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Minireview

Homocysteine and lipids: S-Adenosyl methionine as a key intermediate

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ABSTRACT

An association between hyperlipidemia and hyperhomocysteinemia (HHCY) has been suggested. This link is clinically important in management of vascular risk factors especially in elderly people and patients with metabolic syndrome. Higher plasma homocysteine (Hcy) was associated with lower high-density lipoprotein (HDL)-cholesterol level. Moreover, HHCY was associated with disturbed plasma lipids or fatty liver. It seems that hypomethylation associated with HHCY is responsible for lipid accumulation in tissues. Decreased methyl group will decrease the synthesis of phosphatidylcholine, a major phospholipid required for very low-density lipoprotein (VLDL) assembly and homeostasis. The effect of Hcy on HDL-cholesterol is probably related to inhibiting enzymes or molecules participating in HDL-particle assembly.

1. Introduction

Hyperhomocysteinemia (HHCY) and hypercholesterolemia are linked to the development of atherothrombotic diseases. Few studies have shown that the risk associated with combined HHCY and hypercholesterolemia is greater than that associated with only one of these risk factors [1]. Therefore, an emerging aspect to reduce cardiovascular diseases is maintaining concentrations of plasma total homocysteine (Hcy) as well as that of cholesterol at low levels. The mechanisms linking Hcy and lipid metabolism are not thoroughly known.

Recent invaluable studies strongly demonstrate the importance of the metabolic balance between SAM, SAH, choline, phosphatidylcholine (PC), phosphatidylethanolamine (PE) in Hcy metabolism, hyperlipoproteinemia, liver function, and cardiovascular disease. This review discusses the most important studies linking these physiological pathways in health and disease conditions.

2. Homocysteine metabolism

Homocysteine (Hcy) is a non-essential amino acid that is produced from demethylation of methionine. Hcy can be remethylated into methionine by means of vitamin B12-dependent methionine synthase and 5-methyltetrahydrofolate as a methyl donor. Hcy can be also catabolized into cysteine (the transsulfuration pathway) via cystathionine beta synthase and cystathioninase, both enzymes being vitamin B6-dependent. A third way to remove Hcy is conversion to S-adenosylhomocysteine (SAH). The last reaction is mediated by SAH-hydrolase and favors the SAH formation in case of increased Hcy concentrations. S-Adenosyl methionine (SAM) is a universal methyl donor that is formed from methionine and converted into SAH after donating its methyl group. SAH is a potent inhibitor of most known methyltransferases [2].

3. Lipid metabolism

Plasma lipoproteins are unique particles with a hydrophobic core containing triglycerides and esterified cholesterol and apolipoproteins on the surface. Lipoproteins show some similarity to plasma membrane consisting of a surface layer phospholipids and a core of non-polar lipids. Apo-AI and apoB are the principle apolipoproteins associated with high-density lipoprotein (HDL) and low-density lipoprotein (LDL), respectively. Very low-density lipoprotein (VLDL) is the lipoprotein class of the lowest density and the richest in triglycerides. This particle is formed in the liver

Abbreviations: Hcy, homocysteine; HHCY, hyperhomocysteinemia; SAM, S-adenosyl methionine; SAH, S-adenosylhomocysteine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidyl ethanolamine methyltransferase; LCAT, lecithin-cholesterol acyltransferase; MTHFR, methylene tetrahydrofolate reductase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; BHMT, betaine homocysteine methyltransferase; SREBP-1, sterol regulatory element-binding protein; UPR, unfolded protein response; CYP7A1, cholesterol 7α-hydroxylase

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as a nascent VLDL containing triglycerides, apoE and apoB. VLDL is excreted from the liver in this nascent form, then it is transformed into LDL after gradually losing its content of triglycerides. LDL is taken up by the liver and peripheral tissues via LDL-receptor. In contrast, HDL the lipoprotein responsible for the reverse transport of cholesterol from peripheral tissues to the liver is the one with the highest density and the richest in protein and phospholipids. Additionally, HDL functions in transferring proteins into other lipoproteins, picks up cholesterol from other lipoproteins, and from cell membranes, and esterifies cholesterol by lecithin-cholesterol acyltransferase (LCAT).

Elevated plasma concentration of LDL-cholesterol is a major risk factor for cardiovascular disease. HDL protects from vascular disease by mediating reverse cholesterol transport and by the means of its direct antiinflammatory properties [3]. Low HDL-cholesterol in combination with raised triglyceride levels is considered an atherogenic lipid profile frequently associated with the metabolic syndrome. The metabolic syndrome is a mutual soil for HHCY as well as low HDL resulting in a high risk for coronary diseases. Moreover, clinical studies on patients with coronary diseases documented a negative association between Hcy and HDL-cholesterol [4]. Therefore, interactions between Hcy and HDL-metabolism could be clinically important. For example the cardiovascular benefits of treatment with fenofibrate [5] might be counterbalanced by a sustained increase in the plasma concentration of Hcy.

4. Interrelations of phospholipid and Hcy metabolism

Cholesterol and phospholipids play essential roles in all cellular membranes. The plasma membrane is very rich in cholesterol, the endoplasmic reticulum has an intermediate level, and the mitochondria have the lowest content of cholesterol. The intermembrane asymmetry appears to result, at least partially, from a specific affinity of certain phospholipids for cholesterol in the order sphingomyelin > phosphatidylcholine > phosphatidylethanolamine [6].

In addition to its role in composition of cell membrane and membrane dynamic function, PC is an important player in signal transduction as a source of lipid second messengers [7] thus playing a key roles in several metabolic pathways in the cells.

The role of methyl group metabolism in PC synthesis is summarized in Fig. 1. PC is the methylated product of PE (3 methyl groups are required) (Fig. 1). Phospholipid methylation in mammals is a major SAM-consuming-pathway, since hepatic PC de novo synthesis is responsible for about 50% of plasma Hcy levels [8]. Therefore, synthesis of PC represents a cross point of SAM and phospholipid metabolism.

In the liver, PC can be synthesized via two pathways, both of which are linked to methyl group metabolism. The first pathway for PC synthesis is via the phosphatidyl ethanolamine methyltransferase (PEMT) pathway. The synthesis of approximately 30% of PC in the liver is enhanced by PEMT that involves three sequential

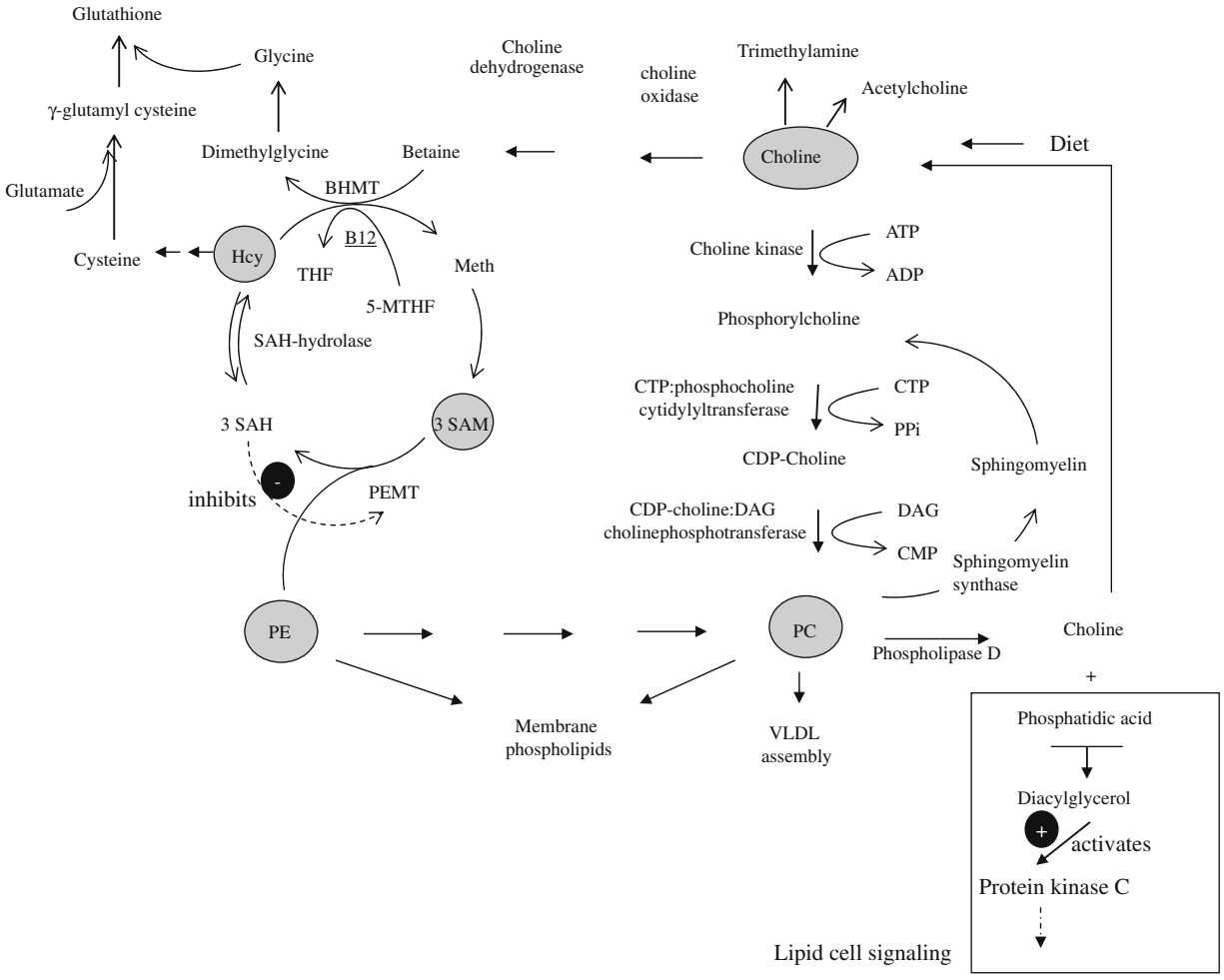

Fig. 1. The metabolic pathways linking Hcy and lipid metabolism. BHMT, betaine homocysteine methyltransferase; Met, methionine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; DAG, 1,2-diacylglycerol; CTP, cytidine triphosphate; CMP, cytidine monophosphate.

methylation steps. The second pathway is responsible for production of 70% of PC in the liver, which can be made through the cytidinediphosphocholine (CDP-choline) or Kennedy pathway (Fig. 1). This biological reaction requires choline that is derived either from the diet or from the catabolism of PC. The CDP-choline and Hcy metabolism are linked via choline, because choline is a precursor for betaine. The last compound is a methyl donor for Hcy methylation via betaine homocysteine methyltransferase (BHMT) in the liver. Therefore, disturbed Hcy metabolism results in decreased SAM or increased SAH thus affecting the synthesis of phospholipids (PC from PE).

The liver is the major site for lipid and Hcy metabolisms. The expression of CBS and BHMT that mediate transsulfuration and remethylation of Hcy, respectively, are especially high in the liver [9]. Fat and cholesterol consumed with the diet are transported to the liver as chylomicrons. In the liver, fat and cholesterol are packaged into VLDL for transport through the blood to tissues that require them. As PC is a component of VLDL particles, inadequate PC can cause accumulation of fat and cholesterol in the liver. Increased hepatic cholesterol biosynthesis, retention of triglycerides in the liver, and decreased plasma HDL and its protein content, apo-AI, have been linked to HHCY in experimental studies despite that plasma total cholesterol levels were either not or only slightly changed [10–14].

A role for PC synthesis via the PE-methylation pathway in the secretion, lipidation, or hydrolysis of VLDL has been suggested [15,16]. 3-Deazadenosine is an SAH-hydrolase inhibitor and an inhibitor of cellular methylation that caused reduction of PE-methylation (thus lower PC; higher PE/PC ratio), decreased VLDL secretion and increased levels of intracellular triglycerides but did not affect the total amount of apolipoproteins (apo-B48, apo-B100) secreted into the medium indicating that the effect was limited to the lipidation of VLDL [16]. Another study documented that mice fed a diet deficient in methionine and choline had lower serum cholesterol (77.2 mg/dl) and triglycerides (46.4 mg/dl) compared to mice fed chew (104.5 and 120.5 mg/dl, respectively) or those fed a methionine and choline supplemented diet (145.5 and 122.9 mg/dl, respectively) [17]. Mice fed a diet completely devoid of methionine and choline had higher liver triglycerides and lower liver cholesterol and phospholipids compared to mice fed a diet enriched with choline and methionine [17]. The authors observed a profound suppression of steoroyl coenzyme A desaturase-1, an enzyme that enhances the metabolic rate [17].

Taken together, these results suggest that when triglyceride transport out of the liver is inactive, the liver might try to produce fewer amounts of the lipids and simultaneously enhance lipid oxidation. Moreover, one can speculate that deficiency of methyl donors (example choline and methionine deficient diet) might be associated with severe changes in lipid homeostasis in the liver and probably the vascular system. It seems that hypomethylation associated with HHCY is responsible for lipid accumulation in tissues. Because Hcy metabolism is not restricted to the liver, one might assume that other tissues such as the endothelial system that are prone to accumulate lipids might be sensitive to HHCY. In contrast, in the endothelial system where the role of BHMT in Hcy metabolism is probably negligible [18], HHCY might enhance the accumulation of lipids.

5. HHCY disturbs lipid homeostasis; clinical observations

In addition to early cardiovascular events, patients with homocysteinuria develop hepatic steatosis or "fatty liver" which is characterized by enlarged, multinucleated hepatocytes containing microvesicular lipid droplets [19]. An association between Hcy and lipids has been also documented in studies on individuals with mild HHCY. In the Hordaland study that included 5917 subjects, a higher intake of saturated fatty acids was positively associated with higher concentrations of plasma Hcy. Concentrations of Hcy were higher (by 8.8%) in the group with the highest intakes of saturated fatty acids compared to that with the lowest intake [20]. These findings are not surprising, recalling the fact that liver is the major organ where Hcy and lipid metabolism take place. The molecular mechanism of fatty liver in HHCY is poorly understood.

Possible mechanism might be that the intake of saturated fatty acids can lead to increased Hcy by increasing the production of PC from PE via the PEMT pathway [20]. PEMT consumes three SAM molecules for transforming PE to PC. The reaction produces three SAH molecules that are hydrolyzed to Hcy via SAH-hydrolase (Fig. 1). Another possible explanation could be that a diet rich in fatty acids might contain more methionine, the precursor of Hcy.

Interesting observations were recently made in few patients with SAH-hydrolase deficiency. This defect was associated with only slightly elevated plasma Hcy (14–16 μmol/L), but a severe elevation in SAH, and less elevation of SAM in blood [21]. Considerably elevated liver enzymes (LDH and AST) have been observed and liver biopsy showed extensive cytoplasmic lipid droplets in electron microscopy [21]. In another patient with SAH-hydrolase deficiency, plasma triglycerides were normal and the liver showed marked structural abnormalities [22]. No specific reference to steatosis has been reported in this case. The role of phospholipids in membrane structure and function might explain the abnormalities in the endoplasmic reticulum of liver biopsy [22]. This disorder was also associated with markedly low plasma concentrations of PC and free choline suggesting that increased SAH inhibited PEMT and thus the production of PC from PE [21,22].

6. Fatty liver and HHCY are mutual findings in chronic alcoholism

Among the many macro- and micronutrients that are negatively affected by chronic alcohol ingestion, are choline, betaine, methionine and B-vitamins which are essential for the generation, transport and transfer of one-carbon units to target molecules such as phospholipids, SAM, DNA, and neurotransmitters [23]. Chronic ingestion of methanol causes disturbances in methionine metabolism in the liver, leading to increased Hcy, higher SAH, and lower SAM [24]. The activity of methionine synthase and methionine adenosine transferase is inhibited by alcohol. In contrast, the activity of BHMT is maintained and this leads to a higher consumption and thus requirements of betaine required for Hcy methylation. Furthermore, low concentrations of folate, vitamin B6 and betaine are common in alcoholism and have been shown to enhance the side effects of alcohol in the liver [25]. Additionally, alcohol consumption causes fatty liver and hepatic steatosis. Hypomethylation (i.e., low SAM/SAH ratio) and reduced PEMT activity in chronic alcoholism may account for lipid accumulation in the liver [26–29] (Fig. 2). In addition, supplementation of betaine or SAM can protect the liver in chronic alcoholism (discussed below) suggesting a role of BHMT and SAM in protecting against the toxic effects of alcohol [26,27].

7. HHCY and dyslipedemia; experimental studies

Both Hcy and lipids are toxic in vascular cells and hepatocytes which could indicate interactions between the two pathways. The interaction between lipids and Hcy metabolism has been tested in several animal models with HHCY and/or hypercholesterolemia (Table 1). The classical animal model for HHCY is homozygous cystathionine beta synthase deficient mice (CBS−/−). These animals developed hepatic steatosis and atherosclerotic lesions

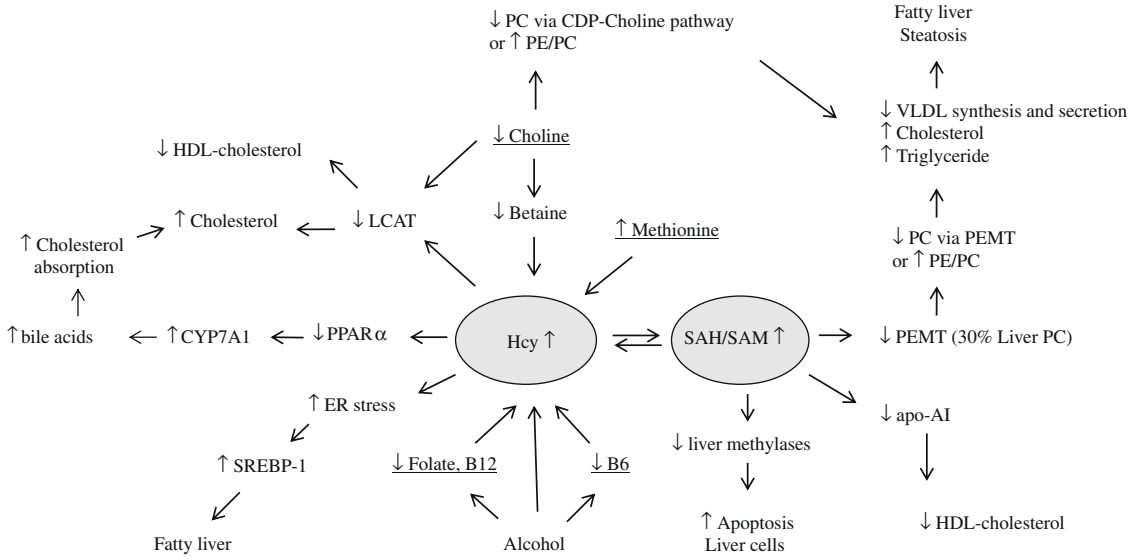

Fig. 2. Possible interactions between HHCY and hyperlipidemia in cell pathology. Hcy, homocysteine; HHCY, hyperhomocysteinemia; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidyl ethanolamine methyltransferase; LCAT, lecithin-cholesterol acyltransferase; CYP7A1, cholesterol 7α-hydroxylase; UPR, unfolded protein response; SREBP-1, sterol regulatory element-binding protein.

Table 1
Studies on the effect of HHCY on blood or tissue lipids.

Conditions Hcy	Plasma Hcy	Blood lipids	Liver/tissue lipids	Reference
Mice fed methionine rich diet	Hcy increased approximately 3-fold	HDL; LDL; VLDL no significant changes	Liver not tested	[35]
A methionine rich diet in mice when added to an atherogenic diet			Increased the size of the atherosclerotic lesions containing macrophage foam cells	
Patients with homocysteinuria	Severe HHCY (>100 μM)		Fatty liver	[20]
CBS−/− mice			Hepatic steatosis	[13,31]
MTHFR−/−	Increased plasma Hcy		Aortic lipid deposition	[32]
ApoE−/− mice fed a diet rich in methionine and deficient in folate, B12 and B6	Increased Hcy		Increased atherogenic lesion compared to apoE−/− mice	[33]
Rabbits fed a cholesterol rich diet 12 weeks			Developed atherosclerosis	[34]
Methionine added to the cholesterol rich diet			Enhance the lesion in animals receiving a cholesterol rich diet	
Betaine supplement 0.5% (W/V) Control rats Ethanol-fed rats	↑ Hepatic SAM (2-fold) ↑ Hepatic SAM (5-fold)		↓ Hepatic triglycerides ↓ Hepatic triglycerides	[109]
Choline, 2 g	Hcy 7.8–7.5, SAM/SAH 3.0–5.2*		RBC membrane ratio of PC/PE was lowered after 14 days	[108]
Lecithin, 2 g	Hcy 8.6–8.5, SAM/SAH 3.9–4.9			
Betaine, 3 g For 14 day to children with cystic fibrosis	Hcy 7.3–6.2*, SAM/SAH 3.4–5.3*			
Methionine rich diet Atherogenic diet + methionine 40 week	Plasma Hcy increased	The methionine did not affect plasma lipoprotein profile	Methionine increased aortic atherosclerotic lesion found in the atherogenic diet group	[35]
CBS−/−/apoE−/− mice vs CBS+/+/apoE−/−	Plasma Hcy increased	Increased total cholesterol, free cholesterol, phospholipids, decreased HDL-cholesterol	Decreased liver apo-AI protein	
		Decreased apo-AI protein in plasma increase LCAT specific activity, decrease LCAT substrate reactivity		[3]
CBS−/−/apoE−/−mice vs CBS+/+/apoE−/−	Plasma Hcy 210 vs 3.8 μM	CBS−/−/apoE−/− mice had lower HDL-cholesterol and lower triglycerides, and higher total cholesterol	Double knock-out mice had more cholesterol ester and triglycerides deposition in the vessel well	[14]
MTHFR+/− mice compared to MTHFR +/+ mice		62% lower apo-AI protein in MTHFR+/− mice	52% lower apo-AI protein in MTHFR+/−mice	[12]
Betaine supplement MTHFR+/− mice compared to MTHFR+/+ mice 1 year treatment	Reduced Hcy	Increased apo-AI		[43]
PC 2.6 g/day or placebo, for 2 weeks to healthy men	Reduced plasma Hcy by 18%, and post-methionine-loading Hcy by 29% relative to placebo	Serum triglycerides increased after PC treatment. serum LDL, HDL, and total cholesterol did not change		[106]

[13,30] which is consistent with observations from human studies on CBS deficient patients [19].

In methylenetetrahydrofolate reductase (MTHFR) deficient mice, decreased methylation capacity and aortic lipid deposition have been observed [31]. Additionally, apoE deficient (apoE−/−) mice fed a methionine rich, folate, B12 and B6 deficient diet showed increased concentration of blood Hcy that was associated with increased atherogenic lesions compared to apoE−/− mice on a control diet [32]. Moreover, methionine rich diet for 12 weeks did not independently cause atherosclerotic lesions but it did enhance the lesions in animals receiving a cholesterol rich diet [33]. A further study confirmed that a methionine rich diet increased the size of the atherosclerotic lesions containing macrophage foam cells in mice when added to an atherogenic diet [34]. One study indicated also that a diet rich in fat did not only elevate total cholesterol but also doubled Hcy levels in animals [14]. These results suggest that high intake of dietary cholesterol and fat might contribute to the increase of Hcy levels, thus further increasing the risk of atherosclerosis. Taken together, HHCY can accelerate atherosclerosis under dietary conditions that elevate plasma lipids and vice versa.

The enzyme SAH-hydrolase plays a major role in Hcy metabolism and cellular lipid homeostasis. Its deficiency results in deregulated lipid metabolism, leading to an imbalance of phospholipids and triglycerides synthesis, with probable implications for mammalian lipid-associated disorders [35]. Furthermore, data on cellular level demonstrated that depletion of SAH-hydrolase in yeast results in massive accumulation of lipid droplets and triglycerides during logarithmic growth [36]. SAH-hydrolase mediates SAH-accumulation after treatment with Hcy and this metabolic condition causes inhibition of PE-methylation to PC, and subsequently triglycerides accumulation [35]. Additionally, inhibition of SAH-hydrolase decreases PC de novo synthesis and is accompanied by an increase in triglyceride levels. Therefore, experimental studies suggest that the conversion of PC to PE and the ratio of PE/PC are key issues in the relationship between HHCY and dyslipedemia.

Phospholipid methylation via PEMT regulates methylation status and vice versa. In line with this, reduced liver methylation was accompanied by alterations in liver phospholipid metabolism in CBS+/− mice [37]. HHCY mice had lower enzymatic activity of PEMT in the liver compared with mice with normal Hcy. Higher levels of PE in liver of HHCY mice led to higher PE/PC ratio, with non-significant differences in PC concentrations between HHCY and control mice. Lower availability of methyl groups and lower activity of PEMT in mice with HHCY might account for increased PE, the demethylated precursor of PC [37].

Using a different approach, a PEMT knock-out mouse model, liver phospholipid metabolism was compromised and the secretion of VLDL from the liver was inhibited. Pemt−/− mice fed a choline-deficient diet develop severe lipid pathologies after 3–4 d [38], had a significant decrease in hepatic and plasma PC levels, and had an increase in hepatic triglyceride levels. In contrast, Pemt−/− mice fed a choline-supplemented diet did not display liver damage and had normal hepatic and plasma PC levels as well as normal hepatic triglycerides level [38]. The addition of a methyl donor, choline, to the diet of Pemt−/− mice was able to rescue the liver damage [38]. Dietary choline can be converted into PC in the liver via the CDP-choline pathway, thus ameliorating the effect of Pemt deletion on phospholipid metabolism.

In a model of phospholipids disorders, Pemt−/− mice show a 70% reduction in apo-B100 secretion from hepatocytes, a 50% reduction in triglycerides secretion, and no change in PC [39]. The authors supposed that the PEMT pathway is essential for secretion of apo-B100-containing-VLDL [39]. Thus lowered apo-B100 in Pemt−/− mice will cause reduction in triglycerides secretion independent of PC amount. In contrast, in choline/methionine-deficient hepatocytes, insufficient PC made by PEMT and CDP-choline pathways caused decreased secretion of both apo-B100 and apo-B48 [40]. Hepatocytes obtained from choline-deficient rats showed impaired secretion of VLDL mediated by low PC synthesis. This disturbance was corrected by the addition of either choline or methionine to the medium as sources for methyl groups [40]. In a further study on choline-deficient animals, analysis of liver phospholipid levels showed that PC/PE ratios had been significantly disrupted in the ER and the Golgi of liver cells in these animals, resulting in abnormal VLDL particles [41]. Choline deficiency in this case results in inhibition of the CDP-choline pathway that is responsible for PC production in the liver. Therefore, available experimental studies agree that a shortage in methyl donors (choline and methionine) is causally related to lipid accumulation in liver cells. The involvement of PC is probable, but some studies did not support this role.

In a study on CBS−/− mice, the levels of choline containing phospholipids were lowered by 68% in mice liver compared to the levels in wild-type mice [13]. In the same study, CBS−/− mice had reduced liver apo-B100 and higher serum VLDL, suggesting that HHCY impairs VLDL hydrolysis rather than enhancing liver synthesis of VLDL [13]. Data obtained in another model of HHCY (CBS+/−, HHCY diet) [11] does not agree with the CBS−/− mice model mentioned above [13]. Increased hepatic VLDL triglyceride secretion rates have been reported in CBS+/− mice fed Hcy-inducing diet [11]. Future studies might test the suggestion that Hcy-induced ER stress causes dysregulation of the endogenous sterol response pathway, leading to increased hepatic biosynthesis and uptake of cholesterol and triglycerides. Such a mechanism might explain the development and progression of hepatic steatosis and possibly atherosclerosis [11]. In addition, because phospholipids have a major role in membrane fluidity and molecule internalization, studies should investigate whether increased PE/PC ratio might cause disturbance in VLDL homeostasis in the liver.

Taken together, impaired methylation of PE to PC can affect VLDL composition and secretion from the liver [40]. Methyl donors, such as choline, methionine, or SAM might interchangeably ameliorate these disorders.

8. Hcy and HDL-cholesterol

Most mechanistic studies relating HHCY to disturbed HDL-cholesterol metabolism or assembly are derived from animal experiments. CBS−/− mice have lower HDL-cholesterol, higher serum apo-B100, lower hepatic apo-B100, lower hepatic LCAT activity, and higher free cholesterol [13]. Moreover, a reduction in HDL-cholesterol has been reported in a double knock-out mice [CBS−/−/apoE−/−] compared to [CBS+/+/apoE−/−] mice (HDL; 19.9 vs 24.4 mg/dl) (Hcy; 210 vs 3.8 μM) [14]. Similarly, a different model of HHCY, MTHFR+/− mice, developed atherosclerosis and had low liver and plasma apo-AI, the protein content of the HDL particles [12,42]. The mechanism by which Hcy can reduce HDL-cholesterol is not entirely clear.

Several studies have tested the effect of Hcy on protein (mainly apo-AI) and lipid contents of HDL particles. Lowered HDL-cholesterol can be related to accelerated catabolism or lower production of HDL molecule. A recent study found that elevated concentrations of Hcy in vivo and in vitro enhanced the expression and the protein level of adiponectin in the epididymal fat pad [43]. Betaine supplemented mice showed increased adiponectin, however no data about HDL-cholesterol was available [43]. Therefore, enhanced adiponectin production might be one explanation for accelerated catabolism of HDL-cholesterol in metabolic syndrome [44], but this has to be further tested.

The hypothesis that HHCY might reduce the production of HDL has been tested in mice fed a methionine rich diet for 7 weeks [45].

The authors suggested mechanisms related to down regulation of key players in HDL production (apo-AI, LCAT, ABCA1) [45]. In line with this, HHCY in mice was associated with a decreased activity of hepatic thiolase and serum LCAT [13], two main enzymes involved in HDL-metabolism. Another line of evidence suggested that Hcy reduces the liver expression of apo-AI and thereby reduces levels of plasma apo-AI and HDL-cholesterol. Accordingly, Liao et al. found that liver apo-AI protein, but not apo-AI mRNA, was low in CBS−/−/apoE−/− mice [4] suggesting an effect on post translational level. In addition, the authors found that Hcy reduced the rate of plasma cholesterol esterification catalyzed by the enzyme LCAT and increased clearance of HDL-cholesteryl esters from plasma [4]. These results were confirmed by Mikael et al., who also found that Hcy reduces the synthesis of apo-AI [12]. Nevertheless, apo-AI reduction in this study was a result of a decrease in apo-AI mRNA [12]. Hcy may decrease apo-AI expression by lowering the transcription factor, peroxisome proliferator receptor alpha (PPAR), that participates in apo-AI transcription [12]. This mechanism seems plausible since fibrates (PPARα agonists) have been found to regulate the expression of apo-AI thus increasing HDL-cholesterol [46]. Accordingly, ciprofibrate has been shown to protect from endothelial dysfunction in HHCY mice [47] probably by mechanisms related to enhancing apo-AI production and HDL particles assembly. Another interesting finding is that PPARα infected HepG2 cells expressed more MTHFR than non-infected cells. Moreover, increasing concentrations of Hcy (0–10 mM) caused decreased expression of PPARα in HepG2 cells [12]. These results have also suggested that higher MTHFR activity in humans would be associated with a higher apo-AI level mediated via lower Hcy and thereby higher PPARα level.

In addition to reducing apo-AI expression, low PPARα is also responsible for increased expression of cholesterol 7α-hydroxylase (CYP7A1), a liver specific enzyme that mediates the 7α-hydroxylation of cholesterol, the rate limiting step in cholesterol conversion into bile acids [48]. Bile acids enhance cholesterol absorption in the intestine. The expression of PPARα might be lowered in HHCY subjects [49] thus probably causing increased CYP7A1 and enhanced intestinal cholesterol absorption. Recent studies observed that the mRNA and the protein levels of CYP7A1 are increased in MTHFR+/− mice compared to wild-type mice [12]. Collectively, available results suggest that in HHCY, a decreased PPARα and an increased CYP7A1 expression can cause enhanced cholesterol absorption in the intestine as a result of increased production of bile acids in the liver.

Paraoxonase-1 (PON1) is a serum HDL-associated phosphotriesterase secreted mainly by the liver and shows a Hcy thiolactonase activity [50] thus protecting from atherosclerosis [51]. Known substrates for PON1 are oxidized phospholipids in HDL and LDL [52], homocysteine thiolactone [53], and platelet-activating factor [54]. Human studies showed that PON1 plays a role in the physiological prevention of cardiovascular disease [55]. Therefore, increased risk for atherosclerosis and dysfunctional HDL particles in HHCY subjects might be related to low activity of PON1 [56].

Extrapolation of animal studies to humans deserves further investigations. For example, there has been no association between MTHFR C677T polymorphism and apo-AI level in a population with a normal folate status [12]. A decreased MTHFR activity in TT subjects can be compensated by sufficient folate. Additionally, experimental studies utilized supra-physiological concentrations of Hcy, but the implication of the mild to moderate elevation of Hcy remains unclear. Obviously, more studies should be conducted to confirm these findings in humans. Future studies should also test whether DNA methylation is involved in altering gene expression of enzymes participating in HDL-metabolism or assembly under HHCY conditions.

Taken together, HHCY is associated with lowered HDL-cholesterol. This association is particularly important in patients with diabetes or those with metabolic syndrome. The effect of Hcy on HDL-cholesterol is probably related to inhibiting several enzymes or steps participating in the assembly of HDL particles.

9. Other molecular mechanisms linking HHCY and dyslipedemia

The sterol regulatory element-binding protein (SREBP-1) is an ER-membrane bound transcription factor that activates genes encoding key enzymes in the cholesterol/triglyceride biosynthesis and uptake pathways [57]. Hcy has been found to enhance the expression of SREBPs and in this way it can enhance intracellular accumulation of cholesterol [11]. Hcy causes protein misfolding in the ER and activates the unfolded protein response (UPR) thus causing increased expression of ER stress-response genes [11,58]. An association between UPR activation and lipid biosynthesis has been demonstrated in yeast [59] and human fibroblasts [60].

Hcy can activate UPR and increase the expression of the SREBP-1 in human endothelial cells [11,58], in cultured human hepatocytes as well as in aortic smooth muscle cells [11]. Another study demonstrated that diet-induced HHCY causes SREBP-1 activation in the livers of apoE−/− mice [11] and alcohol-fed mice [61]. The effect of Hcy on SREBP activation is related to an increased cleavage under HHCY [11,62] and might be corrected by betaine, a Hcy-lowering agent [61]. The observation that diet-induced HHCY did not affect plasma lipid levels [34] might not exclude that the endothelial system might be differently affected by HHCY leading to tissue lipid accumulation.

Global DNA-hypomethylation has been suggested as a mechanism linking Hcy to atherosclerosis in vascular smooth muscle cells [63]. Increased concentration of SAH has been related to the atherosclerotic lesions in apoE−/− mice independent on Hcy levels [64]. Concentration of SAH showed a positive correlation to the area of the aortic disease in apoE−/− mice fed a methionine rich diet. Moreover, SAH levels were negatively related to global DNA methylation in the aorta of these animals [64]. The methylation of genes encoding functional proteins necessary for lipid metabolism has been tested, and was found to be related to the methylation potential (SAH/SAM ratio). For example, Fads2 is the gene encoding Delta6-desaturase, the enzyme that is involved in the elongation and desaturation of linolenic acid and linolic acid. Fads2 gene has been found to be sensitive to methylation status [37].

Oxidative stress is one mechanism by which Hcy might affect lipoprotein particles and thereby damage endothelial cells [65]. Hcy can enhance hydroxyl radical generation and formation of oxidized- [65], nitrated- [66], or homocysteinylated-LDL [67]. The modified forms of LDL are more toxic than the native LDL and are readily taken up by macrophages thus facilitating the initiation and progression of the inflammatory response in the endothelial lesions. Apo E−/− mice treated with Hcy showed increased oxidized-LDL [65]. This might enhance LDL uptake by scavenger receptors in the aortic lesions or on monocyte or macrophages and thus promoting atherosclerosis. Moreover, increased reactive oxygen species under HHCY can stimulate components of the signal transduction thus causing endothelial dysfunction [68]. Some evidence has suggested that the pro-oxidant effect of Hcy involves endothelial nitric oxide synthase (eNOS) [69]. In one study, in vivo supplementation of folate reduced plasma Hcy and protected LDL and VLDL particles from oxidation [70]. The antioxidant effect of folate can be related to Hcy-lowering or can indicate an independent antioxidant effect of the vitamin, since a better effect for 5-methyltetrahydrofolate compared to folic acid has been found [70].

10. Cholesterol-lowering treatment and plasma Hcy

There is some evidence suggesting that the risk associated with HHCY and hypercholesterolemia is higher than the risk associated with one of these risk factors alone [1]. Therefore, it is conceivable to think about using cholesterol-lowering treatment in combination with B-vitamins for the management of at risk patients. In the FIELD study that included more than 9000 coronary patients with type 2 diabetes, fenofibrate caused only 11% reduction in coronary events at the end of the study [5] but levels of Hcy increased by approximately 35% (from 11.2 to 15.1 μmol/L). This might explain why the risk reduction was much less than expected in the study.

In one study on men with mild hypercholesterolemia (mean age 35 years), the effect of pravastatin (40 mg/day) on plasma concentration of Hcy was tested in a randomized, double-blind, placebo-controlled study [71]. The authors found a significant reduction of Hcy after 6-months [71]. Atorvastatin (10 mg/day) induced no significant changes in Hcy after 6 months in patients with primary hypercholesterolemia [72]. In a recent review, Dierkes et al. concluded that HMG-CoA reductase inhibitors (statins) seem not to influence Hcy, fibric acid derivatives seem to increase Hcy, and the effect of nicotinic acid and *n*3-fatty acids are not conclusive [73]. Nevertheless, lipid-lowering drugs (fibric acid derivatives) might have an effect on Hcy independent on their lipid-lowering effect. To resolve this issue, it is important to test whether lowering cholesterol by using a diet with standard energy and low cholesterol contents might reduce Hcy concentrations.

A very interesting aspect of HMG-CoA reductase inhibitors is that this treatment reduces cellular isoprenoids and induces synthesis of phosphatidylcholine [74,75]. Statins may also impact membrane phosphatidylserine that is synthesized from a choline/serine exchange reaction. A recent study found that statins induce drastic changes to the cellular pools of phosphatidylserine, sphingomyelin and ceramid and limits the exposure of phosphatidylserine at the cell surface by restricting the cellular pool of mevalonate and thereby the isoprenoids [76]. Further studies should test whether statins modify SAM levels via increasing PC synthesis that is a major SAM-consuming-pathway.

Therefore, available clinical studies using different lipid-lowering drugs could not confirm a unique effect on Hcy. However, it seems prudent to test for HHCY in patients receiving fibric acid derivatives and to then ensure sufficient status of B-vitamins.

11. Effect of Hcy-lowering treatment on blood lipids

11.1. Folate, vitamin B12, vitamin B6

The hypothesis that Hcy metabolism can affect blood lipids gained further attention in the last few years. Therefore, one might speculate that lowering Hcy by means of the B-vitamins can affect blood lipids. However, there are no well-designed studies that aimed at investigating the effect of vitamin B12, B6, and/or folic acid on blood lipids.

In a 6-week trial, Olthof et al. tested the effect of folic acid (0.8 mg/day) on blood lipids in healthy subjects with Hcy levels below 26 μmol/L [77]. Folic acid supplementation lowered Hcy by approximately 21%. Changes of concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were not significant at the end of the treatment [77]. Another study aimed at investigating the effect of oral folic acid (5 mg/day) for 4 weeks on endothelial function in patients with familial hypercholesterolemia [78]. Supplementation of folic acid improved endothelial function without significant changes in plasma lipids [78].

Few studies were conducted in end-stage renal disease where an elevated plasma concentration of Hcy and dyslipidemia are very common and are recognized risk factors for arteriosclerosis in this group of patients. In a 3-months placebo-controlled study on dialysis patients, folic acid supplementation (5 mg/day) caused Hcy-lowering by 33% [79]. Additionally, levels of total cholesterol, LDL-cholesterol, and triglyceride decreased significantly in patients on peritoneal dialysis but not in those on haemodialysis [79]. In a study on 12 haemodialysis patients, Hcy-lowering therapy using folic acid (5 mg) and vitamin B6 (250 mg) for 26 months caused lowering of LDL-cholesterol, increased HDL-cholesterol, and a slight reduction in total cholesterol and triglycerides [80].

In most of the above mentioned studies, the duration of the vitamin trials was short and probably not sufficient to show an effect [77]. Trials with longer durations were more effective [80], but this has to be confirmed in larger scales. Moreover, most studies included only a small number of patients which might be insufficient for detecting an effect. In addition, most studies excluded patients with HHCY, which might also impact the expected influence of lowering Hcy [77].

11.2. Betaine

Betaine is formed by choline oxidation via choline dehydrogenase in the kidney and the liver [81,82]. Betaine is a methyl donor in the remethylation of Hcy into methionine via BHMT [83]. Accordingly, plasma concentrations of Hcy are negatively related to that of betaine [84], and betaine supplementation can reduce plasma Hcy [77]. In contrast, inhibition of hepatic BHMT in mice by 60–90% by repeated injection of S-(δ-carboxylbutyl)-DL-homocysteine for 1–8 h caused increase in plasma Hcy by 2.7–8-fold and a 65% reduction in SAM/SAH ratio compared to mice given a saline injection [85].

The role of betaine supplementation in lipid metabolism has been investigated in few clinical studies. Betaine seems to be inversely correlated to several components of the metabolic syndrome thus protecting against coronary vascular risk [86]. Plasma betaine was positively associated with HDL-cholesterol and inversely associated with serum triglycerides and non-HDL-cholesterol [86]. Furthermore, in subjects attending a lipid clinic, betaine was inversely related to apo-B and body fat [87]. These results are not consistent however, with those from supplementation studies. Doses of 4–6 g/d betaine raised plasma total cholesterol, and LDL-cholesterol, and did not affect HDL-cholesterol in healthy people [77]. This effect was already evident 2-weeks after starting the supplementation. Therefore, a dual effect for betaine seems to add more complexity to the role of this compound in lipid metabolism.

Animal studies shed more light on the dual effect of betaine. Betaine supplementation improved the atherogenic lipid profile in HHCY mice [42]. In MTHFR+/− and MTHFR+/+ mice, betaine supplementation (450 mg/kg/ day) caused increased apo-AI expression in the liver of supplemented compared to non-supplemented mice [42]. Additionally, mice receiving betaine had lower plasma triglycerides and higher HDL-cholesterol compared to the non-supplemented ones. Moreover, stimulation of BHMT by a methionine restricted, betaine enriched diet caused increased secretion of VLDL and a 45% reduction in liver triglycerides [88]. Increased apo-B mRNA, VLDL apo-B protein content, and triglycerides production, in addition to decreased hepatic triglycerides may suggest that stimulation of BHMT might mobilize hepatic triglycerides by increasing apo-B available for VLDL turn-over [88]. These results support the role of BHMT in lipid metabolism in addition to Hcy metabolism. However, this effect seems to be strictly related to the presence of other methyl donors like methionine in the diet. Betaine supplement in combination with methionine sufficient diet seems to enhance the anti-atherogenic lipid profile.

Betaine supplement in combination with a methionine poor diet causes a negative effect on lipid profile probably related to ineffective SAM production by betaine alone.

Betaine has been often tested in studies on alcoholic liver disease. Animals fed with alcohol develop fatty liver infiltration. Betaine administration (0.5% for 2–4 weeks) doubled the hepatic levels of SAM in control animals and increased the levels of hepatic SAM by 4-fold in the ethanol-fed rats [89–91]. The ethanol-induced infiltration of triglycerides in the liver was also reduced by the feeding of betaine to the ethanol-fed animals. Unlike SAM and betaine, folate treatment was not able to restore the diseased liver in alcoholism [92]. These findings support that betaine can enhance the activity of liver BHMT and provides more SAM for liver methyltransferases such as PEMT. Betaine administration has the capacity to elevate hepatic SAM and to prevent the ethanol-induced fatty liver [90]; probably by normalizing PC synthesis and thereby VLDL homeostasis.

The effect of betaine on blood lipids could be related to increase in synthesis of SAM and, eventually, glutathione, decreasing the hepatic concentrations of Hcy and SAH, and increasing the SAM/SAH ratio. These metabolic conditions lead to the activation of PEMT, increased PC synthesis, and formation of VLDL for the export of triglycerides from the liver to the circulation [92].

Taken together, evidence on the role of betaine in lipid metabolism is currently not sufficient to recommend supplementing this compound. Observational studies suggested a favorable effect, but treatment studies are not conclusive. One factor that should be considered is the dose and the duration of betaine treatment, in addition to other sources of methyl groups in the diet. Short term variations of blood lipids after betaine treatment (2 weeks) might be related to redistribution of lipids between blood and liver. Another factor that might have contributed to variations between the studies is the analytical methods used for quantifying concentrations of betaine and choline.

11.3. Choline

Choline is an important nutrient and a source of methyl groups in the diet. Choline is obtained either from diet or from the catabolism of PC. The most abundant choline derivative, phosphatidylcholine, is formed from PE in hepatocytes via PEMT (Fig. 1). Choline is a precursor for acetylcholine, membrane phospholipids, and lipoproteins thereby serving essential biological functions. Hepatic PC is required for VLDL secretion and constitutes an important component of bile [93]. Choline can also be converted into betaine by means of a mitochondrial enzyme, choline dehydrogenase thus it is considered the metabolic precursor of betaine [94]. Because betaine is a methyl donor for Hcy remethylation via BHMT, it is not surprising that choline, the precursor of betaine, is negatively related to Hcy concentration [77,86].

Choline and folate statuses seem to interrelate. On the one hand, a choline deficient diet for 2 weeks caused 30–40% lower hepatic folate in rats [95]. This was reversed after feeding the animals with choline [95]. Additionally, a combined deficiency of choline and methionine for 5 weeks caused 50% lower hepatic folate [96]. Animal studies have also shown that, choline deficiency caused 50% lower liver SAM [97,98], fatty liver [99], and reduced liver glutathione [100], the most important antioxidant in animal cells. On the other hand, folate deficiency [101] or antifolate treatment (methotrexate) [95,97] caused lowered hepatic choline, especially PC. These results suggest that a greater amount of folate might be required for maintaining essential methylation reactions in the liver when dietary choline is not sufficient. Liver folate can be used for generating SAM and thus enhancing PC synthesis in choline deficient cases. Alternatively, folate deficiency might increase the requirement for choline as a methyl donor.

Choline deficiency interferes also with lipid metabolism and causes non-alcoholic fatty liver [40,102] thus reflecting the role of choline in lipid homeostasis. In accordance with this, a diet deficient in choline caused triglycerides accumulation in the liver of mice fed a low or a high-fat diet [103]. Choline deficiency in rats promoted accumulation of triglycerides in the liver (6.5-fold) and reduction of triglycerides concentration in plasma by 60% [104]. Plasma VLDL levels were reduced in choline-deficient rats, but the concentration of plasma HDL-cholesterol was not affected [104]. A study in humans [105] showed that choline deprived diet (<50 mg/day) for 42 days did not affect serum concentrations of the lipids (total-, LDL-, HDL-cholesterol) or plasma SAM and SAH, but caused fatty liver or muscular damage in the majority of men and post-menopausal women included in the study [105]. The study did not show the response of serum concentration of triglycerides to choline supplementation. These results collectively indicate that choline deficiency causes lower turn-over of hepatic lipids, retention of lipids in the liver, reduced secretion of VLDL from the liver and thereby, lower plasma triglycerides.

Choline administered as phosphatidylcholine is a Hcy-lowering agent [106]. In a study on healthy men, supplementation of phosphatidylcholine equivalent to 2.6 g/day choline for 2 weeks lowered fasting plasma Hcy by 18% and post-methionine-load Hcy by 29% compared to the placebo [77]. In addition, plasma concentrations of choline were positively associated with serum concentrations of triglycerides in a study that included over 7000 middle age and elderly men and women [86]. Moreover, plasma level of choline showed an inverse relation with HDL-cholesterol [86]. This association has been confirmed by showing the effect of choline supplementation on blood lipids. In one study, phosphatidylcholine supplementation increased blood triglycerides concentration without changing concentrations of total-, LDL- and HDL-cholesterol [77]. The observed fatty liver could suggest that choline deficiency can cause reduced PC and thus reduced secretion of VLDL from the liver. In several other studies, lecithin, a choline containing phospholipid, has been used for its cholesterol-lowering effect that is probably related to enhancing the activity of LCAT, the enzyme that removes cholesterol from the tissues [107]. The hypothesis is, choline supplementation can increase PC, thus increasing VLDL turn-over and thereby plasma triglycerides, while increasing HDL-cholesterol because of enhancing LCAT.

In a recent study, the effects of betaine (3 g/d), choline (2 g), and lecithin (2 g) on Hcy cycle metabolites were tested in children with cystic fibrosis [108]. Supplementation with choline or betaine decreased SAH, increased SAM, and glutathione. Supplementation with choline or lecithin increased plasma methionine and SAM [108]. An association between betaine and choline and component of the metabolic syndrome was reported, with only betaine being associated with a better risk profile [86]. However, supplementation studies of both micronutrients are not conclusive. We anticipate that the ratio of choline/betaine could be a meaningful marker in whether one of these micronutrients can shift the risk profile in one direction. This index could be related to the activity of choline dehydrogenase. A higher choline dehydrogenase activity will produce more betaine (lower choline/betaine ratio), increase SAM, reduces Hcy and probably also blood lipids. A low choline dehydrogenase activity will cause a higher choline/betaine ratio, less SAM and probably will drive the choline into PC and production of VLDL.

Obviously, more studies should be conducted on this issue testing for all sources of methyl group donors (folate, methionine, betaine, and choline). Future studies should investigate the effect of one methyl donor on Hcy and plasma phospholipids, keeping the other donors stable. There is currently no recommendation for using choline as cardiovascular protector, before results from such studies are available.

In conclusion, Hcy and lipid metabolism are interrelated at least partly via methyl group metabolism. Strong evidence suggested that deficiency of methyl group donors and HHCY disturb phospholipid metabolism thus affecting the assembly or secretion of VLDL from the liver. Another line of evidence suggested a direct role for Hcy in inducing ER stress or regulating enzymes responsible for HDL-cholesterol metabolism. A very interesting aspect to be tested in future studies is enhancing lipid metabolism via reducing Hcy and increasing the endogenous production of SAM.

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