Dysregulation of 1-carbon metabolism and muscle atrophy: potential roles of forkhead box O proteins and PPARγ co-activator-1α

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Canadian Journal of Physiology and Pharmacology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>cjpp-2019-0227.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Invited Review</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>29-May-2019</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Laha, Anwesha; University of Louisville School of Medicine, Physiology Singh, Mahavir; University of Louisville School of Medicine, Department of Physiology George, Akash; University of Louisville School of Medicine, Physiology Homme, Rubens; University of Louisville School of Medicine, Physiology Tyagi, Suresh C.; University of Louisville, Physiology</td>
</tr>
<tr>
<td>Is the invited manuscript for consideration in a Special Issue:</td>
<td>2018 IACS Slovakia</td>
</tr>
<tr>
<td>Keyword:</td>
<td>homocysteine, hydrogen sulfide, 1-carbon metabolism, transcription factors</td>
</tr>
</tbody>
</table>
Dysregulation of 1-carbon metabolism and muscle atrophy: potential roles of forkhead box O proteins and PPARγ co-activator-1α

Anwesha Laha†, Mahavir Singh†, Akash K. George, Rubens P. Homme, Suresh C. Tyagi

Department of Physiology, University of Louisville School of Medicine,
Louisville 40202, Kentucky, USA

†Correspondence: Mahavir Singh: mahavir.singh@louisville.edu, gene2genetics@gmail.com
Or Anwesha Laha: anwesha.laha@louisville.edu
Abstract

Homocysteine, a non-proteinogenic amino acid but an important metabolic intermediate is generated as an integral component for the “1-carbon metabolism” during normal physiology. It is catabolized to cysteine via the transulfuration pathway resulting in the generation of hydrogen sulfide, a naturally endogenous byproduct. Genetics or metabolic derangement can alter homocysteine concentration leading to hyperhomocysteinemia, a physiologically unfavorable condition that causes serious medical conditions including muscle wasting. Hyperhomocysteinemia environment can derail physiological processes by targeting biomolecules such as Akt however not much is known about regarding the effects of hyperhomocysteinemia on regulation of transcription factors such as forkhead box O proteins. Recently, hydrogen sulfide has been shown to be highly effective in alleviating the effects of HHcy by serving as an anti-apoptotic factor but role of FOXO and its interaction with hydrogen sulfide are yet to be established. In this review we discuss role(s) of HHcy in skeletal muscle atrophy and how HHcy interact with FOXO and peroxisome proliferator-activated receptor gamma coactivator 1-alpha expressions that are relevant in skeletal muscular atrophy. Further, therapeutic intervention with hydrogen sulfide for harnessing its beneficial effects might help mitigate the dysregulated 1-carbon metabolism that happens to be the hallmark of hyperhomocysteinemia-induced pathologies such as muscle atrophy.

Keywords: homocysteine, hydrogen sulfide, metabolism, transcription factors.
Introduction

Hyperhomocysteinemia (HHcy) induced atrophy of the skeletal muscle has been identified as one of the reasons for reduced muscular density especially in the calf muscles (McDermott et al. 2007). As discovered in the past excess mount of Hcy is a risk factor for medical conditions like hypertension, vascular dysfunction, coronary heart diseases and myocardial infarction because of body’s inability to metabolize it (Sen et al. 2010). In this regard, two enzymes that are essentially required for Hcy processing to cysteine are: 1) cystathionine β-synthase (CBS), and 2) cystathionine γ-lyase (CSE). Biochemically, during rate limiting step of the transsulfuration pathway CBS enzyme catalyzes synthesis of an intermediate known as cystathione from Hcy and then the next enzyme CSE converts cystathione to cysteine amino acid, an important precursor for glutathione synthesis. Thus, mutation or variation in the genetic sequence encoding the CBS enzyme can result in the deficiency of CBS activity. Further, hydrogen sulfate (H₂S), generated as a result of cystathione catabolism has been reported to play numerous physiological roles such as angiogenesis, vasorelaxation and also as an anti-apoptotic agent (Jensen et al. 2017). Recently, H₂S has also been shown to be cardioprotective by reversing the detrimental effects of HHcy in cardiomyocytes (Mishra et al. 2010) (Nandi and Mishra 2017) however the underlying molecular mechanism(s) as to how H₂S reverses these pathological effects in the skeletal muscle are not yet fully known.

Since forkhead box class O (FOXO) of transcription factor(s) regulates key genes that are crucial for multiple physiological processes, we surmise that the upregulation of FOXO plays potential role in the biology of skeletal muscular atrophy. It is known that Protein kinase B (also known as Akt, the cell survival signaling molecule) mediates FOXO phosphorylation inhibiting its translocation into the nucleus thus inhibiting the FOXO regulated genes (Carter and Brunet 2007) but FOXO regulation during HHcy in general and the skeletal muscular atrophy in particular is not much studied. We opine that FOXO remain unphosphorylated during HHcy thus
allowing it to translocate into the nucleus and upregulate target genes such as MuRF-1 and MAFbx/Atrogin-1 during muscular atrophy (Bodine et al. 2001). We further hypothesize that H$_2$S helps expedite the FOXO phosphorylation and leads to its proteasomal degradation thus reversing the HHcy mediated pathological process in the muscle. In fact, previous work showed the reduced expression of proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α); the transcription co-activator and a master regulator of several metabolic functions in muscular atrophy indicating that FOXO is inhibited by PGC-1α to alleviate atrophy related symptoms (Sandri M. et al. 2006). By this account HHcy seems to reduce PGC-1α expression in the muscle leading to the atrophy via activation of atrophy related genes through FOXO. H$_2$S in this scenario too is playing its role in upregulating the PGC-1α expression thereby reversing the HHcy influence (Fig. 2B).

**Skeletal muscle atrophy**

Skeletal muscle is the largest organ in human body and makes up almost 40% of the body’s weight. As the name suggests they are attached to the bones and responsible for body’s voluntary mobility (Brooks and Myburgh 2014, Kunkel et al. 2011). The basic unit is the muscle fiber consisting of actin and myosin (Britannica 2018). Actin filaments are associated with myosin and together with the help of ATP they generate the power stroke that is essential for skeletal muscle contraction. The muscle fiber in turn is bound to connective tissue, nervous tissue and blood vessels for oxygen and the nutrient supply (Damjanov 2009). Skeletal muscle atrophy or muscle wasting occurs as a result of muscular disuse or reduced muscular tension due to lack of physical activity from an injury or disease. Patients suffering from muscular atrophy have the affected limb shorter than the normal limb in addition to reduced muscular fiber diameter. Usually muscular atrophy can be reversed to a great extent by proper diet and exercise but a therapeutic form of treatment is still lacking and needs to be identified.

Muscular atrophy occurs primarily due to neurological disorder(s) causing the denervation unlike muscular dystrophy which is more often the result of a genetic mutation that has been inherited by the affected individual. Known neuropathological conditions include amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS). Other causes of muscular
atrophy include bed-rest, aging, cancer and the metabolic disorder. The molecular basis of skeletal muscle atrophy has been attributed primarily to transcription factors that are expressed in skeletal muscles. These factors are upregulated during atrophy phenotype and trigger either the protein degradation or inhibition of the protein synthesis (Bodine et al. 2001; Jackman and Kandarian 2004; Kunkel et al. 2011; Langer et al. 2018) (Fig. 2A). The oxidative stress due to increased concentration of reactive oxygen species (ROS) by HHcy condition has also been shown in contributing to the muscular atrophy phenotype (Majumder et al. 2018b) (Majumder et al. 2018a). Evidence exists indicating that Hcy is responsible for direct toxic effects on the vascular as well as nervous systems (Ansari et al. 2014). Especially in stroke (either ischemic or hemorrhagic) that disturbs flow of the blood to the brain via occlusion or rupture of a blood vessel, the clinical studies have shown that Hcy could serve as a preclinical marker of stroke (Kelly and Furie 2002).

Metabolism of homocysteine, hyperhomocysteinemia and health complications

Hcy is a non-protein amino acid generated in the body during methionine metabolism. Dietary methionine is first converted to S-adenosyl methionine (SAM) and which is then changed to S-adenosyl homocysteine (SAH). SAH leads to the production of Hcy which is further re-methylated to methionine wherein Vitamin B12 serves as a co-factor (cobalamin) and the cycle continues (Fig. 1). Under conditions of low cysteine or saturation of Hcy remethylation, Hcy is further catabolized via the transsulfuration pathway into cysteine. In this rate limiting step, Hcy is first converted to cystathionine through cystathionine β-synthase (CBS) where vitamin B6 (pyridoxine) is an essential co-factor. Cystathionine is further converted to cysteine by cystathionine γ-lyase (CSE). Cysteine has three fates where it is further broken down to L-lysine, pyruvate and hydrogen sulfide (H₂S) (Fig. 1). Thus, Hcy is crucial for the generation of cysteine which is an essential amino acid.

HHcy is a pathophysiological condition in which high levels of homocysteine is encountered in blood and urine due to its inability in getting converted to cysteine. Normal range of plasma Hcy level for young adults (0-30 years) is 4.6 – 8.1 µmol/L and for older adults (30
and above) is 4 -12 µmol/L (Elhomsy 2014). In normal healthy individuals, Hcy level in the blood is maintained due to the elimination of excess Hcy. However, during HHcy, Hcy level tend to increase due to the inability of Hcy to be metabolized into cysteine leading to its accumulation in the body. The ranges for mild HHcy is 12-20 µmol/L, intermediate HHcy is 20–100 µmol/L, and severe HHcy is more than 100 µmol/L (Gonin and Wilcox 2008). Some patients but not all could be treated using folic acid and B-vitamin therapy that helps reduce homocysteine levels however results from randomized placebo-controlled clinical trials testing the effect of vitamin therapy on outcome in these diseases are mixed and have generally fallen short of expectations. These results have led to abandon Hcy monitoring in the management of patients. It should be noted though that these trials included patients with only mildly elevated Hcy levels and have not addressed several clinical scenarios in which Hcy level reduction could have been effective, including the primary prevention of atherothrombotic disease in individuals at low- or intermediate-risk, or those with severe HHcy (Maron and Loscalzo 2009). The congenital HHcy occurs due to homozygous mutations in the CBS gene leading to deficiency of CBS. More recently, a highly promising approach involving the enzyme replacement therapy has been documented to ameliorate multiple symptoms of homocystinuria in a mouse model (Majtan et al. 2018).

**H₂S and Hcy and skeletal muscle atrophy**

Hydrogen sulfide (H₂S) is classically identified as a colorless, gaseous, water soluble compound which is considered to be poisonous and is also responsible for the foul smell that is characteristic of a rotten egg. A temporary overexposure can lead to minor symptoms like nausea, vomiting, dizziness and headache. When inhaled due to extended occupational or otherwise exposure in large amounts then H₂S can result in cardiovascular and pulmonary disorders (Fiedler et al. 2008), stroke, coma and even death (Contaminants and Continuous Exposure Guidance Levels for Selected 2009) (Database 2018). However, recent studies have shown that the endogenous production of H₂S as a result of Hcy catabolism due to CBS and CSE has various neuroprotective (Abe and Kimura 1996, Kimura 2002) and cardioprotective benefits (Lefer 2007). H₂S also promotes angiogenesis especially post ischemic reperfusion and
this angiogenic effect is believed to be Akt dependent (Cai et al. 2007). Thus, intervention or treatment with H₂S the beneficiary effects of this molecule could be harnessed during metabolic derangement as a result of HHcy.

The beneficial effects of H₂S through its antioxidant properties, vasodilation, and a decrease in blood pressure properties have been linked to the activation of CBS by calcium-calmodulin complex. Also, the genetic deletion of CBS or CSE gene in mice markedly reduced H₂S levels in the serum, heart, aorta, and other tissues, resulting in pronounced hypertension and diminished endothelium-dependent vasorelaxation, supporting the concept that H₂S is a physiologic vasodilator and potent regulator of systemic blood pressure (Yang et al. 2008). It has been hypothesized by our group that elevated levels of Hcy may damage nerves especially the vagus nerve and the superior cervical ganglion. Such pathological alteration can affect various G-protein coupled receptors (GPCRs) functions therefore impairing the parasympathetic as well as sympathetic regulation in blood vessels feeding the skeletal muscles (Veeranki S. and Tyagi 2013).

**FOXO and its regulation in skeletal muscle atrophy**

Forkhead Box (FOX) proteins constitute a group of transcription factors with a conserved DNA binding domain and are responsible for regulating a process like cell differentiation, glucose metabolism, apoptosis, oxidative stress resistance and many more. The gene was first identified in drosophila wherein a mutation gave rise to a fork-shaped structure of the head and hence the name. In drosophila they are classified as class A (FOXA). The FOX class O (FOXO) is common in mammals and is regulated by the Insulin/PI3K/Akt signaling pathway. The members of FOXO include FOXO1, 3 4 and 6 (Carter and Brunet 2007). FOXO1 and 3 are widely expressed across all tissues. FOXO4 is expressed primarily in muscles and kidney while FOXO6 is expressed in the brain and liver (Wang et al. 2014). Post translational modifications of FOXO determine its working and therefore, its cellular localization. When FOXO1 (FKHR) is phosphorylated by Akt, it is inactivated and is localized in the cytoplasm, where it is
polyubiquitinated for the proteasomal degradation (Aoki et al. 2004; Matsuzaki et al. 2003). The same is true for Akt mediated phosphorylation of FOXO3 (FKHRL1) (Brunet et al.) and FOXO4 (AFX) (Brownawell et al. 2001) wherein they stay in the cytoplasm and their translocation nuclear is inhibited. However, monoubiquitination of FOXO proteins especially during cellular oxidative stress stabilizes them in the nucleus thus allowing them to carry on their transcriptional activity successfully (Huang and Tindall 2011; van der Horst et al. 2006). Interestingly, all the four isoforms of FOXO are present in skeletal muscles, however, FOXO1 is extensively expressed in the skeletal muscles and FOXO6 is least expressed but its expression is high in the central nervous system (CNS).

Mild to moderate HHcy is a potential risk factor for neurodegenerative diseases since the human studies suggest that Hcy plays a role in central nervous system (brain damage, cognitive and memory decline). Furthermore, findings in recent years that investigated the role of Hcy as a cause of brain damage found out that Hcy itself or folate and vitamin B12 deficiency cause disturbed methylation and/or redox potentials, thereby promoting calcium influx, amyloid and tau protein accumulation, apoptosis, and neuronal cell death. It is noteworthy to mention that Hcy’s effect may also be mediated via activation of the N-methyl-D-aspartate receptor subtype (Obeid and Herrmann 2006). Though FOXO proteins play vital roles in regulating energy metabolism (Sanchez et al. 2014), they also are differentially regulated during skeletal muscular atrophy as they have been shown to be the primary transcription factors regulating MuRF1 and Atrogin-1, the two proteins highly upregulated during atrophy (Sandri Marco et al. 2004). The role of FOXO and its regulation during skeletal muscular atrophy due to HHcy has not yet been fully studied. It is possible that excess Hcy facilitates stabilization of FOXO in the nucleus of muscle cells where they carry out transcription of the atrophy-related ubiquitin ligases like Atrogin-1 and MURF-1. Considering H₂S has the ability to reverse the adverse effects of HHcy, therefore H₂S supplementation could deactivate FOXO through Akt mediated phosphorylation and eventually lead to its proteasomal degradation (Fig. 2B).

PGC-1α and its role in skeletal muscle atrophy
Peroxisome proliferator-activator receptor co-activator-1α (PGC-1α) is a co-activator and the master regulator of peroxisome proliferator-activator receptors (PPARs). PPARs are essential transcription factors which are further classified as PPARα, β/δ and γ and are best known to play their roles in energy metabolism (Laha et al. 2018). They generally heterodimerize with retinoic-X-receptors (RXR) which are further bound to respective corepressors and eventually the PPAR target genes remain inactive (Leonardini et al. 2009). Upon stimulation with (ligands binding to PPARs) or appropriate agonists, the corepressors are released allowing co-activators like PGC-1α and others to be recruited to the target genes and hence promoting PPAR-mediated transcription of genes (Costa et al. 2010; Murphy and Holder 2000). The PGC-1 group of transcription coactivators have three members, PGC-1α, PGC-1β and PRC (PGC-1 related coactivator-1) (Riehle and Abel 2012). PGC-1α is highly expressed in the skeletal muscle since the muscle has a high mitochondrial content and high oxidative capacity (Riehle and Abel 2012). It also plays a key role in maintaining the structure of the skeletal muscles (Liang and Ward 2006) and regulating metabolic balance and insulin sensitivity (Bonen 2009). Deficiency of PGC-1α plays a role in skeletal muscular atrophy and overexpression of PGC-1α alleviate this by mitigating damage accrued as a result of oxidative stress (Kang and Li Ji 2012).

Hcy is negatively correlated with PGC-1α since enhanced PGC-1α expression upregulates genes that are involved in Hcy metabolism (Li et al. 2009). Likewise, Hcy contributes to attenuate the PGC-1α expression leading to mitochondrial dysfunction (Veeranki S. et al. 2015). In HHcy induced skeletal muscular atrophy, there have been reports suggesting that PGC-1α and its downstream effector, PPARγ are downregulated (Veeranki Sudhakar and Tyagi 2015). However, it is not known whether supplementing H₂S can reverse the effects of HHcy induced skeletal muscular atrophy by reducing oxidative stress through increased expression of PGC-1α and therefore PPARγ. PGC-1α and FOXO expression have been shown to be correlated though. In one report, FOXO6 and PGC-1α have been shown to work in tandem to form a regulatory loop for maintaining oxidative metabolism in the skeletal muscle (Chung et al. 2013). FOXO3 and PGC-1α have been shown to be negatively correlated as well.
where PGC-1α suppresses FOXO3 to protect muscle from atrophy (Sandri M. et al. 2006). However, the exact mechanism of FOXO in relation to PGC-1α particularly during HHcy induced skeletal muscular atrophy has not been investigated. We believe that HHcy is possibly downregulating the PGC-1α thus allowing the FOXO to stabilize in the nucleus and therefore transcribe the atrophy related genes. This mechanistic most likely could be successfully reversed by supplementing the H₂S via treatment with appropriate long acting H₂S donor however this hypothesis needs to be analyzed.

**Conclusion**

In this review we offered a potential molecular mechanism of how the dysregulated 1-carbon metabolism leads to HHcy that in turn leads to skeletal muscular atrophy by modulating the expression of transcription factors such as FOXO and PGC-1α. In brief, we discussed possible mechanism(s) that might be involved by providing rationale for the involvement of FOXO and PGC-1α during HHcy and as to how it can possibly be mitigated by H₂S intervention in order to alleviate metabolic dysregulation as instigated by HHcy in susceptible patients. Although much needs to be done but discussion does open up the potential avenue towards exploring the molecular regulatory aspects of muscular atrophy due to HHcy that could be tackled employing the H₂S beneficial attributes as already repaired in numerous studies.

**Conflict of interest**
The authors declare that there is no conflict of interest associated with this work.

**Acknowledgments**
The work was supported by grants from the National Institutes of Health (Nos. R01 HL-74185, R01HL139047-01A1, and R01AR071789-01A1).

**References**


Bonen A. 2009. PGC-1α-induced improvements in skeletal muscle metabolism and insulin sensitivity. This paper is one of a selection of papers published in this Special Issue, entitled 14th International Biochemistry of Exercise Conference – Muscles as Molecular and Metabolic Machines, and has undergone the Journal’s usual peer review process. Applied Physiology, Nutrition, and Metabolism 34:307-314.


Elhomsy G. 2014. Homocysteine. (https://emedicine.medscape.com/article/2085682-overview?pa=vlI3pqAFdEMG7n%2BdAxi0Pj3jrjV0smlW60Pa51FEjPCmCecxxcQSf4KAEFW%2FEgLy6wvQDulPojO6m19fROZ%2BejiC03Rk4DwSd3ZDr5ZwUV%3Dfa1)


McDermott MM, et al. 2007. Elevated levels of inflammation, d-dimer, and homocysteine are associated with adverse calf muscle characteristics and reduced calf strength in peripheral arterial disease. J Am Coll Cardiol 50:897-905.


Figure legends

Fig.1. A schematic representation of methionine metabolism depicting the transmethylation, transsulfuration and remethylation pathways. Dietary methionine is converted to S-adenosyl methionine (SAM) which is further converted to S-adenosyl homocysteine (SAH) in the presence of DNA methyl transferase (DNMT). SAH gets converted to homocysteine (Hcy) that is further remethylated to methionine in the presence of folic acid (Vitamin B12) and the cycle continues. Under low concentrations of cysteine, Hcy is transsulfurated to cysteine wherein cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) act as essential catalysts.

Fig.2. Mitigation of skeletal muscle atrophy by H2S. HHcy-mediated downregulation of Akt leads to FOXO translocation to the nucleus leading to overexpression of the atrogenes that are responsible for skeletal muscle atrophy (A), hydrogen sulfide (H2S) mitigates the deleterious HHcy effects thus alleviating the skeletal muscle atrophy (B).
Fig. 1. A schematic representation of methionine metabolism depicting the transmethylation, transsulfuration and remethylation pathways. Dietary methionine is converted to S-adenosyl methionine (SAM) which is further converted to S-adenosyl homocysteine (SAH) in the presence of DNA methyl transferase (DNMT). SAH gets converted to homocysteine (Hcy) that is further remethylated to methionine in the presence of folic acid (Vitamin B12) and the cycle continues. Under low concentrations of cysteine, Hcy is transsulfurated to cysteine wherein cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) act as essential catalysts.

https://mc06.manuscriptcentral.com/cjpp-pubs
Fig. 2. Mitigation of skeletal muscle atrophy by H$_2$S. HHcy-mediated downregulation of Akt leads to FOXO translocation to the nucleus leading to overexpression of the atrogenes that are responsible for skeletal muscle atrophy (A), hydrogen sulfide (H$_2$S) mitigates the deleterious HHcy effects thus alleviating the skeletal muscle atrophy (B).