High Cholesterol Levels And Chronic Exposure To Toxigenic Molds In Damp Buildings: A High Risk For Cardiovascular Diseases And Stroke

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Abstract

Epidemiological evidence suggests a possible association between chronic exposures to toxigenic molds in damp water-damaged buildings and high levels of cholesterol abnormalities that may represent a high risk for cardiovascular diseases. Although, cholesterol has many useful functions in the body, including its role in membrane structure, brain tissue, fetal development and the biosynthesis of other steroidal hormones, its harmful effects in the body have been widely publicized. Although, the physiological status of cholesterol in animals exposed to toxigenic molds have been reported, there is not enough experimental evidence to support similar occurrence in humans. The purpose of this review is to provide a basis for discussion in the light of on-going research on the topic. The effects of chronic toxigenic mold exposures on individuals in damp buildings are reviewed to provide the background for better understanding of their associations with the observed cholesterol abnormalities. The structure, and the regulatory mechanisms of cholesterol biosynthesis are assessed to identify the important enzymes interact with the toxigenic molds and mycotoxins to which abnormalities are enhanced. It was for this purpose that the effects of mycotoxins on metabolism, cellular supply and utilization of cholesterol and bile acid synthesis and related cardiovascular disease symptomology are evaluated. The outcomes of the reviews showed that, persons exposed to chronic toxigenic molds in damp buildings might be at risk of cardiovascular diseases and other related disorders. This assumption was based on the structural and functional activities highlighted by the evidence from animal models of cholesterolemia: cholesterol LDL-cholesterol/depression, atherosclerosis, premature coronary artery disease (CAD) and neurogenetic aspects of hypercholesterolemia. Given the fact that toxigenic molds release mycotoxins that affect human health, and given the possibility that structurally, and mechanistically, mycotoxins could adversely affect cholesterol metabolism through interactions with the related enzymes and disruption of cellular supply and utilization of cholesterol, and bile acid synthesis, it is most likely that individuals exposed to chronic toxigenic molds might be at risk of neurological disorders including cardiovascular diseases and stroke.

INTRODUCTION

Human exposure to toxigenic molds in damp buildings is a primary environmental health problem associated with adverse health effects, and which costs millions of dollars to insurance companies, homeowners, and schools. There is increasing concern that, a significant number of individuals exposed to chronic mold in water-damaged buildings may be at high risk of cholesterol abnormalities. Cholesterol is an important biomolecular constituent of the cell membrane structure. It serves as a precursor for the synthesis of steroid hormones and bile acids. Both dietary cholesterol and that synthesized de novo are transported through the circulation in lipoprotein particles. The same is true of cholesteryl esters, the form in which cholesterol is stored in the cells. Cholesterol balance is essential to life yet its deposition in arteries has been associated with cardiovascular disease and stroke. Of particular clinical importance is the abnormal deposition of cholesterol and cholesterol-rich lipoproteins in the coronary arteries. Such deposits eventually lead to arteriosclerosis, a contributory factor in diseases of the coronary arteries. Arteriosclerosis is the hardening of the arteries and it is the cause of more than half of all mortality in developed countries and the leading cause of death in the US. More than 90 million American adults, or about 0 percent, have elevated blood cholesterol levels, one of the key risk factors for heart disease, according to the National Heart, Lung, and Blood Institute's National Cholesterol Education Program. When it affects the coronary arteries, it is the underlying cause of most heart attacks and a common cause of cognitive heart failure and arrhythmias.
The pathological process of arteriosclerose begins very early with a fatty streak composed of lipid deposited in the intima of arteries. Modified macrophages known as foam cells accumulate in the plaque region. These foam cells accumulate lipids, especially oxidized low-density lipoproteins. When the lesion becomes infiltrated with fibrous material it protrudes into the lumen of the artery. The lesion itself rarely occludes the artery but rather it is blood clots that form on top of the plaque that close off the channel. Chronic lesions become calcified and the elasticity of the vessel is decreased. This hardening of the arteries causes an increase in resistance to blood flow and therefore an increase in blood pressure. Any vessel in the body may theoretically be affected by arteriosclerosis, but the aorta, coronary, carotid, and iliac arteries are most frequently affected. Arteriosclerose is a common cause of aortic aneurysms (a local abnormal dilation of an artery due to a congenital defect or weakness of the vessel wall) while those in the periphery are usually caused by damage due to trauma or bacterial or fungal infection.

In recent years, these harmful roles of cholesterol in the body have been widely publicized because of the increase in the occurrence of cardiovascular diseases and stroke. Research evidence suggests that high levels of cholesterol are the main culprit. Although, the physiological status of cholesterol in animals exposed to toxigenic molds have been reported, there is insufficient concrete evidence to support similar toxigenic mold-related occurrences in humans. The purpose of this review is to synthesize that could form the basis for discussions in the light of on-going research on the topic. The effects of chronic toxigenic mold exposures on individuals in damp buildings are reviewed to set the stage for better evaluation and assessment of their associations with the observed cholesterol abnormalities.

The structure, and the regulatory mechanisms of cholesterol biosynthesis are assessed, to identify the important enzymatic stages and the rate limiting steps that might be amenable to a possible influence of the mycotoxins consequent upon which abnormalities are enhanced. Invariably, the likely effects of mycotoxins on the stages of cholesterol metabolism steady state of cellular supply, utilization, bile acids synthesis, and the associated cardiovascular diseases are evaluated.

DISCUSSION

The outcomes of the extensive reviews showed that persons exposed to chronic toxigenic molds in damp buildings might be at risk of cardiovascular diseases and other related disorders. These outcomes were based on the structural and functional activities highlighted by the evidence from animal models of cardiovascular diseases and stroke to which cholesterolemia: cholesterol LDL-cholesterol/depression, atherosclerosis, premature coronary artery disease (CAD) and neurogenetic aspects of hypercholesterolemia are significantly associated. It was hypothesized therefore, that given the fact that toxigenic molds release mycotoxins that affect animals and human health, and given the possibility that structurally, and mechanistically, mycotoxins could adversely affect cholesterol at all stages of metabolism through the intercellular interactions, it is most likely that individuals exposed to chronic toxigenic molds might be at risk of neurological disorders including cardiovascular diseases and stroke.

TOXIGENIC MOLD EXPOSURES IN DAMP HOMES

It is widely acknowledged in the indoor air quality (IAQ) research community that biological contamination is one of the important indoor air pollutants to which toxigenic molds are one of the major environmental health concerns, especially, in damp residential and occupational buildings. The airborne toxigenic molds and their metabolites can induce irritational, allergic, infectious, and chemical responses in exposed individuals. The mold spores, because of their size and mass, can easily settle rapidly within the indoor environment and over time they may become nonviable and fragmented by the process of desiccation. Such fragments of molds are common in contaminated environments and can be toxic or allergic, depending upon the specific mold mycotoxins. Molds exhibit their toxigenic effects through the production of mycotoxins, and by contact with humans through inhalation. Although there are several pieces of evidence for a relationship between high levels of inhalation exposure or direct contact to mycotoxin-containing molds or mycotoxins, and demonstrable effects in animals and health effects in humans, the current literature does not provide compelling evidence that exposure at levels expected in most mold-contaminated indoor environments is likely to result in measurable health effects. However, in spite of this, the question of adverse effects is not debatable.

THE STRUCTURE OF CHOLESTEROL

Cholesterol belongs to a large group of biological molecules called steroids that have a similar four-ring structure, a cyclopentanoperhydrophenanthrene ring. Because of the well-established positive association between plasma...
cholesterol concentration and coronary heart disease (CHD), people think that cholesterol is only a harmful biological chemical. However, contrary to that belief, cholesterol plays very important roles the general human physiology especially, in structural integrity of the membranes, production of bile acids, and steroid hormones, including sex and adrenal hormones.

**EFFECTS OF MYCOTOXINS ON BIOSYNTHESIS OF CHOLESTEROL**

To enable a clear understanding of where, how and why abnormal cholesterol occurs in individuals exposed to chronic toxigenic molds, it is appropriate to give a brief review of cholesterol metabolism to appreciate the cellular utilization and transport process. In good health, cholesterol metabolic factors must be at a homeostatic balance in order that the body functions properly. Approximately a half of the cholesterol in the human body is derived from biosynthesis de novo biosynthesis, 10% in the liver, and approximately 15% in the intestines each day. Also cholesterol can be synthesized in the cytoplasm and microsomes from the two-carbon acetate group of acetyl-CoA. The synthesis of cholesterol starts in the cytoplasm with the transport of acetyl-CoA from the mitochondrion to the cytosol. Acetyl-CoA units are converted to mevalonate by a series of reactions that begins with the formation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). Two moles of acetyl-CoA are condensed in a reversal of the thiolase reaction, forming acetoacetyl-CoA. Acetoacetyl-CoA and a third mole of acetyl-CoA are converted to HMG-CoA by the action of HMG-CoA synthase. HMG-CoA is converted to mevalonate by HMG-CoA reductase (this enzyme is bound to the endoplasmic reticulum). HMG-CoA reductase absolutely requires NADPH as a cofactor and two moles of NADPH are consumed during the conversion of HMG-CoA to mevalonate.

The rate-limiting step of cholesterol biosynthesis is catalyzed by HMG-CoA reductase that is responsible for most of the complex regulatory controls. Through three successive phosphorylations, mevalonate is activated to yield 5-pyrophosphomevalonate. Then, an ATP-dependent decarboxylation results in the formation of isopentenyl pyrophosphate, (IPP), an activated isoprenoid molecule. IPP at this point is in equilibrium with its isomer, dimethylallyl pyrophosphate (DMPP). One molecule of DMPP condenses with one molecule of IPP to generate geranyl pyrophosphate (GPP). GPP further condenses with another IPP molecule to yield farnesyl pyrophosphate (FPP). Finally, the NADPH-requiring enzyme, squalene synthase catalyzes the head-to-tail condensation of two molecules of FPP, yielding squalene (squalene synthase also is tightly associated with the endoplasmic reticulum). Squalene undergoes a two-step cyclization to yield lanosterol. The first reaction is catalyzed by squalene monooxygenase. This enzyme uses NADPH as a cofactor to introduce molecular oxygen as an epoxide at the 2,3 position of squalene. Through a series of 19 additional reactions, lanosterol is converted to cholesterol. Intracellularly, the overall reaction for the pathway is represented as follows:

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6 \text{Acetyl-CoA} + 6 \text{Acetoacetyl-CoA} + 14 \text{NADPH} + 14 \text{H}^+ + 5 \text{H}_2\text{O} + 18 \text{ATP} + \text{O}_2
\]

\[
\text{Lanosterol} + 14 \text{NADP}^+ + 12 \text{CoA-S-H} + 18 \text{ADP} + 6 \text{P}_i + 4 \text{PP}_i + 6 \text{CO}_2.
\]

**MYCOTOXINS AND CHOLESTEROL SYNTHESIS REGULATION**

There are several enzymes involved in cholesterol biosynthesis including synthases, reductases, decarboxylases, and transferases etc. some of which could interact with the reactive groups in mycotoxins. Normal healthy adults synthesize cholesterol at a rate of approximately 1g/day and consume approximately 0.3g/day. A relatively constant level of cholesterol in the body (150 - 200 mg/dL) is maintained primarily by controlling the level of de novo synthesis. Although, the level of cholesterol synthesis is regulated in part by the dietary intake of cholesterol, cholesterol from both diet and synthesis is utilized in the formation of membranes and in the synthesis of the steroid hormones and bile acids. The greatest proportion of cholesterol is used in bile acid synthesis. Evidently, mycotoxins have the properties that could disrupt the enzymes that maintain the cholesterol balance in the body. This disruption can be exemplified by some mechanisms that involve the breaking and remaking of bonds.

**PROPOSED MECHANISM OF MYCOTOXIN EFFECTS ON CHOLESTEROL METABOLISM**

Considering the molecular structure of mycotoxins such as aflatoxin B1 and G1, produced by certain species of Aspergillus flavus, and cholesterol, the mechanicic associations of the functional groups, there seemed a possibility of interactions through the breaking and remaking of bonds. Already, it is known that the rate-limiting step of
The exact mechanism for this cholesterol-induced regulation of gene activity is not known, however, mycotoxins could be implicated in this circumstance. Regulation of HMG-CoA reductase through covalent modification occurs as a result of phosphorylation and dephosphorylation. Mycotoxins could modify the enzyme's most active form thereby decreasing the rate of phosphorylation of the enzyme activity. HMG-CoA reductase is phosphorylated by AMP-regulated protein kinase (AMPRK) hence, it is also possible that mycotoxins can deactivate AMPRK via phosphorylation since phosphorylation of AMPRK is catalyzed by kinase.

EFFECTS OF MYCOTOXINS ON CHOLESTEROL BALANCE

The risk of cardiovascular disease associated with cholesterol is dependent on the contrast of balance between the levels of two types of lipoproteins and their quantity in the blood. The first is called the low-density lipoprotein (LDL) form in which cholesterol is carried into the blood and is the main cause of harmful fatty buildup in arteries. The higher the LDL cholesterol levels in the blood, the greater the heart disease risk. The second is the high-density lipoprotein (HDL) that carries blood cholesterol back to the liver, where it can be eliminated. HDL helps prevent a cholesterol buildup in blood vessels. Low HDL levels increase heart disease risk. One of the primary ways LDL cholesterol levels can become too high in blood is through eating too much of two nutrients: saturated fat, which is found mostly in animal products, and cholesterol, found only in animal products. Saturated fat raises LDL levels more than anything else in the diet. Plasma levels of HDL cholesterol are strongly inversely associated with atherosclerotic cardiovascular disease, and overexpression of HDL proteins, such as apolipoprotein A-I in animals, reduces progression and even induces regression of atherosclerosis. Therefore, HDL metabolism is recognized as a potential target for therapeutic intervention of atherosclerotic vascular diseases. The antiatherogenic properties of HDL include promotion of cellular cholesterol efflux and reverse cholesterol transport, as well as antioxidant, anti-inflammatory and anticoagulant properties. The molecular regulation of HDL metabolism is not fully understood, but it is influenced by several extracellular lipases. The effects of mycotoxins on the enzymatic activity of lipase is not known, however, assumed that, since...
mycotoxins are structurally and functionally similar to the compounds that inhibit the enzymatic activity of lipase, have the propensity to influence the role of secreted lipases on HDL metabolism and their relationship to atherosclerosis. In addition to impaired LDL receptor-mediated clearance of low density lipoproteins in FH, evidence from in vitro and in vivo studies suggests that hepatic oversecretion of apoB may contribute to the hypercholesterolemia.

MYCOTOXIN INHIBITORY EFFECTS ON LIPASE
Some toxigenic molds such as trichotheccenes produce mycotoxins and volatile organic compounds (e.g., alcohols) that inhibit cholesterol biosynthesis. The kinetics of alcoholysis showed that the enzyme (lipases B) produced by Candida antarctica has inhibitory effects on cholesterol metabolism. Gastric lipase plays an important role in emulsification and digestion of food fat and since lipids are components of the hydrophobic mucus and mucosa barrier, damage of the gastric mucosa may therefore lead to changes in the lipid content and gastric lipase activity. Although, some molds are shown to prevent the development of premature atherosclerosis, however, the clinical associations others with plaque and coronary heart disease outcomes have been frequently reported in other neurological disorders. Mycotoxins may inhibit the activity of HMG CoA reductase, thereby, affecting the fibrinolytic system of human vascular cells.

EFFECTS OF MYCOTOXINS ON STEROID HORMONES
Steroid hormones are biologically active compounds that are synthesized from cholesterol and have in common a cyclopentanoperhydrophenanthrene ring. Steroid hormones are secreted by the adrenal cortex, testis, ovary, and placenta and include the progestogens, glucocorticoids, mineralocorticoids, androgens, and estrogens. They act by binding to specific receptors to form complexes, which then enhance or inhibit the expression of specific genes. Since the intracellular level of cAMP is regulated by hormonal stimuli, regulation of cholesterol biosynthesis is hormonally controlled. When levels of cholesterol are high, the level of expression of the HMG-CoA reductase gene is reduced. Conversely, reduced levels of cholesterol activate expression of the gene. It is suspected that mycotoxins can bring about the deregulation of cholesterol metabolism by decreasing the level of HMG-CoA reductase synthesis since the rate of HMG-CoA turnover is also regulated by the supply of cholesterol.

EFFECTS OF MYCOTOXINS ON THE UTILIZATION OF CHOLESTEROL
Cholesterol is transported in the plasma predominantly as cholesteryl esters associated with lipoproteins. Dietary cholesterol is transported from the small intestine to the liver within chylomicrons. Cholesterol synthesized by the liver, as well as any dietary cholesterol in the liver that exceeds hepatic needs, is transported in the serum within LDLs. The liver synthesizes VLDLs and these are converted to LDLs through the action of endothelial cell-associated lipoprotein lipase. Cholesterol found in plasma membranes can be extracted by HDLs and esterified by the HDL-associated enzyme LCAT. The cholesterol acquired from peripheral tissues by HDLs can then be transferred to VLDLs and LDLs via the action of cholesteryl ester transfer protein (apo-D) which is associated with HDLs. Reverse cholesterol transport allows peripheral cholesterol to be returned to the liver in LDLs. Ultimately, cholesterol is excreted in the bile as free cholesterol or as bile salts following conversion to bile acids in the liver.

EFFECTS OF MYCOTOXINS ON BILE ACIDS SYNTHESIS AND UTILIZATION
The end products of cholesterol utilization are the bile acids, synthesized in the liver. Synthesis of bile acids is the predominant mechanisms for the excretion of excess cholesterol. However, the excretion of cholesterol in the form of bile acids is insufficient to compensate for an excess dietary intake of cholesterol. Bile acids are carried from the liver through these ducts to the gallbladder, where they are stored for future use. The ultimate fate of bile acids is secretion into the intestine, where they aid in the emulsification of dietary lipids. In the gut the amino acid (glycine and taurine) residues are removed and the bile acids are either excreted or reabsorbed by the gut and returned to the liver. This process is termed the enterohepatic circulation. At high bile-salt-secretion rates the biliary transport allows peripheral cholesterol to be returned to the liver in LDLs. Ultimately, cholesterol is excreted in the bile as free cholesterol or as bile salts following conversion to bile acids in the liver.

EFFECTS OF MYCOTOXINS ON CHOLESTEROL METABOLISM
Individuals with toxigenic mold infection (e.g., candidiasis) showed low values of cholesterol and high values or levels of LDH, urea N, creatinine and total bilirubin and the
common sites of infection are the respiratory system, esophagus, stomach, and intestinal tract. In the presence of granulocytopenia and immunodeficiency, tissue invasion become severe and associated with vascular invasion,\textsuperscript{27} Animal study,\textsuperscript{28} of the effect of oyster fungus (Pleurotus ostreatus) on the serum and liver lipids of growing male Syrian hamsters showed an increase in serum cholesterol, triacylglycerol (TG) and phospholipid (PL) concentration, 40 - 60% of which was accounted for by an increase in the HDL concentration. The proportion of VLDL in the lipoprotein pool rose by almost 15%, whereas the proportion of HDL fell. Both the serum TG and the VLDL concentration fell by 30%, but neither the chemical composition nor concentration of the HDL nor the cholesterol concentration were affected. The addition of the fungus to the diet completely abolished the increase induced in the liver cholesterol and TG concentration by the chronic intake of alcohol.\textsuperscript{29} The animals fed on fumonisin B1 diet (EFD) and mixtures of mycotoxins diet (EMD) showed marked abnormal values cholesterol, alkaline phosphatase (ALP), calcium, triglycerides, and aspartate transaminase (AST), with severe lesions in the liver.\textsuperscript{29}

**CLINICAL SIGNIFICANCE**

There are three major cellular mechanisms by which a steady state of cholesterol is maintained in the body systems. These mechanisms include: the regulation of HMG-CoA reductase activity, regulation of excess intracellular free cholesterol through the activity of acyl-CoA-cholesterol acyltransferase (ACAT), and the regulation of plasma cholesterol levels via LDL receptor-mediated uptake and HDL-mediated reverse transport. The possible overall clinical significance of all the effects of mycotoxins on the cholesterol metabolism include: hypertension or high blood pressure, atherosclerosis (the hardening of the coronary arteries) the underlying cause of most heart attacks and a common cause of congestive heart failure and arrhythmias. The pathological process starts very early with a fatty streak composed of lipid deposited in the intima of arteries.

Venous thrombosis and thrombophlebitis (may be permanent or temporary) are another likely consequences of cholesterol disregulation due to mycotoxins. The obstruction of a portion of the trunk or main branches of the veins causes vessels distal to the point of obstruction to dilate and can result in permanent damage to valves and vessel walls due to pressure, hypoxemia, stretch, and malnutrition. Edema may also result from damage to peripheral vessels. Bile acids perform four physiologically significant functions: their synthesis and subsequent excretion in the feces represent the only significant mechanism for the elimination of excess cholesterol; bile acids and phospholipids solubilize cholesterol in the bile, thereby preventing the precipitation of cholesterol in the gallbladder; they facilitate the digestion of dietary triacylglycerols by acting as emulsifying agents that render fats accessible to pancreatic lipases, and they facilitate the intestinal absorption of fat-soluble vitamins.\textsuperscript{11}

Total cholesterol and LDL-cholesterol levels in the blood sample from persons with chronic toxic mold exposures were determined and correlated with the ECG findings. Although, these individuals had no specific diet or medication that could influence the levels of lipids in the their serum, they showed evidence of depression according to DSM-IV definition. Correlations between the level of total cholesterol and severity of depressive symptoms and between total serum cholesterol and LDL-cholesterol and chronic toxic mold exposures have been reported.\textsuperscript{20-21,52-53} The early lesions of atherosclerosis in youth are strongly related to antemortem levels of total and LDL cholesterol, VLDL cholesterol, and triglyceride to ponderal index and to blood pressure.\textsuperscript{34} The presence of functionally abnormal monocytes in hypercholesterolemia suggests the importance of such cells in the premature atherosclerosis that occurs in these individuals.\textsuperscript{35}

**CONCLUSIONS**

The structure, synthesis, and utilization of cholesterol and the regulatory processes that prevent over-accumulation and abnormal deposition within the body and the associations with cardiovascular diseases have been reviewed. High cholesterol levels in individuals with chronic exposures to toxigenic molds in damp buildings were observed. Whether the observed cholesterol levels in living under chronic toxigenic molds are related to mycotoxins is yet to be determined. However, based on the findings in the literature and considering the structural disposition of cholesterol that has mechanistically functional affinity to that of mycotoxin, it is predicted that the abnormal cholesterol levels in individuals presenting with chronic toxicogenic mold exposures might be related to adverse interaction between cholesterol and mycotoxins. If the prediction is confirmed, then, high cholesterol levels in individuals with chronic exposures to toxigenic molds in damp buildings may lead to a high risk for cardiovascular diseases and stroke.
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