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REVIEW

Lifestyle diseases and cardiovascular risk factors are interrelated to deficiencies of major substrates in ATP synthesis

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Abstract: Recent studies on diabetes and metabolic syndrome indicate a common disturbance of inorganic phosphate (Pi) metabolism. Pi is an important substrate in the formation of adenosine triphosphate (ATP), and many lifestyle diseases and cardiovascular risk factors similarly show deficiencies in either 1 or 2 major components of ATP synthesis. Age, male gender, hypertension, obesity, hypertriglyceridemia, metabolic syndrome, and diabetes mellitus are all associated with hypophosphatemia. In addition, tobacco smoking, hyperchylomicronemia, hypertension, and diabetes may involve defects in tissue oxygen delivery. Hypophosphatemia may lead to a critical decrease in intracellular Pi and to mitochondrial dysfunction, which might be counteracted by the pharmacological use of fructose 1,6-diphosphate.

Keywords: hypophosphatemia, hypoxia, diabetes, metabolic syndrome, hypertension

Introduction

Inorganic phosphate (Pi) is the ubiquitous anion required for intermediate metabolism and energy-transfer mechanisms. Pi is a vital component of DNA and RNA, and it is also present in phospholipids in membranes. Pi participates in both glycolysis and oxidative phosphorylation, the 2 major sources of adenosine triphosphate (ATP). In glycolysis, Pi is a substrate for glyceraldehyde-3-phosphate dehydrogenase and stimulates the activity of hexokinase and phosphofructokinase. In oxidative phosphorylation, Pi is a putative signaling molecule and takes part in the phosphorylation potential ATP/ADP × Pi. Bose et al[1] have demonstrated in a multiparameter monitoring system applied to heart and skeletal muscle mitochondria that Pi controls the oxidative metabolism in a balanced fashion. Optimal amounts of both Pi and oxygen are required for a continuous supply of free energy according to the classical equation for oxidative metabolism:[2] $3ADP + 3Pi + 1/2O_2 + NADH \rightarrow 3ATP + NAD^+ + H_2O$. If the production of ATP is interrupted or stopped for any reason, it may have serious consequences and may possibly lead to cell injury or cell death.

Plasma Pi is held within narrow limits through a complex interplay between intestinal absorption, exchange with intracellular and bone storage pools, and renal reabsorption. Intestinal absorption is mediated by sodium-phosphate cotransporter protein NaPi-IIb, which can be up-regulated and down-regulated when needed to maintain and exchange with intracellular and bone storage pools and renal reabsorption. The kidneys are the major regulators of Pi homeostasis, controlled by both hormonal and nonhormonal factors, and can increase and decrease Pi reabsorption capacity to accommodate the Pi need. The critical regulated step in this process is the transport of

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Pi across the luminal membrane of the renal proximal tubules by the sodium phosphate-dependent cotransporter proteins that are bound to the brush border membrane, and the last intracellular loop of NaPi-IIa contains sequence information that confers parathyroid hormone (PTH) sensitivity.[3] Because of the extensive literature, it is beyond the scope of this article to go further into details in the regulation of Pi homeostasis by PTH, vitamin D, phosphocalcins as fibroblast growth factor 23, phosphate regulating and Klotho genes, and more, but readers are referred to excellent reviews.[4,5]

Recently, we demonstrated a paradoxical metabolic imbalance in Pi from the early onset of diabetes, which may lead to a reduction in high energy phosphates and tissue hypoxia.[6,7] This imbalance may be associated with the risk of late diabetic complications including cardiovascular disease. Hypophosphatemia, likewise, has been reported in studies of large numbers of individuals with metabolic syndrome.[8,9] As metabolic syndrome also has been shown to be closely associated with cardiovascular diseases,[10,11] we were stimulated to examine through Medline (PubMed), Embase, and reference lists whether other lifestyle diseases and risk factors may show similar biochemical alterations.

In this article, we will present evidence suggesting that many lifestyle diseases and risk factors are interrelated to deficiencies in 1 or 2 major components for optimal generation of ATP, eg, phosphate and/or oxygen.

Increasing age and male gender

More than 80% of deaths from coronary heart disease occur in patients of 65 years or older, an age at which female heart attack patients are more likely to die than men despite the fact that men have a greater risk of heart attack and suffer heart attacks earlier in life. Even after menopause, when women's death rate from heart disease increases, it is not as great as men.[12]

The concentration of plasma Pi and the maximal renal tubular reabsorption of phosphate (T_{mPO4}/GFR) are closely related to age and sex, with the highest values occurring in childhood. In adults, plasma Pi in men declines with age almost linearly, whereas in women under the age of 45, the values overlap those of men and then increase between 45 and 54 years before declining thereafter. The practical implication is that phosphate-depleting disorders, such as diabetes and metabolic syndrome, might induce hypophosphatemia more easily in older persons because of the diminished tubular capacity for phosphate reabsorption before the development of such disorders.[13]

Diabetes mellitus

Cardiovascular diseases are the leading cause of diabetes-related deaths. Substantial clinical and experimental evidence suggest that both diabetes and insulin resistance cause endothelial dysfunction, which may diminish the antiatherogenic role of the vascular endothelium. In this respect, it is of interest that hypophosphatemia has been shown to increase insulin resistance and induce glucose intolerance.[14–17] Hypophosphatemia is a common finding in both type 1 and type 2 diabetes, and several investigators have found decreased concentration of Pi in poorly regulated diabetic patients and slightly elevated levels when optimally controlled.[6]

Oxyhemoglobin dissociation is a measure of the ability of the red cells to release oxygen as they pass through the microcirculation. The position of the oxyhemoglobin dissociation curve (ODC) is often expressed by the P_{50}, (oxygen tension at 50% oxygen saturation). The position of the ODC is dependent of red cell 2, 3-diphosphoglycerate (2, 3-DPG) concentration. Oxygen release to tissues can be increased by interaction with a number of organic phosphates, primarily 2, 3-DPG. In a variety of situations where tissue oxygenation is impaired, the 2, 3-DPG level rises, producing a proportional increase in P_{50} (a right shift of ODC). Such changes have been reported in anemia,[18,19] cardiac failure,[20,21] and cardiopulmonary insufficiency.[22,23] Conversely, impaired synthesis of 2, 3-DPG is associated with a left shift of the ODC leading to a decreased delivery of oxygen to the venous end of the microvasculature (venous part of capillaries and venules).

In newly diagnosed, nonacidotic, type 1 diabetic patient's plasma, the Pi concentration was normal at admission, lower on the day after initial insulin administration, and slightly above normal on the day when the best metabolic control is achieved. Red cell 2, 3-DPG exhibited the same fluctuating pattern, and Pi correlated closely with 2, 3-DPG ($r = 0.61$, $P < 0.001$). Red cell 2, 3-DPG correlated equally well with P_{50} of the ODC.[24,25]

To clarify the underlying mechanism leading to hypophosphatemia, a study was performed in comparable groups of ambulatory, nonacidotic, insulin-dependent diabetic and healthy children. The average plasma Pi was significantly lower in the 26 children with diabetes compared with 28 healthy children (1.36 vs 1.48 mmol/L, $P < 0.005$). In children with diabetes, the urinary phosphate excretion rate was significantly elevated (1.19 vs 0.43 mmol/h, $P < 0.001$), and phosphate excretion rate positively correlated with the urinary excretion rate of glucose ($r = 0.53$, $P < 0.01$) and with the blood glucose ($r = 0.52$, $P < 0.01$). The renal

threshold concentration of phosphate (T_{mPO4}/GFR) was significantly suppressed in the children with diabetes (1.23 vs 1.73 mmol/L, $P < 0.001$). This disturbance was related neither to changes in serum PTH nor to changes in growth hormone, but inversely correlated with the degree of hyperglycemia ($r = 0.61$, $P < 0.001$). The study demonstrates an abnormality in tubular phosphate reabsorption, which is related to glycemic regulation.[26]

However, in ambulatory subjects with juvenile diabetes with no evidence of vascular complications, despite an almost 30% increase in the concentration of 2, 3-DPG at the same hemoglobin content, the P_{50} of the ODC was not increased.[27] This may be explained by the fact that hemoglobin A_{1c} has increased the oxygen affinity and may react less readily with 2, 3-DPG compared with regular hemoglobin A.[28]

Therefore, in patients with both type 1 and type 2 diabetes, there is a close correlation between the Pi concentration in plasma and improved diabetes control. In order for patients with diabetes to achieve optimal oxygen delivery to tissues, the concentration of 2, 3-DPG and Pi must be higher than in healthy persons, partly because the red blood cells of patients with diabetes contain higher concentrations of hemoglobin A_{1c}.

Obesity

Obesity is a major risk factor for cardiovascular diseases. Lindgärde and Trell[29] found an inverse correlation between plasma Pi and body weight in 752 men born in 1926. Other parameters of minerals with possible relevance to their findings were not found. In 194 subjects with a wide range of body mass index (BMI), Lind et al[30] found that plasma Pi inversely correlated with BMI and fat distribution. PTH was not significantly correlated with any obesity parameters. Håglin et al[8] conducted a large study of 1,272 women in whom serum phosphate inversely correlated with BMI.

Dyslipidemia

In apparently healthy persons, total cholesterol and low-density lipoprotein cholesterol positively correlated with increasing Pi levels, and triglyceride concentration negatively correlated with plasma Pi levels.[9]

Hypertriglyceridemia and especially hyperchylomicronemia have been found to interfere with tissue oxygen delivery. A marked left shift of the ODC has been demonstrated in familial type 1 hyperlipoproteinemia (familial combined hyperlipidemia), in diabetic and nondiabetic persons with hyperlipoproteinemia type V (mixed hypertriglyceridemia), and in blood mixed with lipid emulsions.[31–33] The reason for this negative effect on tissue oxygen delivery might be an abolishment of the pH difference across the erythrocyte membrane resulting in a displacement of the ODC to the left by the Bohr effect.[34]

Tobacco smoking

Acute myocardial infarction or sudden death in patients with coronary artery disease is among the disorders strongly associated with cigarette smoking.[35]

Cigarette smoking is associated with increased levels of carboxyhemoglobin in the blood. This leads to a decrease in available hemoglobin for oxygen transport and will shift the ODC to the left, decreasing the volume of oxygen that can be unloaded to the tissue at any given P_{O_2}. Carbon monoxide also reduces the formation of 2, 3-DPG by inhibiting glycolysis in the erythrocytes. The P_{50} of the ODC was decreased 4 mmHg in a study of cigarette smoking.[36,37] Smoking also releases the sympathetic neurotransmitter norepinephrine and the adrenomedullary hormone epinephrine and may thereby increase tissue oxygen demand.[38] The oxygen availability or demand ratio will, therefore, decrease and the oxygen deficit may participate in adverse changes in the cardiovascular system.

Alcohol abuse

The metabolic effects of alcohol in man are complex since some are due to the direct action of ethanol or its metabolites, whereas some are related to the changes in the redox state or to nutritional factors.[39] Hypophosphatemia and phosphate depletion are well recognized consequences of acute and chronic alcohol abuse.[40] Hypophosphatemia has been suggested to cause myopathy, rhabdomyolysis, and cardiomyopathy.[41] Hypophosphatemia may be caused by a reduced renal threshold concentration of phosphate, but this might be only a part of a more complex tubular dysfunction.[42,43] The presence of glycosuria and aminoaciduria with reduced renal threshold concentration of phosphate suggests a generalized reduction in the reabsorption ability of the proximal tubules. These data are supported by experimental studies indicating that ethanol interferes with the carrier function of the tubular cells by decreasing Na^+/K^+-ATPase activity.

Red cell 2, 3-DPG has been found to be increased, which has been suggested to be caused by a decrease in CO_2 production (cellular oxidation of alcohol produces 33% less CO_2 than cellular oxidation of glucose) leading to increased red cell glycolysis compensatory to the left shift of the ODC due

to the Bohr effect.[44] The similarities of alcohol intoxication and oxygen deprivation warrant further studies.

Hypertension

Ljunghall and Hedstrand[45] conducted a population study of more than 2,000 men aged 49–50 years and showed that plasma Pi, within normal range, was inversely related to blood pressure. The presence of hypophosphatemia in hypertension has repeatedly been confirmed.[46,47] Interestingly, a significant correlation was found between the rise in the initially low plasma Pi and the lowering of the initially high arterial blood pressure in overweight patients with essential hypertension.[48]

In spontaneous hypertensive rats, it was shown that the Pi and ATP in the vascular walls were reduced and that this reduced energy availability may contribute to a reduced response to vasoconstrictor agonists (noradrenaline and angiotensin II).[49] Bindels et al[50] in their study of hypertensive rats found that a disturbance in phosphate metabolism was already present at 6 weeks of age and that hypophosphatemia and hypophosphaturia were accompanied by an adaptive change in the transport capacity of Na^+-dependent phosphate transport in brush border membranes from renal cortex. Older animals, at age 20 week, with marked hypophosphatemia showed reduced content of red cell 2, 3-DPG.

Epinephrine is a hypophosphatemic hormone in men,[51] and patients with hypertension have often been found to have increased plasma catecholamine concentrations consistent with the theory of a pathophysiologic role for increased sympathetic activity in this disease.[52]

Metabolic syndrome

There are different definitions of the metabolic syndrome. According to the Adult Treatment Panel III guidelines, metabolic syndrome is based on the presence of 3 or more of the following criteria: hypertension, impaired glucose tolerance, abdominal obesity, and dyslipidemia involving hypertriglyceridemia and decreased high-density lipoprotein cholesterol values.[53]

It was recently suggested that a disturbance in Pi metabolism may be a basic and fundamental representative of this metabolic syndrome. Håglin[54] studied 2,752 consecutive patients (1,190 men, 1,562 women) admitted during the years 1986–1996 to the patient education centre of Vindeln, a small community in northern Sweden. It was found that a low plasma Pi was associated with high BMI, high blood glucose, high systolic and diastolic blood pressures, but low serum high-density lipoprotein and serum magnesium levels. Another study by Kalaitzidis et al[55] on 254 persons with a diagnosis of metabolic syndrome was based on Adult Treatment Panel III guidelines. Subjects with fewer than 3 criteria served as controls. Patients with metabolic syndrome showed significantly lower plasma Pi and magnesium levels than controls. Because the fractional excretion of phosphate was similar in both groups, it was assumed that hypophosphatemia in patients with metabolic syndrome was attributable to decreased dietary intake and internal redistribution of this element.

Discussion

Harden and Young[56] in 1906 were the first to note that phosphorus played an important role in carbohydrate and energy metabolism, and the importance of phosphorus has been looming larger ever since. They furthermore found in living tissue a sugar-phosphate ester consisting of fructose with 2 phosphate groups, fructose diphosphate, also called Harden–Young ester.[56] Pi is an important substrate for ATP formation both by oxidative phosphorylation and by glycolysis. Because phosphate is a component of ATP, it also plays a pivotal role in the energy-related processes that take place in the endothelium and muscle cells of the cardiovascular system.[57] Furthermore, without optimal oxygenation to the terminal electron acceptor in a reaction that is carried out by cytochrome c oxidase in the electron transport chain in mitochondria, the consequence of mitochondrial dysfunction may be endothelial dysfunction, increased endothelial permeability, or cell lyses.

Our study indicates that age, gender, hypertension, obesity, diabetes, hyperglyceridemia, and the metabolic syndrome all are associated with hypophosphatemia and increased risk for cardiovascular diseases. In addition, tobacco smoking, diabetes, and hyperchylomicronemia show defects in tissue oxygen delivery (Figure 1). In hypertension, microcirculation plays a critical role in that the increase in peripheral resistance underlying the raised blood pressure is localized to a narrowing of small arteries and precapillary arterioles with rarefaction of capillaries possibly leading to ischemic hypoxia. In diabetes, affinity hypoxia may be related to the increased levels of glycosylated hemoglobin, relative or absolute hypophosphatemia, and red cell 2, 3-DPG content. In diabetes mellitus, a major disturbance in phosphate handling occurs in the kidney tubules, where the excessive sodium-dependent glucose reabsorption in patients with diabetes depolarizes the electrochemical sodium gradient. Since Pi uses the same driving force but has less binding ability to sodium than glucose and aminoacids, such as

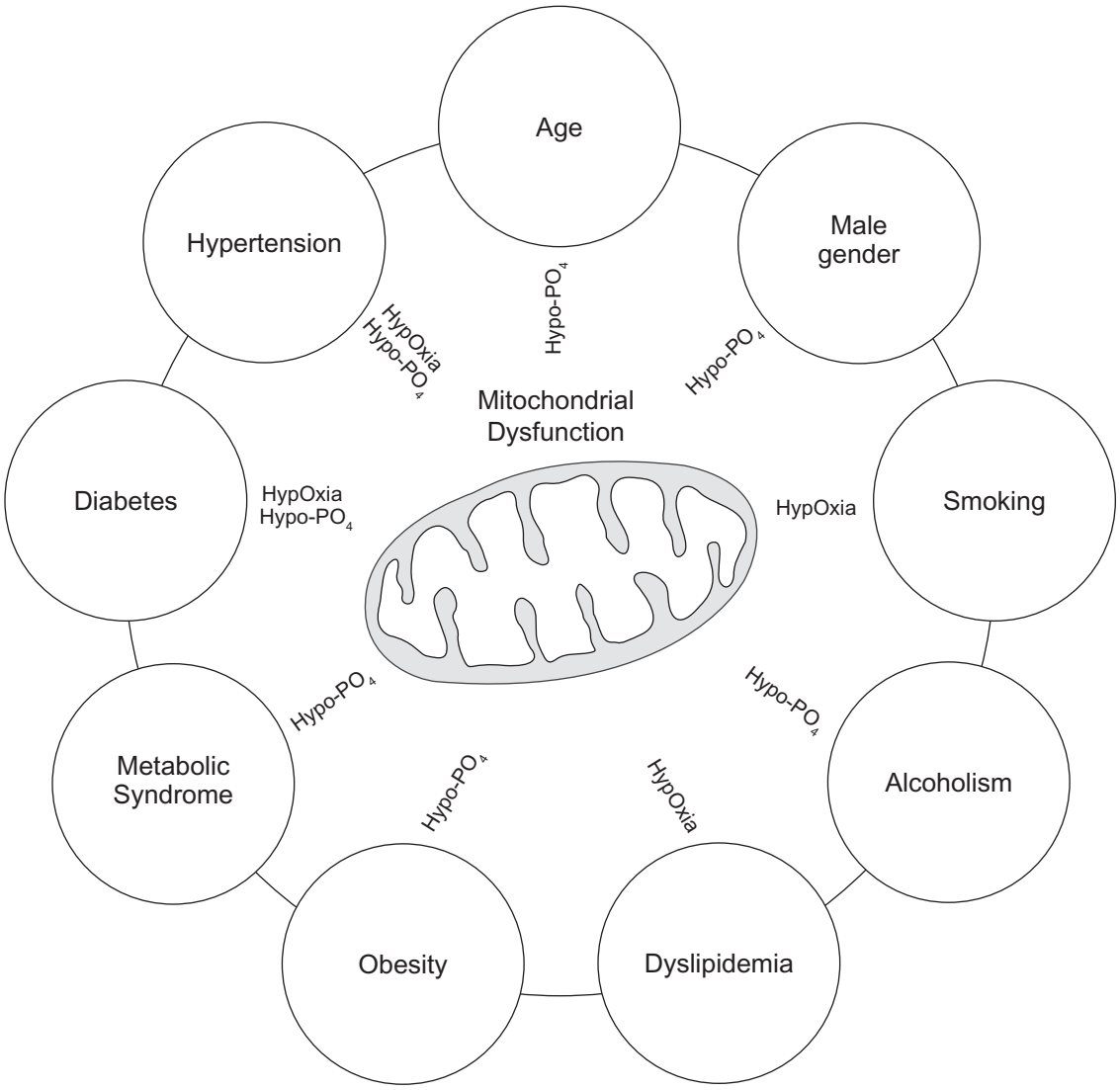

Figure 1 The present concept indicates that the risk factors to cardiovascular disease lead to mitochondrial dysfunction due to either hypophosphatemia and/or hypoxia (see text).

alanine, the Pi reabsorption, particularly in poorly regulated patients, become impaired. Sodium-dependent phosphate transport has been described in many epithelial cells and may be a widespread process.[58] It occurs in cells in which the entrance of glucose is not controlled by insulin and the result of hyperglycemia is increased sodium-dependent glucose transport. Besides the renal tubular cells, this may occur in the cardiovascular endothelium.[59]

In a double-blind study over 1 year to assess the effect of addition of 2 g of calcium diphosphate to the 3 main meals to 43 patients with juvenile diabetes, no increase was seen in the concentration of Pi (active = 19, 1.33 mmol/L vs placebo = 24, 1.42 mmol/L; P = ns). In the treatment group, the fasting urinary phosphate excretion significantly increased and the threshold concentration of Pi (T_{mPO4}/GFR) was suppressed. The threshold concentration was not related to the level of PTH or to growth hormone, but inversely correlated with the degree of hyperglycemia. Therefore, the normalization of blood glucose levels leads to an improved capacity of the kidney tubules to reabsorb Pi and a subsequent increase in plasma Pi concentrations.[6,60]

The carbon monoxide, a byproduct of smoking, leads to a decrease in oxygen carrying capacity, a left shift (decreased P_{50}) of the ODC, and a decrease in oxygen availability or demand ratio.

The large study by Park et al[9] supports the findings of Håglin et al[8] and that serum phosphate levels showed a negative correlation to age, BMI, fasting blood glucose, triglyceride levels, and systolic and diastolic blood pressures. However, it was also shown that it may be important to maintain an appropriate level of phosphate for the prevention of cardiovascular events and metabolic syndrome.

It is well established that patients and animals with very low plasma concentration of Pi have abnormal function of red blood cells, kidneys, brain, myocardium, pancreas, muscles, and nerves.[61,62] The relationship between extracellular and intracellular availability of Pi is uncertain because the availability of Pi in various intracellular compartments is not well known. Freeman et al[63] used nuclear magnetic resonance to provide an estimate of free Pi in intact renal cortex of rats. From their data, they calculated the intracellular concentration of free Pi to be 0.6 mmol/L. Brazy and Mandel[64] used this estimate in combination with their own data relating phosphate dependence with oxidative phosphorylation in renal tubules and concluded that the availability of Pi in cortical renal tubules may be a factor in regulating rates of oxidative phosphorylation. They further showed that transepithelial phosphate transport provides Pi for use within cells and that intracellular metabolic processes compete for Pi. Perfusion of proximal convoluted tubules from rabbit kidney with phosphate-free medium containing glucose resulted in complete inhibition of the fluid absorption. It was suggested that as glycolysis increases, there is not enough intracellular phosphate for both glycolysis and mitochondrial respiration, and the rates of respiration decrease, thereby reducing the tissue content of ATP. Thus, these and other studies[65,66] indicate that pathways of intracellular metabolism may depend on and compete for intracellular phosphate.

Plasma Pi concentration appears to act as a double-edged sword. Several epidemiological studies have indicated that calcium, phosphate, and calcium–phosphate product may also be positively associated with increased risk of cardiovascular diseases.[67,68] Interestingly, vitamin D levels that are directly correlated with plasma Pi have been found to be inversely related with the risk of cardiovascular diseases.[69] However, in end-stage renal failure, a U-shaped association of Pi and PTH to cardiovascular disease, with high risk in both hypophosphatemia and hyperphosphatemia, has been demonstrated.[70,71] So, although we are dealing with complex processes, an explanation could be that hypophosphatemia leads to mitochondrial dysfunction and, in diabetes, to affinity hypoxia, whereas hyperphosphatemia promotes calcification of the vessel walls leading to ischemic hypoxia by narrowing or causing mechanical occlusion of both the macrovasculature and the microvasculature.[72–74]

The negative effect of hypophosphatemia might be abrogated by increasing intracellular phosphate, which may be possible by using the key intermediate fructose 1,6-diphosphate (FDP). Natelson et al[75] showed that orally administrated FDP as calcium salt was absorbed directly by the intestinal tract without splitting the phosphate linkage and that 6 g led to an increase of plasma Pi averaging 15%, citric acid 10.7%, and nonprotein organic phosphate, as much as, 173%. Further preclinical and clinical data indicate that FDP can enter cells and serve as a metabolizable substrate of glycolysis. FDP acts as human bioenergy. It can transport phosphorous intracellularly and can deliver 4 mol of ATP per mole of FDP. FDP can be given orally and intravenously in humans and is well tolerated at pharmacological doses. Although FDP appears to be efficacious, no controlled study has been reported with this key intermediate in patients with cardiovascular risk factors.

Disclosure

The authors report no conflicts of interest in this work.

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