfuration pathway and has potential neuroprotective effects as an endogenous gas with physiological activity [200–202].

H<sub>2</sub>S could alleviate the impairment of cognition in part by vasoprotection, promotion of autophagy, and reduction of apoptosis. Cerebrovascular aging leads to cognitive impairment, whereas it is possible that H<sub>2</sub>S could protect cerebrovascular vessels from aging [203]. H<sub>2</sub>S is a vasoactive factor that plays a role in vascular contractility [204]. MR leads to increased H<sub>2</sub>S levels, which may partially inhibit mitochondrial electron transport and oxidative phosphorylation before mediating proangiogenic effects [205].

Damage to cerebral vessels can lead to activation of astrocytes and microglial cells [206]. H<sub>2</sub>S effectively inhibited reactive glial responses and synaptic damage and induced autophagic flux, thus improving behavioral outcomes [207]. NaHS as an H<sub>2</sub>S donor ameliorated diabetes-associated cognitive decline (DACD) and postoperative cognitive dysfunction, with involved mechanisms being improvement of autophagic flux [208], regulation of mitochondria-mediated apoptotic pathways [209], suppression of endoplasmic reticulum (ER) stress [210], enhancement of synaptic plasticity, and neurogenesis in the hippocampus [211].

In addition, H<sub>2</sub>S attenuates cognitive dysfunction induced by the systemic pro-aging factor  $\beta$ 2-microglobulin (B2M) [211]. Furthermore, in the same study it was shown that the H<sub>2</sub>S donor NaHS recovered autophagic flux in the hippocampus of B2M-exposed Sprague-Dawley rats, as evidenced by decreases in the ratio of autophagosomes to autolysosomes and the expression of p62 protein in the hippocampus. NaHS could also attenuate the development of early brain injury and cognitive dysfunction induced by subarachnoid hemorrhage via Akt/ERK-related anti-apoptosis pathway, and upregulating BDNF-CREB expression in Wistar rats [212].

In streptozotocin (STZ) induced diabetic rats H<sub>2</sub>S not only activated the hippocampal PI3K/AKT pathway, as evidenced by the increase of phosphorylated AKT, but also favorably reversed STZ-disturbed hippocampal neurogenesis and subsequently mediate antidepressant- and anxiolytic-like effects [213]. In an AD mouse model, pathological features such as excessive A $\beta$  accumulation [214] and tau hyperphosphorylation [215] were closely connected to the methionine metabolism. H<sub>2</sub>S as a product of methionine metabolism, decreased extracellular levels of A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub>, resulting in improved spatial learning and memory acquisition in APP/PS1 mice [216]. Presumably, H<sub>2</sub>S may also sulfhydrylate GSK3 $\beta$ , inhibit tau hyperphosphorylation, and reduce neurotoxicity in the 3xTg-AD mouse model [217].

In addition, H<sub>2</sub>S attenuates oxidative stress associated with cognitive impairment. The exact etiology of neurodegenerative diseases is not understood but oxidative stress, inflammation and synaptic dysfunction are primary hallmarks. Oxidative stress leads to free radical attack on neural cells contributing to protein misfolding, glia cell activation, mitochondrial dysfunction, impairment of DNA repair system and finally cellular death [218]. In the brains of LPS-induced AD mice, H<sub>2</sub>S reversed the increased MDA and decreased GSH levels [219]. H<sub>2</sub>S may protect neurons from oxidative stress by increasing GSH levels and inhibiting ROS overproduction in both primary cortical neuronal cells and glial cells [220,221].

Furthermore, it is possible that the Nrf2 system is involved in cognitive decline in multiple diseases [222]. Nrf2 regulates, among others, the catalytic and modifying subunit of glutamate cysteine ligase (GCL) to increase GSH levels [223]. Interestingly, it was observed that the expression of GCL, the biosynthetic enzyme for H<sub>2</sub>S, was reduced in the cerebral cortex and hippocampus of 3xTg-AD and human postmortem samples [217,224]. Similarly, brain H<sub>2</sub>S levels, CBS, and GCL are also significantly decreased and peroxidative markers are increased in chronic hypobaric hypoxia-, chronic osteoarthritis pain- [225] and hyperhomocysteinemia-induced cognitive dysfunction [226]. H<sub>2</sub>S can modulate the Nrf2 and glutathione systems in the kidney-brain axis [227], suggesting that H<sub>2</sub>S regulates Nrf2 to maintain redox balance in the brain. Also, Hyperhomocysteinemia in late postnatal life is often associated with severe oxidative stress, leading to developmental disorders and lower H<sub>2</sub>S and CBS levels in the offspring. Surprisingly, H<sub>2</sub>S donors are able to prevent anxiety-like behaviors, spatial memory decline, and oxidative stress (lipid peroxidation and activity of glutathione peroxidase) in the offspring [228].

A specific H<sub>2</sub>S concentration as well as activated H<sub>2</sub>S synthesizing enzymes may be also a potential biomarker for Alzheimer's disease and other dementias [229]. Total plasma H<sub>2</sub>S was shown to be a strong indicator for AD, and partially drove the relationship between cognitive dysfunction and white matter lesion volume, an indicator of microvascular disease.

Changes in dietary patterns appear to activate H<sub>2</sub>S production. Late-life every-other-day (EOD) intermittent fasting drives renal H<sub>2</sub>S production, and may modulate age-related frailty, including cognitive deficits [230].

In conclusion, increasing H<sub>2</sub>S levels by MR may have certain beneficial effects on cognition and emotion, which may depend on the antioxidant and autophagic flux-promoting properties of H<sub>2</sub>S.

## 4. Dietary recommendations for MR and future studies

### 4.1. Dietary recommendations for MR

Methionine is found in higher levels in animal foods such as pork, beef, dairy products and eggs compared to a plant based diet. The average daily requirement of methionine is 10.4 mg/kg body weight/day [231]. Both excessive and too low methionine in the diet can cause adverse effects.

The digestion efficiency of plant protein is lower than that of animal protein. In general, the utilization rate of animal protein is at least 90%, whereas it is only 80% for plant protein [232–235]. Consequently, omnivores have a higher protein/methionine intake than vegetarians/vegans. This means MR can be achieved with vegan or Mediterranean diets in particular, but there are some recommended foods and supplements. For vegans, isolated soy protein is of high nutritional quality, comparable to that of animal protein sources, and the methionine content is not limiting for adult protein maintenance [234].

Dietary recommendations for MR should be based on individual health status. Certain populations, such as pregnant women, adolescents, and athletes, are not advised to restrict methionine. Methionine, as a nutrient of one-carbon metabolism (OCM), is involved in the methylation pathway.

**Table 2**  
Effects of MR in human studies.

Studied condition	n value	Met concentration	Age (yr)	Sex (M/F)	BMI (kg/m <sup>2</sup> )	Intervention duration	Effects	Ref.
Obese subjects	26	33 mg Met/kg body weight/day	44–53	6/20	32.9–38.9	16 wks	Fat oxidation ↑; intrahepatic lipid content ↓; energy expenditure -	[114]
Adenocarcinoma of the colon or rectum	11	Free	48–78	8/3	24.6 ± 3.1	8 wks	Fed state plasma methionine ↓; BMI -; plasma albumin -; plasma prealbumin concentration -	[258]
Metastatic solid tumors	12	2 mg Met/kg/day	Not mentioned	Not mentioned	Not mentioned	8–39 wks	Plasma methionine ↓; BMI ↓; plasma albumin -	[264]
Healthy, normal-weight subjects	14	0.93 g Met/Cys for women, 1.19 g Met/Cys for men	20–40	4/10	20–25	7 days	Plasma methionine ↓; urinary cysteine and taurine ↓; plasma SCD -; total cholesterol ↓	[265]
Overweight or obese subjects	20	Met/Cys 1.6 g/day	23–40	0/20	24.7–34.7	7 days	Subcutaneous adipose tissue ↓; serum FGF21 ↑	[266]
Healthy, normal-weight subjects	14	0.93 g Met/Cys for women, 1.19 g Met/Cys for men	20–38	4/10	21.0–26.6	7 days	Fatty acids ↑; median glucose concentrations ↓	[267]
Healthy subjects	6	2.92 mg/kg/day	49–58	1/5	27.6 ± 4.32	3 wks	Plasma methionine, NAC and glutathione ↓	[259]
Overweight and obese subjects	23	1.4 g Met/day	37.8–42.2	6/17	30.3–31.1	6 months	Vitamin B <sub>12</sub> intake ↓; folate intake ↑; homocysteine concentrations -	[244]
Normal-weight subjects	72	Vegan/vegetarian diets	32–46	36/36	21.9–26.1	At least 1 yr	Plasma FGF21 ↑; leptin/adiponectin concentrations -	[48]
T2D	37	Plant protein diets	62.2–66.4	24/13	28.4–31.8	6 wks	Uric acid ↓; glycated haemoglobin ↑; diastolic blood pressure ↑; fasting non-esterified fatty acid ↑; insulin sensitivity -; fasting glucose -	[102]
Not mentioned	48	Lacto-ovo vegetarians	19.8–37	16/32	Not mentioned	2–29 yrs	Erythrocyte SOD activity ↓; serum vitamin B <sub>12</sub> ↓; serum MDA ↓	[268]
Healthy subjects	61	Lacto-ovo vegetarians/vegan	25.3–43.9	61/0	19.8–26.4	Not mentioned	Plasma homocysteine ↑; plasma vitamin B <sub>12</sub> ↓	[245]

a Symbol: ↑ means increase; ↓ means decrease; - means no effects.

b M means male; F means female.

Although there is no evidence that OCM nutrient intake has significant effects on fetal growth [235], pregnant women are not recommended to use MR. However, the rate of transsulfuration of methionine appears to be higher in the first trimester, suggesting a higher demand for methionine. The high rate of transsulfuration could be also aimed at providing cysteine and glutathione for the fetus [236].

Brain methionine levels increase physiologically after eating as a result of changes in the serum amino acid pattern [237]. Plasma methionine levels tend to be naturally lower when dietary methionine intake is restricted. School-aged children (9.1 ± 2.2 years old) had similar total sulfur amino acid (TSAA) requirements as adults [238]. Moreover, the TSAA levels of children with chronic renal failure did not differ from those of healthy children; in fact, the minimum requirement for methionine was higher in children with chronic renal failure (7.3 mg/kg/day) [239]. This implies that humans may not benefit from a low-methionine diet during development.

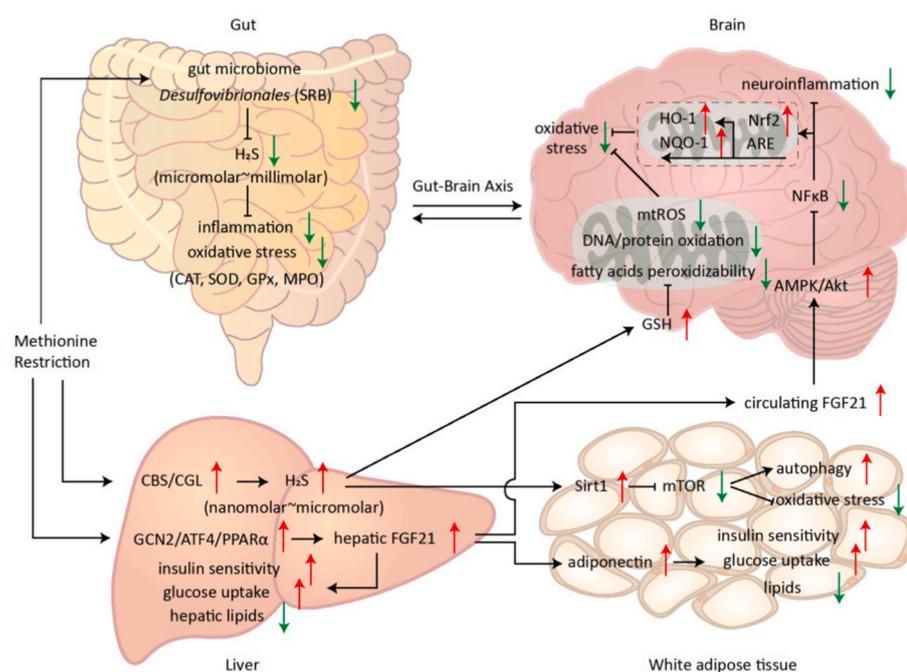
Although MR may improve skeletal muscle health in obese and aging mice and, when combined with endurance training, may increase intrinsic bone strength [240,241], this dietary pattern is not recommended for athletes. Methionine intake appears to be positively associated with lower limb muscular fitness in men [242]. Carnitine is necessary for muscle development during exercise [243] and methionine plays a role in endogenous carnitine synthesis and is not sufficiently present in the MR diet to meet the requirement.

In addition, osteoporosis is a significant problem in aging. MR has been shown to improve cognitive impairment in aging mice, while it also decreases bone mass, trabecular bone volume, bone mineralization

activities, and bone mineral content in rats with a decrease in serum osteocalcin and C-terminal telopeptide of type 1 [241]. MR may therefore negatively affect the bone growth and development process. To prevent the development of osteoporosis, MR should also be used with caution in the elderly. But as described in the previous chapters, animal studies show that MR has beneficial effects on disorders of glucose and lipid metabolism (obesity, diabetes, CVD), cognitive decline, and life expectancy; therefore, MR should be considered in certain pathological conditions, but with consideration of the adverse effects. More importantly, micronutrient supplementation is required during MR. Dietary vitamin B12 intake was reduced after a 6-month MR intervention [244] and in ovo-lacto vegetarians/vegans [245]. Vitamin B12 should be supplemented at the same time since it is mainly derived from milk, dairy products, meat, and fatty fish [246]. Some vegan cases show that iron intake may be inadequate as well [247]. In addition, a plant based diet tends to result in lower calcium intake [248]. In conclusion, the above factors should be considered by vegans/vegetarians and the principles of a reasonable diet and balanced nutrients must be followed when performing MR.

#### 4.2. Future studies

There have been many reports regarding the benefits of MR in health and longevity, but despite many studies, the mechanisms of lifespan regulation by CR and PR remain incompletely understood. Interestingly, lifespan of *Drosophila melanogaster* was extended by MR under conditions of low amino acid status, while MR did not work under conditions



**Fig. 3. The possible antioxidant mechanisms of MR in aging and aging-related diseases.** [1] FGF21. MR may increase the expression of FGF21 by activating GCN2/ATF4/PPAR $\alpha$  nutrition signaling in liver, thereby improving insulin sensitivity, promoting fat oxidation and glucose metabolism in both liver and WAT. Although FGF21 has been reported to induce the adiponectin expression and secretion in WAT, the positive benefits of glucose metabolism induced by MR may be independent of adiponectin and FGF21. Circulating FGF21 released by the liver enters the BBB, regulates AMPK/Akt and inhibits Nf $\kappa$ B, further attenuating neuroinflammation and oxidative stress [2]. H<sub>2</sub>S (nanomolar to micromolar concentrations) released by the liver. MR produces more H<sub>2</sub>S by enhancing the CBS/CGL in trans-sulfuration pathway. On the one hand, H<sub>2</sub>S activates autophagy and inhibits oxidative stress via regulating Sirt1 and mTOR in the WAT. On the other hand, H<sub>2</sub>S increases the GSH and improves mitochondrial oxidative damage, including mtROS, DNA/protein oxidation and fatty acids peroxidizability in the brain [3]. Gut microbiome. MR balances the gut microbiome, especially suppresses the *Desulfovibrionales*, a kind of SRB, which could reduce sulfate to produce H<sub>2</sub>S (high micromolar to low millimolar concentrations). Lower H<sub>2</sub>S decreases the inflammation and oxidative stress in the gut. Moreover, gut and gut microbiome/metabolite homeostasis affects brain function, which is considered as gut-brain axis. FGF21 = fibroblast growth factor 21, WAT = white

adipose tissue, BBB = blood brain barrier, CBS = cystathionine- $\beta$ -synthase, CGL = cystathionine- $\gamma$ -lyase, H<sub>2</sub>S = hydrogen sulfide, GSH = glutathione, mtROS = mitochondrial ROS, SRB = sulfate-reducing bacteria, CAT = catalase, SOD = superoxide dismutase, GPx = glutathione peroxidase, MPO = myeloperoxidase.

of high amino acid status, from which may be concluded that certain conditions must be met for the beneficial effects of MR [58]. For that reason, future studies may benefit from the use of diverse combinations of methionine and other nutrients. In addition, further studies are needed to better understand the molecular mechanisms involved and apply these principles to human nutrition to positively impact aging and/or age-related chronic diseases.

However, humans have considerable difficulties remaining compliant to strict dietary changes. MR is usually implemented with diets consisting of elemental amino acids (AA) that reduce methionine content to ~0.17%. Therefore, practical implementation of MR with diets based on elemental AA is difficult because of poor palatability. The development of methods for the production of highly palatable, low-methionine proteins is a better and innovative approach, as it will solve the problem of compliance and should therefore be a future task. Additionally, a comparison of the physiological responses to different dietary methionine levels or incremental restriction should be a future objective, since it was reported that MR also produces hyperphagia [110,249].

Another experimental strategy to better understand the complex responses to MR is to study the temporal response. In addition, a better understanding of how MR improves tissue-specific and overall insulin sensitivity should also be a focus. The overall metabolic phenotype elicited by MR appears to be the product of a number of responses that require further investigation.

Furthermore, the protective effects of dietary MR on age-related gut barrier dysfunction still remain mainly unclear. Interestingly, the gut microbiome can rapidly respond to diet alterations [250] and has the potential to modulate inflammation and oxidative stress. Inflammatory bowel disease (IBD) is often associated with a severe imbalance of redox homeostasis in the intestine, whereas MR is able to modulate corresponding markers such as catalase (CAT), SOD, GPx activities, and myeloperoxidase (MPO) [251]. In addition, recent evidence suggests that intricate and crucial links between the gut microbiota and the brain

involve multiple biological systems and may contribute to neurological disorders [252]. It was shown that time-restricted fasting (TRF) and intermittent energy restriction (IER) prevents colitis mice from gut microbiome composition disorder and gut leakage, thereby reducing oxidative damage in both colon and brain [253]. In addition, immune hemocytes act as signal transducers in the gut and brain, which may also affect chemokines in the brain in response to ROS stress [254]. The above studies show that the balance of the gut microbiome and the integrity of the gut barrier are closely related to redox homeostasis in the brain. A research focus should be on the fact that MR seems to alter gut microbiome/metabolites [255,256] and improve the gut barrier [257]. The interaction of gut and brain function has to be taken into consideration in future MR research.

More importantly, MR not only has beneficial effects on metabolism, but has also been shown to support the feasibility and good tolerability (nutritional status and toxicity) of cancer treatment regimens [258]. MR can specifically affect one-carbon metabolism and redox metabolism in tumor cells involved in chemotherapy and radiation. The response to MR appears to be conserved between humans and mice [259]. This is the basis that MR is likely to be a dietary intervention for adjuvant treatment of diseases such as cancer in the future. In addition, race and region should be better considered in clinical trials in the future. For example, Indian women have different serum OCM markers after methionine exposure than American women [260]. The effects of MR already found in a multitude of human studies are summarized in Table 2.

## 5. Prospects

The impact of nutrition on longevity and health will continue to be one of the most important issues facing society in the future. Caloric intake and protein intake are of crucial importance here. In the context of sustainability and planetary health, new factors are evolving that are important to be considered. This also affects protein and methionine intake since new protein sources are opened up [261], and new

nutritional habitus will evolve [262]. The possible antioxidant mechanisms of MR in aging and diseases are summarized in Fig. 3, which involves multiple targets. However, whether this leads to a health promotion remains to be elucidated due to many other components of such a diet. One of the future developments with regard to protein and in particular, methionine restriction will be the investigation of the mechanism of action of these diets and the translation of this theoretical knowledge into human studies. However, it must be under the guidance of professionals if implemented.

#### Declaration of competing interest

The authors Yuyu Zhang, Julia Jelleschitz, Tilman Grune, Weixuan Chen, Yihang Zhao, Mengzhen Jia, Yajie Wang, Zhigang Liu and Annika Höhn declare no conflict of interest.

#### Data availability

No data was used for the research described in the article.

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