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ADIPOSE TUSSUE AND HOMOCYSTEINE METABOLISM

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Homocysteine (Hcy) is a non-protein aminoacid which is an intermediate of methionine metabolism. Elevated homocysteine level (hyperhomocysteinemia) is a risk factor of cardiovascular diseases. In addition, obesity and metabolic syndrome are associated with greater prevalence of atherosclerosis. The purpose of this article is to discuss the relationship between adipose tissue and Hcy metabolism. All enzymes involved in the synthesis and metabolism of Hcy are expressed in adipose tissue, and recent studies suggest that adipose tissue may be an important source of circulating homocysteine. The effect of obesity on Hcy level is controversial, however, most experimental and clinical studies indicate that Hcy is elevated in the metabolic syndrome in the absence of diabetes. Homocysteine elevation in the metabolic syndrome may result from either hyperinsulinemia or the impairment of renal function. In contrast, plasma Hcy is reduced in both type 1 and type 2 diabetes if renal function is normal, but becomes elevated when diabetic nephropathy develops. Leptin, which is markedly elevated in obese patients, has no effect on total plasma Hcy, but increases the level of homocysteine thiolactone – a cyclic thioester of homocysteine which plays an important role in complications of hyperhomocysteinemia. The effect of leptin is accounted for by the inhibition of paraoxonase 1 (PON1) – the HDL-associated esterase, which hydrolyzes homocysteine thiolactone to Hcy. In addition, recent studies indicate that homocysteine may induce insulin resistance in adipose tissue by promoting endoplasmic reticulum stress and/or disrupting adipokine production; e.g. enhancing resistin and suppressing adiponectin. ***Biomed Rev 2009; 20: 7-15.***

Key words: obesity, metabolic syndrome, leptin, adipokines, hyperhomocysteinemia, insulin resistance

INTRODUCTION

Homocysteine (Hcy) is a non-protein sulfur-containing aminoacid (Fig. 1). Homocysteine is derived from the metabolism of methionine – the essential aminoacid which must be provided in the diet (Fig. 2). First, adenosine is transferred by methionine adenosyltransferase (MAT, SAM synthetase) from ATP to methionine to form S-adenosylmethionine (SAM). SAM is the universal donor of methyl groups for methylation reactions catalyzed by various methyltransferases. When $-CH_3$ group is transferred by methyltransferases to the respective acceptor, SAM is converted to S-adenosylhomocysteine (SAH) that is subsequently hydrolyzed by SAH hydrolase to adenosine and homocysteine (1). Homocysteine is metabolized in two pathways referred to as remethylation and transsulfuration. Remethylation of Hcy to methionine is mainly catalyzed by methionine synthase which uses 5-meth-

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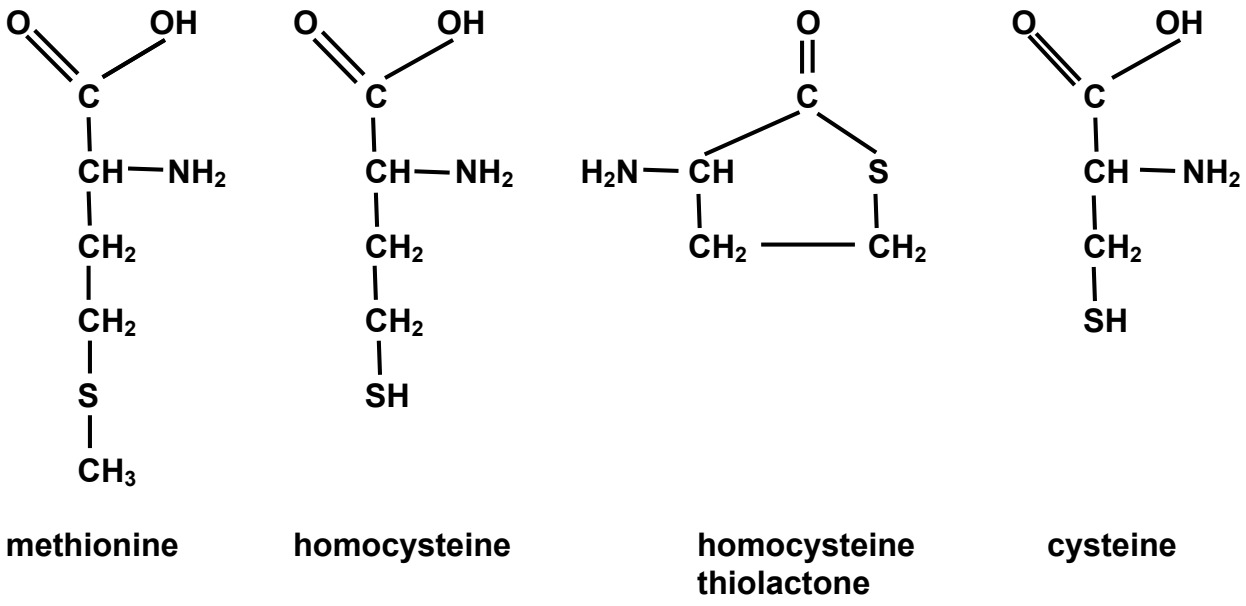

Figure 1. *Structure of sulfur-containing aminoacids.*

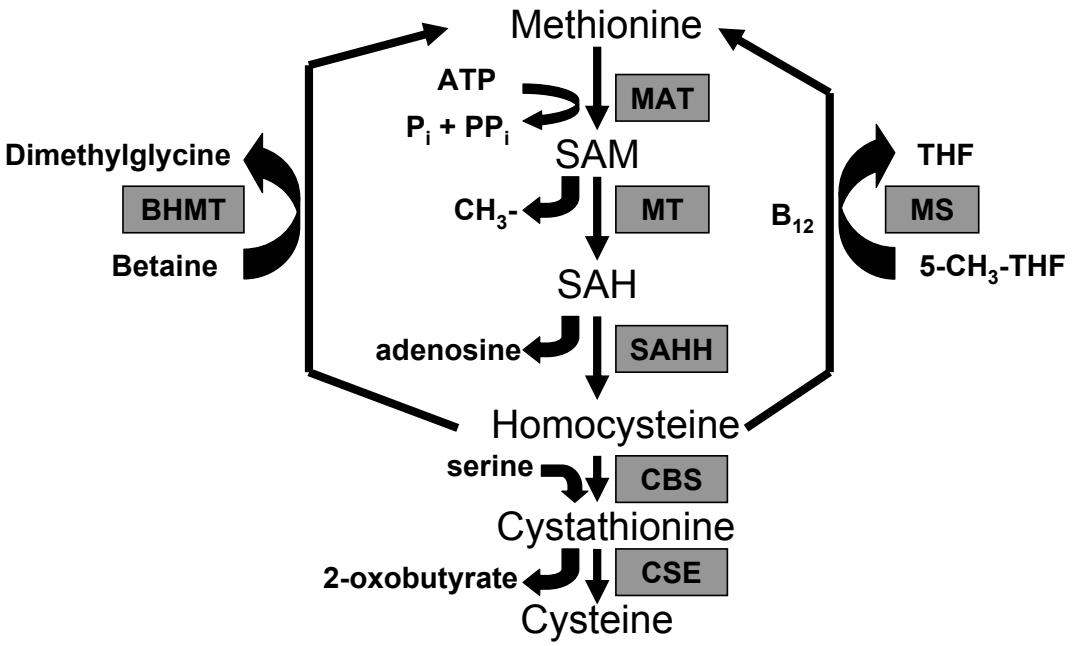

Figure 2. *Pathways of homocysteine synthesis and metabolism. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; P_i, inorganic phosphate; PP_i, inorganic pyrophosphate; THF, tetrahydrofolate; 5-CH_3-THF, 5-methyltetrahydrofolate; MAT, methionine adenosyltransferase; MT, methyltransferases; SAHH, SAH hydrolase; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; MS, methionine synthase; BHMT, betaine:homocysteine methyltransferase.*

yltetrahydrofolate and vitamin B_{12} as cofactors. Alternatively, some Hcy may be remethylated by betaine:homocysteine methyltransferase (BHMT) which uses betaine as a methyl donor. In the transsulfuration pathway, Hcy condenses with serine to form cystathionine in the reaction catalyzed by cystathionine β-synthase (CBS). Cystathionine is then converted to cysteine and 2-oxobutyrate by cystathionine γ-lyase (CSE) (2). Recently, it has been demonstrated that homocysteine may also be the substrate for CSE (3). Indeed, CSE may condense two molecules of homocysteine to form homolanthionine (Hcy-S-Hcy) and hydrogen sulfide (H_2S) – a novel physiologically relevant gasotransmitter (4). Both CBS and CSE use pyridoxal 5-phosphate (an active form of vitamin B_6) as a cofactor.

Homocysteine circulates in the blood at a concentration of ~10 μM. In plasma, about 2/3 of total Hcy is bound to protein cysteine residues by disulfide bridges (Hcy-protein disulfides) and most of the remaining 1/3 circulates in oxidized form as low-molecular weight disulfides, homocystine (Hcy-S-S-Hcy) or Hcy-S-S-cysteine. Only about 1% of total Hcy circulates in free reduced form (1, 2).

It was noted in 1960s that the risk of atherosclerosis is markedly increased in patients with homocystinuria – the inherited disease resulting from homozygous CBS deficiency which is characterized by episodes of thromboembolism, mental retardation, lens dislocation, hepatic steatosis and osteoporosis. In these patients, plasma Hcy usually exceeds 100 μM. Much more frequent (5-10% of the general population) is mild to moderate hyperhomocysteinemia (plasma Hcy 15-40 μM), which also increases the risk of atherosclerosis. The most common causes of hyperhomocysteinemia include heterozygous CBS deficiency, thermolabile variant of methylenetetrahydrofolate reductase (MTHFR) resulting from C677T polymorphism of *MTHFR* gene, deficiency of vitamins involved in Hcy metabolism (folate, B_6 and B_{12}), renal impairment and high methionine intake (5). Apart from atherosclerosis, hyperhomocysteinemia is also associated with several other health burdens including Alzheimer's disease and other forms of dementia, osteoporosis, cancer, as well as complications of pregnancy such as intrauterine growth restriction, preeclampsia, miscarriages and neural tube defects in the neonate.

Metabolic syndrome (MS) is a cluster of pathologies which directly or indirectly result from obesity. Several components of the metabolic syndrome such as insulin resistance and compensatory hyperinsulinemia, hypertriglyceridemia, low HDL-cholesterol, arterial hypertension, proinflammatory and prothrombotic state, impaired glucose tolerance or type 2 diabetes are independent risk factors of atherosclerosis. In addition, MS is associated with the dysregulation of adipose tissue-derived signaling proteins collectively termed adipokines (6). Some of adipokines such as leptin, resistin and visfatin promote atherosclerosis, whereas adiponectin, interleukin-10, nerve growth factor and brain-derived neurotrophic factor are atheroprotective mediators (6). Due to high prevalence of both obesity/MS and hyperhomocysteinemia, the relationship between adipose tissue and homocysteine metabolism is of great significance.

The purpose of this review is to summarize the current knowledge about role of adipose tissue in homocysteine metabolism, the effect of obesity/MS on homocysteine, and the effect of hyperhomocysteinemia on adipose tissue.

ADIPOSE TISSUE AS A SITE OF HOMOCYSTEINE SYNTHESIS AND METABOLISM

Methylation reactions are essential for the metabolism of lipids, proteins and nucleic acids, and methionine-to-homocysteine pathway (Fig. 2) is operative in all tissues including the adipose tissue. Recent studies (7, 8) indicate that histone methylation is essential for the expression of two transcription factors crucial for adipogenesis, peroxisome proliferator-activated receptor-γ (PPAR-γ) and CCAT enhancer binding protein-α (C/EBP-α). Adipose tissue also contains protein methyltransferases involved in formation of asymmetric dimethylarginine (ADMA) – an endogenous nitric oxide synthase inhibitor – from its precursor, L-arginine (9), and phospholipid methyltransferases (10). Recently, Riederer *et al.* (11) have demonstrated that nicotinamide N-methyltransferase (NNMT), which methylates nicotinamide and nicotinic acid (vitamin B_3) is expressed in adipocytes. Interestingly, the expression of NNMT was higher in white adipose tissue than in the liver which was previously considered as the main NNMT-containing organ. NNMT is also expressed, although at lower level, in brown adipose tissue. NNMT expression and activity markedly increased during the differentiation of preadipocytes to mature fat cells. In addition, homocysteine was produced and secreted to the medium by 3T3-L1 preadipocytes and its release increased six-fold during their differentiation to adipocytes (11). Chronic treatment with nicotinic acid increased NNMT expression and activity in murine adipose tissue but not in the liver. Obesity induced by high-fat diet also increased NNMT in murine adipose tis-

sue. Moreover, nicotinamide increased, whereas NNMT inhibitor decreased homocysteine production in adipose tissue by about 50%, indicating that NNMT is an important source of homocysteine in adipocytes (11). These data indicate that adipose tissue may be an important source of circulating homocysteine.

It is unclear if remethylation pathway is operative in adipocytes. However, both CBS and CSE are expressed and active in perirenal, epididymal and perivascular white adipose tissue, as well as in brown adipose tissue (12, 13).

EFFECT OF OBESITY/METABOLIC SYNDROME ON PLASMA HOMOCYSTEINE LEVEL

The impact of obesity/metabolic syndrome on homocysteine metabolism is controversial and most likely reflects the complex, sometimes oppose, effects of various MS components. Fonseca *et al* (14) demonstrated that experimental obesity induced by high fat and high sucrose feeding resulted in almost 50% elevation of plasma homocysteine in the rat. In this model, plasma glucose was normal but hyperinsulinemia and insulin resistance were observed. Hepatic CBS expression and activity were reduced and negatively correlated with both plasma insulin and homocysteine. In contrast, hepatic MTHFR activity was higher in obese animals, indicating that remethylation pathways is intact or even improved and that reduced transsulfuration is responsible for hyperhomocysteinemia. Similarly, metabolic syndrome induced by high-fructose feeding, which is associated with severe insulin resistance, hyperinsulinemia, hypertriglyceridemia and arterial hypertension but not hyperglycemia or obesity, resulted in 70% elevation of plasma homocysteine in the rat (15). In addition, homocysteine level positively correlated with plasma insulin and triglycerides. Hyperhomocysteinemia in these animal models may be associated with high insulin level. Indeed, insulin reduces CBS activity in the liver (16, 17). Streptozotocin-induced diabetes – the state of insulin deficiency – is characterized by high CBS and CSE activities in the liver and low plasma homocysteine level, and insulin supplementation corrects these abnormalities (18).

In contrast to these studies, plasma homocysteine was normal in Zucker fatty rats – an experimental model of obesity and insulin resistance associated with the mutation of the leptin receptor (19). However, a peroxisome proliferator activated receptor gamma (PPAR-γ) agonist, troglitazone, which increases insulin sensitivity and reduces fasting insulin level, decreased plasma Hcy and increased CBS activity in the liver of obese rats (19). Wijekoon *et al.* (20) found that plasma Hcy was 25% lower in Zucker diabetic fatty (ZDF) rats at 5th week of age when these animals are insulin resistant and hyperinsulinemic but still nonrmoglycemic. In that study, increase in the activities of several enzymes involved in Hcy metabolism including CBS, CSE, MTHFR and BHMT in the liver was observed (20). In contrast, methionine synthase activity was slightly but significantly reduced in these animals. However, in parallel with increased metabolism, Hcy formation might also be enhanced since MAT activity was greater in ZDF than in control lean rats (20). Furthermore, insulin sensitizer, rosiglitazone, has been show to reduce Hcy level in animal models of insulin resistance or hyperhomocysteinemia (21, 22).

Data about the relationship between metabolic syndrome and homocysteine in humans are also controversial. Homocysteine was positively correlated with body mass index (BMI), absolute fat mass, percentage of fat mass, and fasting insulin level in obese children and adolescents (23). In the Framingham Offspring Study including 2011 nondiabetic subjects, plasma homocysteine was higher in those with hyper- than in those with normoinsulinemia (24). Sanchez-Margalet *et al.* (25) observed that plasma Hcy was higher in hyperinsulinemic than in normoinsulinemic obese non-diabetic males, although folate and vitamin B_{12} levels were not different between groups. In addition, fasting homocysteine significantly correlated with fasting insulin in these patients (25). An independent association between insulin resistance and plasma Hcy was also observed in healthy non-obese subjects (26). De Pergola *et al.* (27) compared Hcy levels in apparently healthy normal weight, overweight and obese premenopausal women, and found gradual increase in Hcy from normal weight to obese group. However, in multivariate analysis only insulin resistance but not BMI, age or waist circumference was independently associated with Hcy level. In addition, plasma Hcy was higher in 80 obese than in 50 non-obese subjects, however, there was no correlation between Hcy and plasma insulin, BMI or waist-to-hip ratio (WHR) in either non-obese or obese group (28). Recently, Bukhari *et al.* (29) reporter that Hcy was much higher in 160 obese than in 160 lean patients in the Pakistani population. Positive association between Hcy and insulin levels was also observed in insulin-resistant women with polycystic ovarian syndrome (30) and preeclampsia (31).

In contrast, Abbasi *et al.* (32) observed no relationship between insulin-induced glucose disposal and Hcy in healthy

volunteers. Similarly, Gómez-Ambrosi *et al.* (33) observed no difference in Hcy levels between lean and obese males. Godsland *et al.* found no relationship between plasma Hcy and insulin sensitivity in healthy men (34), whereas Rosolová *et al.* (35) found the inverse correlation between homocysteine and insulin resistance. Bar-On *et al.* (36) demonstrated a significant negative correlation between plasma Hcy and insulin in an epidemiological cross-sectional study.

These discrepancies may be accounted for by several factors. First, taking into account that insulin reduces hepatic CBS activity (16), the extent of hyperinsulinemia and insulin resistance is significant. If insulin resistance is confined to its effect on glucose disposal in peripheral tissues whereas hepatic effect of insulin is intact, hyperinsulinemia will impair Hcy metabolism in the liver. However, if the liver is insulin resistant, the inhibitory effect of insulin on hepatic CBS would be impaired, and Hcy metabolism might even be accelerated. Second, insulin has the anabolic effect on protein metabolism and may reduce intracellular methionine available for Hcy production. Third, alimentary intake of methionine, folate, vitamin B_6 and B_{12} may determine the effect of hyperphagia and obesity on Hcy level. Fourth, the kidney is one of the major organs involved in homocysteine metabolism (37). Obesity and metabolic syndrome are often associated with glomerular hyperfiltration, which may enhance renal Hcy clearance (38). On the other hand, obesity and metabolic syndrome may impair renal function even in the absence of other risk factors such as diabetes or hypertension (39). Impaired renal function is always accompanied by hyperhomocysteinemia (40). Fifth, Hcy level depends on whether diabetes is present or not in patient with the metabolic syndrome. While metabolic syndrome with normoglycemia may be associated with hyperhomocysteinemia, experimental and clinical studies consistently show that plasma Hcy is reduced in both type 1 and type 2 diabetes mellitus if there is no nephropathy (18, 41), and increases in diabetic patients only when nephropathy develops (42, 43). Finally, it cannot be excluded that insulin resistance is the consequence rather than the cause of hyperhomocysteinemia (see below).

LEPTIN, HOMOCYSTEINE THIOLACTONE AND PARAOXONASE 1

Multiple studies performed during the last decade indicate that adipokines play an important role in the pathogenesis of metabolic syndrome and its complications. We examined the effect of experimental hyperleptinemia induced in lean rats by the administration of exogenous leptin at doses, which elevate its level to the range observed in obesity. This model allows studying the effects of leptin excess isolated from other abnormalities observed in obesity. We found that leptin had no effect on total plasma homocysteine in the rat. However, leptin increased the amount of homocysteine thiolactone bound to plasma proteins (44). Homocysteine thiolactone is a cyclic thioester of homocysteine formed when Hcy is nonspecifically bound by methionyl-tRNA synthetase. Although Hcy may bind methionyl-tRNA synthetase, it is not incorporated into proteins but is converted to Hcy-thiolactone. Thiolactone is probably one of the most detrimental forms of Hcy. Indeed, Hcy-thiolactone binds to ε-NH_2 groups of protein lysine residues even at very low concentrations, whereas many other toxic effects of Hcy are observed only at high concentrations, markedly exceeding its physiologic and even pathologic levels. Homocysteinylated proteins have altered properties and impaired biological activity, and may also induce the immune response. Augmentation of protein homocysteinylation was observed in patients with hyperhomocysteinemia, renal impairment or coronary heart disease (45). Importantly, protein-bound Hcy-thiolactone is omitted by most routine methods of plasma Hcy assay, because its measurement requires prior hydrolysis of plasma proteins. Hcy-thiolactone is hydrolyzed to open-form Hcy by two enzymes: extracellular paraoxonase 1 (PON1), synthesized in the liver and circulating in high-density lipoproteins, and intracellular cysteinyl protease, bleomycin hydrolase (BLH). We found that experimental hyperleptinemia decreased plasma PON1 activity, which resulted in almost two-fold elevation of protein-bound Hcy-thiolactone despite normal total Hcy level (44). PON1 deficiency was observed in obesity/metabolic syndrome (46) and, in some studies, negative correlation between PON1 and leptin was observed in obese subjects (47). In addition, multiple experimental and clinical studies indicate that hyperleptinemia promotes atherogenesis (48). Taken together, these data indicate that leptin-induced PON1 deficiency impairs metabolism of Hcy-thiolactone, leading to the enhancement of protein homocysteinylation. This mechanism may contribute to atherogenesis in obese subjects.

EFFECT OF HYPERHOMOCYSTEINEMIA ON ADIPOSE TISSUE

Not only the amount of adipose tissue in patients with the metabolic syndrome may affect Hcy metabolism but also

vice versa, hyperhomocysteinemia affects adipose tissue function. Indeed, it has been demonstrated that Hcy and Hcy-thiolactone impair insulin signaling by inducing oxidative stress and reducing insulin receptor tyrosine kinase activity (49,50). Golbahar *et al* (51) have shown that experimental hyperhomocysteinemia induced in Sprague-Dawley rats by the administration of homocysteine in the drinking water for 50 days induced insulin resistance, as evidenced by higher fasting insulin level and HOMA index, whereas plasma glucose and body weight were not altered. Authors suggested that Hcy-induced insulin resistance could have been associated with impaired endothelial function. Hyperhomocysteinemia induces endothelial dysfunction, and impaired endothelium-dependent relaxation of the skeletal muscle microvasculature is well known to contribute to insulin resistance by impairing glucose disposal. In support of this hypothesis, lowering plasma Hcy with folate and vitamin B_{12} improved insulin sensitivity in humans (52).

Recently, Li *et al* (53) have demonstrated that hyperhomocysteinemia induced by a 4-week oral administration of homocysteine induced insulin resistance in mice. Interestingly, in that study plasma Hcy was increased less than three-fold but Hcy in adipose tissue was about seven-fold higher than in control normohomocysteinemic animals. Body weight, plasma triglycerides and HDL cholesterol were similar in normo- and hyperhomocysteinemic mice, but total cholesterol was increased in hyperhomocysteinemic animals. Moreover, hyperhomocysteinemia reduced tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1), but increased serine phosphorylation of IRS-1 in adipose tissue. Phosphorylation of IRS-1 by various serine/threonine protein kinases impairs its phosphorylation by the insulin receptor tyrosine kinase. and is a common mechanism of insulin resistance. Phosphorylation of protein kinase B/Akt, a downstream kinase of IRS-1, was also impaired in adipose tissue of hyperhomocysteinemic animals (53). These data indicate that homocysteine disrupts insulin signaling in adipose tissue. Furthermore, Hcy inhibited insulin receptor-IRS-1-Akt signaling pathway, increased serine phosphorylation of IRS-1, and reduced 2-deoxyglucose uptake by cultured rat adipocytes *in vitro* (53). Interestingly, hyperhomocysteinemia increased resistin production both *in vivo* and *in vitro*. Resistin expression in epididymal adipose tissue at both mRNA and protein level was increased in hyperhomocysteinemic mice as was plasma resistin concentration. Hyperhomocysteinemia was also associated with the up-regulation of tumor necrosis factor-α (TNF-α) and plasminogen activator inhibitor-1 (PAI-1) in adipose tissue, whereas leptin expression was reduced in homocysteine-treated mice. Furthermore, it was demonstrated that Hcy induced resistin expression in adipocytes by increasing intracellular formation of reactive oxygen species (ROS), stimulating protein kinase C (PKC), and increasing DNA-binding activity of nuclear factor-κB (NF-κB) (53). These data suggest that resistin may be involved in Hcy-induced insulin resistance, although oxidative stress, PKC and NF-κB may also directly impair insulin signaling. Song *et al.* (54) have demonstrated that Hcy decreased adiponectin synthesis in isolated rat adipocytes, and that high methionine diet-induced hyperhomocysteinemia suppressed adipose tissue adiponectin production and reduced plasma adiponectin concentration in the rat. In addition, homocysteine-induced hypoadiponectinemia resulted in the reduction of AMP-stimulated protein kinase (AMPK) which mediates beneficial effects of adiponectin on carbohydrate and lipid metabolism in the liver. Effect of Hcy on adiponectin production was mediated by the inhibition of extracellular signal-regulated kinases (ERKs), since homocysteine reduced ERK activity in adipocytes, and ERK inhibitors, U0126 and PD98059, mimicked the inhibitory effect of Hcy (54). In addition, it has been demonstrated that homocysteine induced endoplasmic reticulum (ER) stress in adipocytes, as evidenced by the induction of ER stress marker, C/EBP homologous protein (CHOP). The ER stress inducer, thapsigargin, also reduced adiponectin production. ER stress not only suppresses adiponectin, but also stimulates resistin production (55), induces adipose tissue inflammation (56), and impairs insulin signaling in adipocytes (57). Adipocyte ER stress is observed in obesity (57) and is reduced by weight loss (58). Thus, ER stress may be a common mechanism of adipocyte dysfunction in both obesity and hyperhomocysteinemia. Last not least, Hcy and folic acid are also implicated in the regulation of coronary circulation as well as the pathogenesis of coronary artery disease (59).

CONCLUSIONS

Obesity/metabolic syndrome and hyperhomocysteinemia are two common risk factors of cardiovascular disorders. Recent studies indicate that bidirectional relationships between adipose tissue and homocysteine metabolism exist. Adipose tissue may be the source but also the site of metabolism of homocysteine. Plasma homocysteine is increased in obese insulin resistant patients who are not diabetic. Adipokines have

an important impact on homocysteine metabolism. For example, leptin has no effect on total plasma homocysteine but, by reducing PON1 activity, impairs the metabolism of homocysteine thiolactone and enhances homocysteinylation of plasma proteins. On the other hand, hyperhomocysteinemia has pleiotropic effects on adipose tissue, i.e. induces insulin resistance, ER stress, and dysregulates adipokine secretion.

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