Journal of Advanced Veterinary and Animal Research
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2017.d230
*A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)*

**December 2017**
Vol 4 No 4, Pages 319-332.

Review Article

# Homocystein: A new biochemical marker in livestock sector

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• **Received:** June 28, 2017 • **Revised:** July 17, 2017 • **Accepted:** July 19, 2017 • **Published Online:** Dec 21, 2017

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## ABSTRACT

The livestock sector is making great contributions to the world economy. Many different diseases, such as cardiovascular diseases, kidney and mineral substance insufficiency, cause huge losses in yield and production in the livestock sector. Early diagnosis is essential to combat these diseases. Today, homocysteine levels are used as biochemical markers in the diagnosis of the functions and diseases of many different organs in human medicine. Homocysteine is an amino acid that occurs in the process of methionine metabolism and does not enter the primary structure of proteins. Homocysteine is a biochemical marker used in the assessment of cardiovascular and renal diseases as well as other organ functions. In this review, homocysteine determination methods and detailed information about which organ and system diseases can be used in livestock sector will be given.

## KEYWORDS

Disease; Homocysteine; Livestock

**How to cite:** Kozat S, Okman EN. Homocystein: A new biochemical marker in livestock sector. Journal of Advanced Veterinary and Animal Research. 2017; 4(4):319-332.

## INTRODUCTION

Homocysteine is a non-proteinogenic sulphur-containing amino acid obtain from chemical process of methionine synthesis. There are so many data show that Hcy has a main role for overall health status (Barbosa et al., 2008). Disruption of Hcy metabolism, i.e. increase in Hcy in the plasma may bring about the oxidative stress and imbalance causing in increase carbohydrate, nucleic acids, oxidation of proteins and metabolism process of lipoperoxides, which are accepted to be contribute in cytotoxicity. There is the relationship between Hcy metabolism and numerous disease, like as neurological problems, cardiovascular disease, cancer, chronic kidney figure, skeletal tissue damages, gastric and intestinal problems, and connate abnormalities (Barbosa et al., 2008). Many researches show a few simplified steps on the importance of methylation and appropriate homocysteine levels and their connection to certain metabolic processes like glutathione synthesis, mitochondrial ATP production, oxidative stress and detoxification (Barbosa et al., 2008; Zhou et al., 2012). Elevated levels of homocysteine are known as a risk factor marker heart related problems. Numerous research articles have also shown that elevated homocysteine levels are linked to certain genetic traits (Zhou et al., 2012).

### 1. Homocysteine and methionine

Homocysteine is an amino acid sulfhydryl-containing generate from the metabolic process of methionine which can stand autooxidation biologically reactive yields (Bostom et al., 1999). Homocysteine take part in stimulate directions combined with elevate cellule toxicity. It has been knew as a subscriber to four important mechanisms of problems: oxidation stress (Bostom et al., 1999), thrombosis (Lubos et al., 2007), apoptosis (Rounds et al., 1998) and cellular proliferation (Welch et al., 1998). Homocysteine metabolism happens with three directions; a) Hcy remethylation to produce methionine by methionine generate in a process of cobalamin and folate-related passivity, b) in transsulfuration direction, cystathionine β-synthase (CBS) produced by converting Hcy to cystathionine, in certain organs like renal and liver tissue and , c) methionine is generated by remethylation process of homocysteine with betaine : Hcy methyltransferase (BHMT), as soon as the surplus of a serine (Finkelstein, 1998). Homocysteine is a mediocre of methionine metabolism. Impair in the cystathionine β-synthase genes mutations and mutation in 5 and 10 methyl-tetrahydrofolate reductase mar homocysteine generation to cystathionine and methionine. As an alternative, increase in amounts of homocysteine can outcome nutritional shortage of folic acid, vitamins $B_6$, $B_{12}$ which are cofactors essential for natural metabolism of homocysteine (Maron and Loscalzo, 2007). Folic acid refills the Tetrahydrofolate Lake and is needed for natural activity of methionine metabolic process; but also elevates *S*-adenosyl-methionine. Methyl transferor is a pioneer for unsymmetrical dimethylarginine, synthase suppressor of endothelial nitric oxide and it can separate enzyme, reduced nitric oxide beside vessels endothelial dysfunction. Eventually, the antioxidant glutathione is last crop of cysteine metabolism; wrong transulfuration crossing activity has been implicated in reduced cell antioxidant defence, can increased Hcy amounts serve as a successor for downstream functions which characterized the oxidative physiology of different malady due to hyper homocysteinemia (Maron and Loscalzo, 2007). One of the important amino acid is methionine which is generating in the shape of proteins intake as nutrition. In digestive system protein is converted to amino acid. Methionine, proteins produced methionine (met) which is absorb by the epithelium of the intestinal and released to blood flow and all organs. Metabolism of met in cells: (A) as an important factor to make fresh proteins via synthesis ribosome (B) in a metabolic process of S-adenosylmethionine (AdoMet (Maron and Loscalzo, 2007). Exposure homocysteine metabolism in a contact with vitamin $B_{12}$, methionine and folat in metabolic pathways (A) and (B), Met is combination with ATP although in a crossing-specific state: the carboxyl factor of Met is lunched in crossing (A), provided the thioether sulfur is lunched in crossing (B). Met usage crossing (A) which is finally converts to Homocysteine. Due to transfer of its methyl group, AdoMet is changed to AdoHcy. The only source of homocysteine in the Human or animal body is reenzymatic hydrolysis of AdoHcy. Amounts of Homocysteine are adjusted by remethylation to methionine, MS is responsible for enzyme catalysed. transsulfuration of cysteine: the first step is procedure of cystathionine γ-lyase (CSE), while the next stage is procedure of the enzyme CBS. Vitamin $B_{12}$ and 5, 1 methyltetrahydrofolate (CH3-THF) which are important to catalysed. The remethylation MS, generated by MTHFR (Rosenblatt and Fenton, 2001). Next remethylation passage is altered by betaine–Homocysteine methyltransferase (BHMT) (Bostom et al., 1999). Homocysteine I a relationship oxidatively with another thiols, specifically sulfhydryl classes of proteins in extracellular, to generate blended disulfides (S-Hcyprotein). The transsulfuration needs vitamin $B_6$, along remethylation metabolic process via MS happens in entire of body, remethylation by BHMT and transsulfuration by CBS are bounded with renal and liver tissue (Jakubowski, 2002). Thioester Hcy-thiolactone which is produced by methionyl-tRNA synthetas is responsilble for rest of

Homocysteine metabolizing (Jakubowski, 2004, 2006a). The circulation via the Hcy-thiolactone passage is elevate by large amount of Met nutrition intake (the final process of Homocysteine), insufficient store of CH3-THF, or disorder in transsulfuration or remethylation reactance by genetic changings of enzymes, like MTHFR ,MS or CBS. At normal pH (pKa ¼ 6.66-6.67) Hcy-thiolactone is neutral (Jakubowski, 2006b) hence collect mainly in the extracellular fluids (Ecf). by two there are directions of Hcy-thiolactone metabolized: (A) a hydrolytic passage that brings Homocysteine-this direction is done by intra and extra cellular Hcythiolactonases which is called as bleomycin hydrolase (Zimny et al., 2006) and paraoxonase 1 (Jakubowski, 2000) direction(B) a synthetic direction in which lysine (protein) rests reacts with Hcy-thiolactone generating N-Hcyprotein (Jakubowski, 2002). N-Hcy-protein Proteolytic degradation provides N-Hcy-Lys, initially detected in a physiological apparatus when N-Hcy-hemoglobin was incubated with hepatic tissue extracts (Bostom et al., 1999). Renal disease can elevate in it and also CBS deficiency. Genetic shortage in Hcy and folate metabolism and a hyperhomocysteinemic large amount of Met nutrition intake bring about increase of Nε-Hcy-Lys in mice. The plasma levels of Nε-Hcy-Lys are positively matched via nitric oxide synthase suppressor unsymmetrical dimethylarginine, which is include with their usual obtain as yields of protein revenue (Bostom et al., 1999).

## 2. Homocysteine, folic acid and Vitamin $B_{12}$

Amino acid Methionine is produced from diet protein intake. A part of methionine is changed in to Hcy. Amino acid help to converts lots of homocysteine in to methionine. In a case of $B_{12}$ deficiency, homocysteine levels will enhancement because of defect in reaction (Blom and Smulders, 2011). Both sufficient $B_{12}$ and folic acid (folate) levels indispensable to keeping Hcy at status associated with less rates of problems as well as inadequate vitamin $B_6$ levels brings about increased Hcy in animals (Blom and Smulders, 2011).

**2.1. Metabolism of folic acid (folate):** Folic acid and vitamin $B_9$ are another name of folate which is belong to B vitamins family group. Daily intake level of folate is nearly 400 micrograms from foods or dietary supplements. Metabolism process of folate is the basic metabolism in which carbon units are tended to Hcy, more over amino acids and purine-pyrimidine nucleotides are generated (Bostom et al., 1999).

**2.1.1. Folic acid:** Water-soluble folate which is known as folic acid and pteroyl monoglutamate (PteGlu) is belonging to vitamin B groups (vitamin $B_9$). Chemical appearance of it includes of 3 portion: a pteridine hoop, glutamic acid and para-aminobenzoic acid. considering that oxidation situation of the pteridine hoop ,folic acid are classified, the rest of glutamic acid and one-carbon units at the N5 and N10 status .folic acid and Hcy metabolism and there complications are explained beside descriptions why disturbed Hcy and folic acid are Elevated risk in lots of various diseases, consisting connate birth faults like heart problems, cleft palate and lip the residues of glutamic acid, neurodegenerative, cancer, late gestation, osteoporosis and neurodegenerative which suggests a potential role for folate in reducing probability of related diseases (Bostom et al., 1999).

**2.1.2. Vitamin $B_{12}$ metabolism:** Vitamins like $B_{12}$, $B_6$ and riboflavin are contributed in the metabolism of an S-including amino acid, Hcy. Methionine and folic acid cycle links Hcy metabolism. Vitamin $B_{12}$ is indispensable for methylation processes which is necessary for cell metabolism and deoxyribonucleic acid (DNA) reactions , so any shortage may leads to interruption of cell metabolism and DNA so may causes important clinical problems (Stabler, 2013).

Succinyl-CoA is generated by Methylmalonic acid and vitamin $B_{12}$ plays as a cofactor role for this process. Vitamin $B_{12}$ transformation to two active coenzymes, methylcobalamin in the cytoplasm and adenosylcobalamin in mitochondria which is essential for methylmalonic acid and Hcy homeostasis. Hcy again synthesised into methionine or changed into amino acid cysteine. In a case of vitamin $B_{12}$ level shortage Hcy and methylmalonic acid are elevated. There is a potent relationship between Hcy levels and methylmalonic acid, and the sensitivity of Hcy levels are the worth marker (more than 95%) for recognizing vitamin $B_{12}$ shortage. Patient's response to treatment beside measurement of folic acid, vitamin $B_{12}$ and creatinine levels will illustrate the reason of the elevated Hcy amounts (Refsum et al., 2004).

On the alternative, with the assist of vitamin $B_{12}$ Hcy can be changed to methionine (Swart et al., 2013). In a case of increase methylmalonic acid and Hcy levels, it means that only with Hcy (sans methylmalonic acid) deficiency of vitamin $B_{12}$ can be determined and its answer to therapy. Folic acid, vitamins $B_6$ and $B_{12}$, has been demonstrated in several surveys to help decrease Hcy amounts. L-methylfolate (active form of folic acid), can increase plasma folic acid levels up to 700% much more than synthetic folate, so this may be more useful decreasing Hcy amounts (Refsum et al., 2004; Swart et al., 2013).

## 4. Hyperhomocysteinemia (hyper-Hcy)

Protein (mainly albumin) is the main form of Plasma Hcy which is considered 85-90 % of total Homocysteine.

Hcy-Hcy or disulfides of Hcy-Hcy oanther oxidized forms which are considered 10–15% of total Hcy, the rest of Hcy less than 1% is called Free Homocysteine (reduced Homocysteine). Total Hcy (tHcy) testing is done in most clinical labs (Ueland and Refsum, 1989). tHcy refers to the total of protein bound, oxidized and decreased Homocysteine, and is expressed as total homocysteine amount elevating in Hcy in the blood it can taking part to plaque producing by hurting arterial and vein vessels (arrest and heart attack, lung embolism and vein thrombosis) blood clots can damage the lining of blood vessels also animal homocysteine level in the plasma is increasingly being recognised as a risk agent for disease and consider as a potential health problems like cardiovascular disease, brain vessels disease, dementia-type disorders, and osteoporosis-related fractions (Ueland and Refsum, 1989).

**5. Factors contribute to high homocysteine levels**

**5.1. Vitamin and mineral shortage**: In Hcy metabolism Folate deficiency, vitamins(B2, B6 and B12), betaine, and magnesium which are important (Ueland and Refsum, 1989).

**5.2. Enzyme shortage**: genes encoding abnormality for MS and MTHFR as they take part in the Homocysteine metabolism directions Genetic variant that bring about disorder in metabolism of folate by folic acid (Ueland and Refsum, 1989).

**5.3. Diseases**: low levels of thyroid hormone, renal abnormalities (kidney failure can elevate Hcy amounts due to lowered kidney eliminate and ruin metabolism), psoriasis and some medications can increased homocysteine levels.

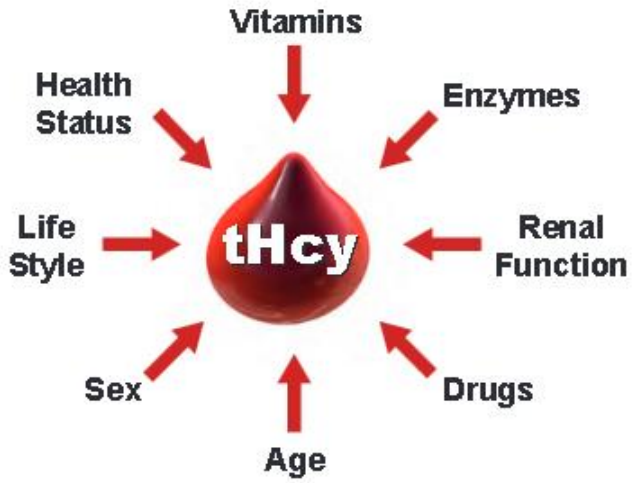


**Figure 1:** Factors that cause elevation total homocysteine

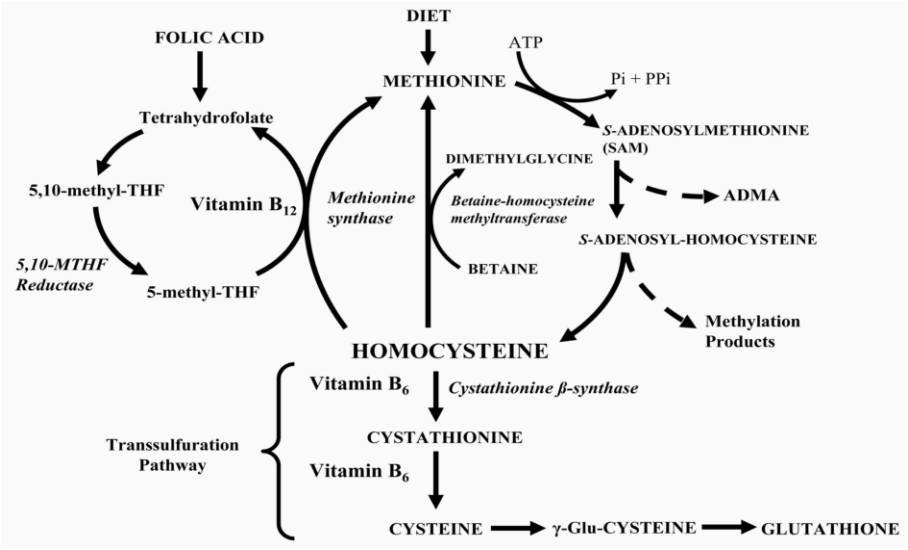


**Figure2**. The metabolic cycle of homocysteine

**5.4. Drug interaction:** Certain drugs such as sulfasalazine, nitrous oxide, levodopa, metformin, fenofibrate, cholestyramine, colestipol, methotrexate, niacin, pemetrexed, phenytoin acauses elevation of Hcy (Desouza et al., 2002) (**Figure 1**).

**5.5. Age faktor:** It Hcy mounts tend to elevate with age (Ueland and Refsum, 1989).

**5.6. Sex:** as human also in animal population homocysteine mounts in males are much more than females.

**5.7. Life procedure***:* Obesity, stresses which can elevate the requirement for folate), physical inactivity, large amount of methionine nutrition intake (dairy products and meat) and drinks containing caffeine drinking cause elevation of Hcy (Ueland and Refsum, 1989).

**5.8. Health factor***:* it is demonstrated that health factor has main relationship with low Hcy levels because it is elevated in suboptimal health conditions (Ueland and Refsum, 1989). Hyperhomocysteinemia will also provoke the risk of birth defects and bone fractures. usual causes of hyperhomocysteinemia consist kidney disease, defect of B vitamins (like vitamins ($B_{12}$ and $B_6$) and folic acid) in the foot regime, hypothyroidism, and certain medications. There is epidemiologically connection based between arteriovascular malformation, vein thromboembolic problems and hyperhomocysteinemia. indicative physiological mechanisms of the result of Hcy consist elevated peroxidation damage, promotion of clotting, elevation of monocytes chemotaxis, proliferation of smooth vessel, increased inflammation and cell toxicity, prevention of anticoagulated process, effects on cell endothelium and platelet lunching association (Abramson and Abramson, 2001).

**6. The kidney and homocysteine metabolism**

Connection between hyperhomocysteinemic and renal disorders exhibit unusually high rates of cardiovascular morbidity problems which may lead to sever status and death. Present information explain that the accurate renal function play basic role in Homocysteine clearance. This vitnessing and the reverse connection between Homocysteine amounts and GFR signify the renal as a serious take part in Hcy metabolism. Also accurate renal function has a basic role in plasma amino acid clearance (Bostom et al., 1999). The existence in the renal of specific homocysteine grasp mechanisms and Homocysteine metabolizing enzymes explains that this role develops to Homocysteine. Retained uremic inhibitory substances and Decrease clearance of plasma Homocysteine in disease related to kidney problems lead to hyperhomocysteinemia and this lower may be attributable to disorder kidney clearance and extra renal clearance. Hyperhomocysteinemia is related to decrease in kidney homocysteine metabolism and clearance. Homocysteine amounts elevate as kidney operation impairment, patients show resistant to the normal homocysteine decreasing remedies (Bostom et al., 1999).

**6.1. Amino acid metabolism:** As mentioned before kidneys contribute as a main part in the metabolism of amino acid. Amino acids are easily transforming in glomerulus, in this place the filtered load reverberates the plasma amino acid GFR and doping. Daily amino acids filtered are Almost 450 mmol (Silbernagl, 1998). Majority amino acids are absorbed again along the proximal loop of Henle and just almost 5 mmol being finally ejected in the urine (Silbernagl, 1998; Kopple et al., 1978). Another mechanism of cell absorbing is through the basolateral surface tubular cells. This happens basically in distal tubular cells, perhaps produce metabolic substrates to cellules in which luminal amino acid transfer is lowered. In this way Homocysteine may be picked up also, because amino acid cysteine is demonstrated to be picked up in this way (Silbernagl, 1998). These tubular metabolic procedures can be quite complicate, with tubular cellules generating and sending out amino acids while altogether picking up and reducing others (Kopple et al., 1978).

**6.2. Metabolic procedure of homocysteine:** One of the renal functions as does other amino acids is capable metabolization of Homocysteine. Molecular mass of 135 D is one part of Homocysteine (House et al., 2000) and filtration well in accurate glomeruli. Considering in a normal GFR range of 125 mL/min and plasm fHomocysteine concentration of 3 µM (Refsum et al., 1985) the daily quantity of Homocysteine filtration could be almost 0.5 mmol. Like other amino acids, lots of evidence demonstrate that Homocysteine filtration is eagerly absorbed again and excreted so less via urine urination (6 µmol/d, or 1%) (Refsum et al., 1985; Ueland, 1995; Bostom et al., 1995) **(Figure 2)**.

As mentioned before, it is depreciation of the true Homocysteine filtration bar. A host of studies have demonstrated that animal renal system has the necessary metabolized Homocysteine enzymes compared with hepatic system, the kidney has lots betaine/ Homocysteine methyltransferase enzyme (300%) and low cystathionine and methionine synthase (30% and 50%) (Selhub, 1999). Evaluations of the cystathionine-synthase enzyme in 2 tissue paradigms demonstrated less amounts, contrasted with hepatic tissue. But, cystathionine-

synthase gene expression has been performed in the renal tissue (Bao et al., 1998).

Although both enzymatic directions can scientifically be applied, *in vivo* and *in vitro* rat experiences illustrated that homocysteine was metabolized initially through transculturation (House et al., 1998). Kidney may make up for alters in glomerular filtration by moving up and down regulating these bio physiologic directions, therefore holding constant the level of Homocysteine flowed to plasma (Wollesen et al., 1999). GFR increasing would lead to elevated Homocysteine filtration and tubular again absorption. Therefore, kidney Homocysteine metabolizing enzymes would be increased. Opposite of this, in a case of GFR lowering , renal Homocysteine metabolism also would wrack so any alter in Homocysteine filtration do not change kidney Homocysteine transport to plasm. Briefly, normal kidney has the ability to filtration, reabsorption, and metabolization of Homocysteine. In addition to the filtration bar, Homocysteine absorb may also happen on the basolateral surface of tubular cells (Kopple et al., 1978).

**6.3. Dietary intake on homocysteine filtration process:** Experimental data suggest that food intake affects Hcy protein binding and, consequently, Hcy glomerular filtration. These results may help explain how diet-related increases in Hcy levels are normalized. Dietary methionine-containing protein loads increase plasma levels of Hcy and cysteine. Disulphide exchange reactions and competition for protein binding cause a disproportionate increase in fHcy levels, allowing excess Hcy in the free form to be filtered by the kidney (Guttormsen et al., 1996). The increase in fHcy levels may also accelerate extrarenal uptake. Therefore, a combination of increased renal Hcy filtration and possibly upregulation of intrarenal degradative pathways would lead to increased plasma clearance. evidence not show an elevate in postprandial free homocysteine (fHcy**)** or total Hcy (tHcy) levels were limited by dietary protein load size or inadequate follow-up periods or lacked fractionated Hcy measurements (Hultberg et al., 1993). The rate of daily homocysteine filtration at the glomeruli should be requirement to measure again. Protein nutrition severely elevate f-Homocysteine amounts and also increase in GFR itself, elevating f-homocysteine clearance. Neurohormonal and hemodynamic are more likely responsible for recent mechanism process (King and Levey, 1993).

## 7. Diseased kidneys and homocysteine

**7.1. Relationship between GFR and homocysteine amounts:** Association between GFR and Homocysteine amounts are belonging to the importance of healthy kidneys in plasma Homocysteine maintenance. Homocysteine amounts elevate in kidney abnormalities and development of end stage renal diseases (ESRD), with the vast majority (more than 90%) patients eventually experiencing mild-to-moderate hyperhomocysteinemia (Foley et al., 1998). This cause disorder in Homocysteine protein binding via food intake or medicines, can momentary elevate f-homocysteine amounts, leading to enhancement renal metabolism also glomerular filtration. There is completely fixed inverse connection between Homocysteine amounts and kidney metabolism. This connection indicate increased Homocysteine amounts in kidney abnormalities are literally lead to renal metabolism (Burgunder et al., 1989).

**7.2. Plasma protein binding:** Kidney Disease bring about elevate Absolute fHcy, bHcy, and total Hcy, its known ratio of f-Homocysteine lowering quite less (Hultberg et al., 1995). So, uremic animal which characteristic with hypoalbuminemic show larger ratio of protein-bound Homocysteine. Uremic plasm proteins contribute to binding Homocysteine. What's more surveys are demanded to corroborate the ratio of f-Homocysteine, the performs of maintained uremic solutes on Homocysteine binding, as well as back demand toxicities of decreased Homocysteine, f-Homocysteine, and b-Homocysteine in the uremic surroundings (Hultberg et al., 1995).

**7.3. Metabolic procedure of amino acids:** Amino acids usage and removal are deeply changed due to uremic induced undernourishment, toxin preserved, decreased hormonal amounts, elevated elimination of uric amino acid (Fermo et al., 1995). Decrease in plasm amounts of indispensable amino acid, like valine, tryptophan, isoleucine, lysine and leucine elevates in amounts of cysteine, methyl histidine, hydroxyproline, citrulline, glycine and all dispensable amino acids are usually apperceived kidney abnormalities (Silbernagl, 1998). Obviously elimination of amino acids via urine is underestimate while elevates with severe kidney disorders. Increase plasm concentrations of certain amino acids bring about elevates GFR and following amount of filtrate circulation in the trace remaining running renal unites (Bostom et al., 1999). In This recent process contiguity of amino acid and renal tubular cells are decrease eventually it can bring about disrupting the capability of them to picking up, metabolizing and filtration of amino acids. Elevated discharging of tubular amino acid can take apart to the large quantity of elimination amount (Bostom et al., 1999).

**7.4. End-stage renal disease and extrarenal metabolism of homocysteine:** Hyperhomocysteinemia

in renal problems as a result of faulty elimination of Homocysteine from plasma more than elevate transfer to plasma was proved in kidney failure which was used intravenous and oral homocysteine bars (McCully, 1969). Plasma Homocysteine elimination was decreased in contrasted with vitamin $B_{12}$ and folic acid shortages. consequence attribute to a lower in intrarenal Homocysteine filtration due to decreasing running kidney mass and a lower in extra renal filtration, probably ascribable to preserved uremic solutes which can prevent metabolism of Hyperhomocysteinemia (McCully, 1969) has been provided that increased total plasm amounts of the atherothrombotic sulfur amino acid Hcy (McCully, 1969), which can cause CVD risk factors increase in ESRD. In ESRD patients Increased fasting plasm total homocysteine amounts is possible takes part freely to their extra occurrence of deadly and non-deadly CVD results (Bostom et al., 1995).

Demonstration of these prospective findings in more sizeable ESRD groups is immediately needed. There is few knowledge about Homocysteine derivation and metabolism by renal abnormalities which bring about an inconceivable to deterministic recognize the origin of the clearance imperfection. Hopefully data show that potential role of healthy kidneys in clearance and metabolism of amino acid and Homocysteine. The entity of Homocysteine metabolizing enzymes and kidney tubulate cellules absorbing procedure has been corroborated, and Homocysteine extraction surveys in animal renal recorded significant Homocysteine absorbing (Bostom et al., 1995; House et al., 1998). Folate delivery was prevented in the uremic state decreased folic acid L-folinic acid and L-5 methyl tetrahydrofolate decreased Homocysteine amounts in ESRD patients discovered no data of mutilated folate absorption (Bostom et al., 2000).

**7.5. Hcy-lowering examinations:** Connection between GFR and Homocysteine amounts is inverse and related overall a nonuremic level of renal running process, confirms the presupposition that it is decreased kidney running process, except cumulation of uremic toxins which can bring about Homocysteine amounts to elevated. Kidney abnormalities and hyperhomocysteinemia are tendentiously resistant to all existence therapies, such as vitamin $B_{12}$, folate and its decreased forms, vitamin $B_6$, serine and betaine. Beside refractory to treatment increases as renal function decrease (Bostom et al., 1999).

**7.6. Relationship between chronic renal disease and hyperhomocysteinemia:** Hcy lowering intervention relating surveys implemented in keeping ESRD patients hve not been controlled, open label studies (Bostom et al., 1999; Arnadottir and Hultberg, 1997), with a single exception (Bostom et al., 1996), it is important following results could be drawn from these surveys:

1. The addition of folate to a baseline regimen consisting decreases fasting t-homocysteine amounts by approximately 25 to 30 %.
2. folate-based B vitamin regimens, appear to lower fasting t-Homocysteine amounts by almost 30% to 50%.
3. Folate intake, more than half of patients with empty stomach or not, t-Homocysteine amounts will carry out remain t-homocysteine amounts raising or at this quantity.
4. A lack of studies on independent effect of cobalamin on empty stomach t-Homocysteine amounts.

**8. Effect of homocysteine on thromboembolism:** The connection between arteries abnormality and higher hyper-Hcy was initially offered by McCully (1969) who perceived post- mortem examination demonstration arteries atherosclerosis and thromboembolism in a homocystinuric patient with damaged vitamin b12 metabolism that was equal to CBS shortage (Mudd et al., 1964). Increased plasma Hcy amounts are participate in the improving of venous thromboembolic also atherosclerotic abnormalities. Venous thromboembolism (VTE) Meta-analyse beside the hyper-Hcy was carried out (Fermo et al., 1995).

Probability of disease return in patients with hyper-Hcy is not clears, so the ideal therapy for these patients after serious venous thromboembolism is unclear. The most currency of hyperhomocysteinemia in thrombosis patients combine with the elevate risk of repetition warrants broad patient screening. The effect on the risk of repeating of prolonged anticoagulation, supplementation of folate and vitamin $B_{12}$, or both have to be surveyed. Title deed supporting the role of mild hyper-Hcy in the improvement of immature and recurrent disease relating vein thromboembolism. One of the main risk factor ford deep-venous thrombosis is a large amount of plasma Hcy amounts (Den Heijer et al., 1996). A remarkable risk for VTE in the attendance of hyperhomocysteinemia seemingly presence between patients with repetitive vein thromboembolism and this remarkably manifests for patients with VTE with aging (Bostom et al., 1999). These informations are combined with the concept that mild hyper-Hcy, as such, is a separate risk factor for vein and arteries thromboembolism (Ahlner et al., 1991). Many surveys must be performed relating to pathophysiology of thrombosis in hyperhomocysteinemia also clinically effects of Hcy lowering by means of vitamin supplement also on the interplay of this very usual abnormality with acquired

thrombogenic defects (Ahlner et al., 1991; Mangge et al., 2014).

**9. Relationship between homocysteine and cardiovascular disease**

Negative effects of Hcy on smooth muscle cells and cardiovascular endothelium with outcome changes in subclinical arterial structure and function is a responsible for cardiovascular related diseases. Cardiovascular diseases (CVD) consist of disorders of the heart and blood vessels (Mangge et al., 2014). It is hard to say that CVD function is specific. Importance of coronary artery disorders could be divided to single, double and triple vessel disorders (Shenoy et al., 2014). It has known that Hcy played specific role in atherosclerotic vascular abnormalities since 1990s (McCully, 1996). Evidence has indicated a connection between mild increased Hcy amounts also possibility of CVD (heart, peripheral artery, coronary and cerebrovascular disorders) (Shenoy et al., 2014). Arteriosclerosis is defined as a consecutive inflammatory hurt to intima of artery with increase discharging of plasma, sedimentation of plasma lipids in fibrosis and plaques grading (Schaffer et al., 2014). Hyper-Hcy and atherosclerosis connection was explained more than four decades ago. Atherosclerotic diseases are the usual pathophysiological procedure which can be responsible for cardiovascular disorders like heart ischemia, myocardial infarction (MI), stroke and etc (Mangge et al., 2014).

There is lots of reports which are remarked a clear relationship between total serum Hcy and the outbreak of cardiovascular system disorders (Okura et al., 2014). A systemic review was done to find the benefit of Hcy decreasing interpositions. Regrettably, Hcy lowering interpositions has no efficacy on stroke, MI, or death by any cause it comes contrasted to a placebo (Okura et al., 2014). Hcy can intercede the process of cardiovascular disorders by lots of various functions like negative efficacy on vessels endothelium also smooth muscular cellules with outcome changes in subclinical arterial function and construction (Zhang et al., 2014). Elevation of, oxidative damage, vessels smooth muscle cellules, endothelial dysfunctionan elevate is produce of collagen and decline of artery wall elastic material to name a few of these effects (Zhang et al., 2014). Trial of the efficacy of Hcy on the expression of CRP and examination on the process in vascular smooth muscle cellules (VSMCs) disclosed that Hcy remarkably persuaded mRNA and protein manifestations of CRP in vascular smooth muscle cellules. Hcy boosted Number 1 sub unit (of N-methyl-D-aspartate receptor (NMDAr) manifestation, while MK-801 decrease Hcy provoked CRP expression in vascular smooth muscle cellules. There is evidence that Hcy has basic role in commence an inflammatory reaction in VSMCs by provoke CRP generation, which is intercede NMDAr-ROS-ERK1/2/p38-NF-$\varkappa$B signal direction which is demonstrate the role of Hcy in incidence of atherosclerosis in body (Pang et al., 2014). Hcy amounts connected remarkably with boosting intensity of cardiovascular disorders. Lots of reports expressing Hcy was demonstrated to operate increase of VSMCs. What's more it is responsible for elevating the activity of HMG-CoA lowering hence increases cholesterol generates (Shenoy et al., 2014).

Hcy serum amounts were considered remarkably high amount in coronary arterial disease than in non-coronary arterial disease cases. Boosted Hcy amounts effected intensity of Coronary arterial disease (Shenoy et al., 2014). Homocysteine has effect on endothelial malfunction and is consider to be intercede by factors consisting nuclear factor-kb (NF-kb) activation, provention of endothelial nitric oxide synthase, oxidative stress and inflammation (Basu et al., 2014). It is known that in a case of atherosclerosis, Carotid intima-media thickness is non-invasive factor (Basu et al., 2014). There is less dependence between total Hcy amounts and carotid intima media sickness in the nondiabetic groups, also it's known that the connection in the base of diabetes mellitus (DM) (Basu et al., 2014). Researchers have demonstrated a considerable connection of serum Hcy amounts with several indicators of arterial stiffness like pulse pressure and aortic hardness as evaluated by carotid-femoral Pulse Wave Velocity (Zhang et al., 2014). Boosted Hcy amount increase platelet accumulation in the endothelial cellules and has also been connected with high amounts of hypercoagulability agents such as, tissue plasminogen promoter, β-thromboglobulins (Zhang et al., 2014). These cause the exacerbation of thrombus formation. Moreover, it is augmented arterial hardness in hyper-Hcy might be ascribed to Hcy related low density lipoprotein atherosclerosis, like tiny size of low density lipoprotein and its oxidative turnover (Zhang et al., 2014).

Hcy therapy dose dependently increased phosphatidylserine offering and as a result the procoagulant activity of red blood cells (Xie et al., 2014). Studies demonstrate adverse effect of Hcy on endothelial, boosting clotting, and increasing the number of smooth muscular cellules. But, the given Hcy in lots of studies is much more than pathological Hcy amounts (Xie et al., 2014). the carotid resistance index as a successor sign of encephalic peripheral vessels resistance and indicated a remarked connection between the Hcy amounts and index in aged patients with high blood pressure (Okura et al., 2014). This shows that elevated serum homocysteine may has

role in elevate in resistance index exclusively in aged patients with high possibility of stroke (Okura et al., 2014). The connection between Hcy and cardiovascular disorders may result from inadequate of vitamin B or it may only change vessels reactivity when folic acid is synchronously decrease (Xie et al., 2014). But, folic acid is related with changing in vessels reactivity without alteration of Hcy levels (Faeh et al., 2006; Xie et al., 2014). It is known that hypertension bring about cardiovascular problems. Relationship of blood pressure and Hcy is important because blood pressure may intercede part of the cardio toxic process of Hcy (Faeh et al., 2006). Several mechanisms like effect on vessels endothelial integrity are responsible of Hypertension related to Hcy (Faeh et al., 2006).

Connection between blood pressure and Hcy demonstrate a rising in blood pressure due to induced hyper-Hcy (Faeh et al., 2006). As mentioned before, Homocysteine performance hurts endothelial cellules in vitro and in animals. Hcy provoked oxidative stress to endothelium as well as available nitric oxide decreasing (a potent vasodilator) (Faeh et al., 2006) is homocysteine a risk factor or a biomarker. There is no data for classified Hcy as cardiovascular disease risk factor. Hcy is a novel marker that performs the criteria to categorize (Veeranna et al., 2011). Although decreasing Hcy amounts in patients with cardiovascular disorders has no effect, drugs as part of initially inhibitions strategy need to be measured much for verification (Veeranna et al., 2011). Outlook of Hcy as a risk factor for cardiovascular problems due to its role in decrease plasm Hcy amounts have not shown a positive consequence regarding the risk of cardiovascular problem event (Veeranna et al., 2011).

## 10. Homocysteine and liver disease

The role of liver in the metabolism and synthesis of Hcy is clear, therefore, liver problems may effect the Hcy amount also it represents expression of genes involved in Hcy metabolism and methionine (Cravo et al., 1996). Liver is responsible for large amounts of methionine metabolism (more than 80% of the whole body Trans methylation). The previous data reporting the near connection between serum levels of homocysteine and liver disorders (Finkelstein, 1998). Homocysteine levels were noted in all individuals with chronic liver disease or liver mass. There was a orientation towards boost Hcy concentrations in more advance levels of liver problem (Cravo et al., 1996). Relationship between increased Homocysteine and atherothrombotic vascular disorders is not clear in the pathophysiological survey (Finkelstein, 1998). Endothelial hurt, which can cause changed NO generation also devastated platelet acting, has been proved (McCully, 1996). Moreover, Hcy encourage DNA generation and collagen generation in VSMCs, (Majors et al., 1997) cholesterol production by hepatic cells (McCully, 1996) and the loss of the methyl group in the 5-methylcytosine nucleotide. These are certificate that Homocysteine beside vascular may happen on a variety of cellular contests. Cirrhosis is another problem related to Disruption of Homocysteine metabolism connected to reduce presence or usage of folic acid, vitamin$B_6$ and vitamin $B_{12}$. Homocysteine and vitamin $B_{12}$ levels were remarkably elevated in liver cirrhosis and liver cancer compared to chronic hepatic related disease (Karmin et al., 1998).

**10.1. About fatty liver disease:** Non-alcoholic fatty liver disease expression is referring to fatty liver which may affect liver tests and constant liver abnormalities due to the cumulation of lipids and triglycerides in the liver cellules and its known that factors such as insulin resistance, diabetes, hyperinsulinemia, obesity, hypertriglyceridemia and hypertension are the main responsible for liver metabolic disorders (Vanni et al., 2010). Non-alcoholic steatohepatitis and steatosis are types of non-alcoholic fatty liver disease. Simple fatty liver is another name of Steatosis in a case of excess of liver fat does not cause inflammation .In a case of developing non-alcoholic steatohepatitis the possibility of liver cancer and cirrhosis and is high. Extra liver fat has caused inflammation and if proceed it can bring about non-alcoholic steatohepatitis which can hurt hepatic cellules. Because of connection between Non-alcoholic fatty liver disease and hyper-Hcy, evaluation of their blood amounts is valuable (Lazo et al., 2011).

## 11. Diagnosed of measured homocysteine

Hcy amounts are evaluated through blood analysis. Researchers suggest the test in heart disease like high blood pressure or high cholesterol. Total Hcy is described as the aggregate of whole Hcy species serum or plasm, consisting free form and protein-bound form (Boushey et al., 1995). Measured amounts of circulating Hcy elevate risk potential of increasing coronary atherothrombotic diseases, circumferential vascular disease, myocardial disruption, and stroke (Boushey et al., 1995). Ion-exchange chromatography methods used to evaluate amino acids in lots of lab shows the main boosts in the amounts of homocystine (Boushey et al., 1995). In current years, but, it has been demonstrated that much less boosts in tHcy levels are detected in cobalamin , pyridoxine, and folic acid shortages and, in this circumstances, in subjects with a heat labile form of Methylene tetrahydrofolate reductase. also, just a lightly elevated total Homocysteine amount has improving role in cerebral, peripheral vascular and coronary disease, also venous thrombosis , as well as increasing risk of diabetes,

hyperlipidemia and hypertension. Laboratory studies are recognize between normal total homocysteine doping and risk factors that put individuals in these situations. In the following existence methods to do this recognition, so they come up with rules to explain and manage hyper-Hcy (Boushey et al., 1995).

**11.1. Measurement of homocysteine:** Homocystine (homocysteine-homocysteine disulfide) almost 70% of the Hcy in serum and plasm is combined with rest of cysteine in plasm proteins like albumin and just small levels presence as free Hcy and homocysteine-cysteine disulphide (Ueland et al., 1993). most labs have not enough ability to find the small levels of free amino acids in normal blood also they cannot help to find tiny elevate of those that may put individuals at the risk for cardiovascular problems. Dithiothreitol or beta-mercaptoethanol is available method to detect this process by addition of a lowering agent , to fresh obtained plasm, to detach Hcy from thiols and plasm proteins, before direct evaluation of tHcy with normal values of 5–15 μmol/L (or μM) (Araki and Sako, 1987; Ueland et al., 1993).

Reports indicate Homocysteine amounts using stable isotope–dilution gas chromatography–mass spectrometry (GC-MS) or high-performance liquid chromatography (Ungvari et al., 2003). However methods to evaluate total homocysteine by high-performance liquid chromatography are growing, lots of them are planned to find fluorescent derivatives of Hcy bound covalently to a chromophore. Simple automatized of these uses sample decreasing with tributylphosphine, follow by chromatograph technique of sulfhydril-consisting compositions like the **s**ulfobenzooxadiazole derivatives (Beetstra et al., 2008). Another High Performance Liquid Chromatography based techniques, like those that use mercury using gold electrodes to find not derivatized thiols or that change Hcy to S-adenosylmethionine, are used less frequently. Another altenative is fluorescent derivatives which is handling, however the o-phthaldehyde derivative of Hcy is labile and should be perfused into the High-performance liquid chromato-graphy slowly after it is produced, and monobromo-biamine, another derivatization chemical agent (Jacobsen et al., 1989), is itself fluorescent and can generate chromatographic summits that might entangle the examination. Despite the expensive price of this system, it is useful to make differential diagnosis between methylmalonic acid methionine, and cystathionine and also measured them at the same time (Stabler et al., 1987). Hcy is evaluated after capillary isotope–dilution GC-MS, by evaluation of the extent to which the internal standard is diluted by homocysteine in the sample and Total homocysteine levels might be evaluated by constant isotope–dilution GC-MS with opted ion monitoring which is deuterated Hcy is added to the sample as an internal standard before derivitization (Stabler et al., 1987).

**12. Hyper-Hcy therapy**

As mentioned before, a lot of surveys have determinate hyper-Hcy as an independent risk factor for disease related to cardiovascular problems (Johnson et al., 1991). Homocystinuria or severe hyper-Hcy is a rare autosomal recessive problem specified by intense increases in urine and serum Hcy levels. Clinical sings of homocystinuria such as impaired growth, thromboembolism, osteoporosis and intense coronary-artery disease (McCully, 1996; Eikelboom et al., 1999). High homocysteine levels can see in deficiencies of vitamins ($B_6$, $B_{12}$) and folate. However combined folate and vitamin B treatment essentially decreases Hcy amounts, the effect of vitamin therapy in placebo-controlled studies are mixed, but do not meet prospects (Eikelboom et al., 1999). Hyper-Hcy is typically run with folate, vitamins ($B_6$, $B_{12}$). As well as taurine supplementation has been detected to decrease homocysteine levels (McCully, 1996).

## CONCLUSION

Hyperhomocysteine is associated with a different of physical health problems, such as coronary artery disease, strokes. Increased Hcy level have a notably elevated risk of fatality. So as human it has important to consider Hcy as a main risk factor of various disease in animal and the role of homocysteine must consider as a one of the predominant diagnostic parameters as well as, it can be used as a therapeutic process to aid veterinarians in the treatment of related diseases.


## ACKNOWLEDGEMENT

Nothing to disclose.


## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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