# Mitochondrial reactive oxygen species, endoplasmic reticulum stress and phosphate toxicity in impairment of pancreatic cells

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**Inorganic phosphate ($P_i$) is necessary for cellular structure and energy metabolism. However, excessive $P_i$ causes harmful effects, including vascular calcification, cell death, defective insulin secretion and impaired insulin synthesis. $P_i$ uptaken into mitochondria generates reactive oxygen species (ROS), which leads to reticulum endoplasmic stress (ER stress) and mitochondrial dysfunction via opening of mitochondrial permeability transition pore (mPT) pore. These two main sequences of $P_i$ overload have important roles for mentioned detrimental effects in hyperphosphatemia. This review provides an insight into the relationship of $P_i$ overload, mPT pore and ER stress in pathologic conditions.** 

***Key words:*** *Reactive oxygen species; Endoplasmic reticulum stress*

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## 1. PHOSPHATE HOMEOSTASIS AND ITS PATHOPHYSIOLOGICAL FUNCTIONS

Inorganic phosphate ($P_i$) plays a critical role for cell structures and functions. It is an essential component in bone metabolism [1], phospholipids in the plasma membrane, cell signaling, and energy metabolism [2–4]. Plasma $P_i$ level is tightly regulated in the range of 2.5 4.5 mg/dl (0.8 1.5 mmol/L) [5, 6] by four main organs, including the gastrointestinal system, parathyroid glands, bone, and kidneys [7]. The $P_i$ demand of body is recommended between 580-700 mg daily, which is mainly provided in diet [8]. About 85% of total $P_i$ of the body is stored in the bone as hydroxyapatite crystals [8, 9] and nearly 90% of filtered $P_i$ is excreted via kidneys [5]. $P_i$ homeostasis balance is maintained by the regulation of PTH, vitamin D, and FGF23. Increasing the $P_i$ loading through diet or decreasing $P_i$ excretion leads to hyperphosphatemia with many complications, including vascular calcification [6, 9], sudden cardiac death [6, 10], defective insulin secretion, and aging [4, 11, 12]. Only 1% of total $P_i$ is present in the extracellular compartment [5, 9]. From that 1%, 0.255% of $P_i$ is circulating in the form of inorganic $P_i$. However, this tiny bioactive portion of $P_i$ must be tightly regulated otherwise cells would be exposed to high $P_i$ conditions leading to $P_i$-induced toxicity even in the high range of normophosphatemia [9]. Western diet supplies 1500 mg $P_i$ daily [8], almost twice higher than daily demand. In the researches of Shegal's group, they found that in the 18- to 44-year-old hemodialysis patients, who consumed 0.75 fast food meal per day, had significantly higher plasma $P_i$ level [13, 14]. These results implicated that there is an easily absorbed $P_i$ form in the food additives or preservatives of processed foods and that the absorbed $P_i$ is underestimated, leading to the fact that plasma $P_i$ level does not correctly reflect the total $P_i$ level. A number of prospective cohort studies showed that even in individuals with non-chronic kidney disease, plasma $P_i$ level in the high normal range (> 3.5 mg/dl) cor-

relates with cardio-vascular events or mortality [5]. In addition, many reports stated that acute kidney injury (acute kidney nephropathy) follows use of an oral sodium phosphate (OSP) bowel purgative [15–17]. The higher prevalence of acute nephropathy occurs in older people, whose kidney function is slightly reduced, females, and those who have hypertension, chronic kidney disease, and diabetes [15]. In 1966, Goldsmith and his group reported that patients who were administered inorganic $P_i$ in the form of disodium or dipotassium salt ($Na_2HPO_4$ or $K_2HPO_4$) either orally or intravenously to treat hypercalcemia developed diarrhea, pulmonary edema, acute myocardial infarction, and ectopic calcification [18]. Those reports revealed the acute toxic effects of $P_i$. Chronic increase in plasma $P_i$ level is observed in chronic kidney disease (CKD), when the excretory function of the renal system is significantly reduced. Monckeberg's sclerosis, the medial calcification of arteries [19], which is different from atherosclerosis due to the accumulation of lipids and inflammation, is often observed in CKD patients. Disturbance of the metabolizing of phosphate, calcium, and vitamin D have been suggested as the underlying mechanism for medial calcification [20]. However, ectopic calcification observed in ob/ob-Klotho-/- mice with marked hyperphosphatemia could be eliminated by reducing plasma $P_i$ level even in the presence of high calcium and vitamin D [12]. Another model, feeding FGF23 null mice with a vitamin D-deficient diet could normalize plasma level of circulating vitamin D but could not ameliorate vascular calcification or plasma $P_i$ level. When these FGF23 null mice were fed with a $P_i$-restricted diet, ectopic calcification and mortality were reduced, even though the plasma level of vitamin D and calcium were high [21]. Similarly, when $NaPi_2a^{-/-}$/$Klotho^{-/-}$ double knockdown mice were fed with a high $P_i$ diet, premature aging phenotype including injury in the alveolar leading to emphysema, atrophy of intestinal wall and skin, ectopic calcification reappeared [12]. Those experiments implicate that $P_i$ itself, not calcium or vitamin D, induced toxicity. The $Klotho^{-/-}$ mouse, whose significantly high plasma $P_i$ level was accompanied [10], showed a substantial insulin content reduction [11] with ill-defined mechanism. Massry described that reduced glucose-stimulated ATP content and impaired insulin secretion were observed in pancreatic islets with chronic renal failure [22]. Taken together, it implicates that both impaired insulin secretion and attenuated insulin content exist when $P_i$ level is increased.

## 2. MITOCHONDRIAL PERMEABILITY TRANSITION (MPT) PORE OPENING AND APOPTOTIC CELL DEATH

Mitochondrial permeability transition has been believed as the critical role in apoptosis. The prompt changes in the inner mitochondrial membrane permeability have been identified by the sudden loss of mitochondrial membrane potential leading to equilibrate small molecules up to 1.5 kDa with cytosol [23–25]. Even though there have been intensive studies on the regulation and genetic dissection of mPT pore, but the molecular identity of this pore is still not defined yet. Some proteins, like cyclophilin D (Cyp-D), adenine nucleotide translocator (ANT), VDAC, hexokinases, peripheral benzodiazepine receptor (PBR), Bcl-2, and Bax, have shown their important roles in the structure of mPT pore [25]. Mitochondrial $Ca^{2+}$ overload has been widely accepted as the main trigger to increase mPT [23, 24, 26, 27]. $P_i$ was proved to activate mPT pore opening by shrinkage and swelling assay [28]. Recently, PiC was reported to interact with Cyp-D in a CsA dependent manner [28, 29]. This implicates that PiC may be a component of mPT. In $\beta$-cells, the phenomenon, known as phosphate flush, that stimulation of insulin secretion by high glucose is accompanied with reduction of 50% of cellular $P_i$ level, has been observed. Koshkin et al. argued in his model for the role of mPT in $\beta$-cells that phosphate flush during insulin secretion helps to increase the free cytosolic $Ca^{2+}$, which stimulates insulin secretion, but an increase in free cytosolic $Ca^{2+}$ also results in mitochondrial $Ca^{2+}$ overload leading to flickering open mPT [30]. However, prolonged mPT pore opening causes mitochondrial dysfunction [31] leading to impair glucose-stimulated insulin secretion [32].

## 3. UNFOLDED PROTEIN RESPONSE AND DIABETES

$\beta$-cells are often suffering from the fluctuation of blood glucose. When blood glucose level is increased, the demand for insulin ($\beta$-cell produces 1 million proinsulin molecules/min in response to blood glucose stimulation) [33] poses a heavy burden on the endoplasmic reticulum (ER). Proinsulins synthesized in the ER go into a process to produce active form and then stored in the granules. A huge proinsulin

mRNA (20% of total mRNA) level has been found in the cytoplasm of $\beta$-cells [34]. Therefore, the translation of proinsulin in response to glucose stimulation may cause an excessive protein folding load on ER, resulting in ER stress, leading to activation of some signaling pathway though ER stress sensors, called unfolded protein response (UPR) [35]. A line of evidence revealed the adaptive role of UPR to overcome ER stress [36] by enhancing the folding capacity via increased chaperones and protein processing enzymes [37]. If the UPR can clear misfolded/unfolded proteins, ER homeostasis is restored; if not, it will switch to programmed cell death, usually via apoptosis [38]. Under ER stress condition, some $\beta$-cells die, and the remaining residual $\beta$-cell population increase ER workload. However, when ER stress prolongs, more $\beta$-cells die, leading to a decreased $\beta$-cell population and causing diabetes [37]. The ER transmembrane proteins, including protein kinase RNA (PKR)-like ER kinase (PERK), activating transcription factor-6 (ATF-6) and inositol-requiring enzyme-1 (IRE1), are activated [38, 39]. Recent evidence focused on the important role of PERK in the phosphorylation of eukaryotic initiation factor 2 Alpha-subunit (eIF2Alpha) in translation attenuation to prevent oxidative stress and maintain the function of $\beta$-cells [34]. In unstressed condition, the binding of immunoglobulin proteins (BiPs) bind to PERK to prevent them from dimerization. Once misfolded/unfolded proteins are accumulated in ER, BiPs are released allowing the dimerization of PERK, in turn, phosphorylates eIF2Alpha, results in translational attenuation [39]. There are several causes that induce ER stress, including hypoxia, high glucose, accumulation of free fatty acids and insulin mutations [37]. ROS is also suspected to cause ER stress and disrupt insulin production [35]. Taken together, these implicate that ROS may initiate ER stress, leading to reduced translation of insulin. In addition, ROS may affect cell death-related ER stress, resulting in a decreased $\beta$-cell population, and influence insulin synthesis.

## 4. PHOSPHATE TOXICITY IN INSULIN SECRETING CELLS

Physiological intracellular $P_i$ level in cardiac muscle detected by $^{31}P-NMR$ was reported to be 0.8 mM, but elevated under ischemic or hypoxic conditions [40]. In pancreatic -cells, intracellular $P_i$ is partially lost during nutrient stimulation, a phenomenon which is referred to as 'phosphate flush' [41]. Total islet $P_i$ was also shown to be reduced during glucose-stimulated insulin secretion [42]. Analysis of the cytosolic and mitochondrial $P_i$ content in insulin-secreting cells revealed that the drop in mitochondrial $P_i$ is lower than in the cytosol due to $P_i$ uptake during mitochondrial activation [43]. The molecular mechanism and functional implication of cellular $P_i$ loss in response to nutrient stimulation in insulin-secreting cells remains to be investigated. In contrast, excessive $P_i$ load elicits cytotoxicity in a number of cell types in vitro [44–46]. Serum $P_i$ levels are maintained at about 0.8 1.5 mM in healthy individuals [47], but may increase to higher than 2 mM in some pathogenic conditions, including chronic kidney disease [48, 49]. Elevated serum $P_i$ levels can lead to cellular $P_i$ overload inducing osteogenic differentiation and calcification in vascular smooth muscles. A strong positive correlation between serum $P_i$ levels and cardiovascular morbidity and mortality has been reported [5, 6]. High $P_i$-induced vascular calcification was proposed to be mediated by oxidative stress. This is supported by data showing that $P_i$ increases the release of reactive oxygen species (ROS) from isolated mitochondria [50]. Pancreatic -cells display low expression level of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase [51]. Therefore, insulin secreting cells are highly sensitive to oxidative stress [52], and elevated $P_i$ may be particularly harmful to the pancreatic -cells. Consistent with this possibility, in chronic kidney disease associated with hyperphosphatemia, insulin secretion is impaired [53]. An epidemiologic study also shows that individuals with higher plasma level of $P_i$ have an increased risk of developing Type 2 diabetes [54]. Intriguingly, in 'klotho'-deficient mice (kl/kl) with pronounced hyperphosphatemia [11], insulin content of the pancreas is significantly lowered compared to wild-type mice.

## CONCLUSIONS

Appropriate provision of $P_i$ for mitochondria is indispensable for energy homeostasis and -cell metabolism-secretion coupling. However, $P_i$ overload causes oxidative stress and mitochondrial dysfunction, which may be sequent in ER stress, finally result in impairment of -cell performance and viability. Controlling the $P_i$ in the daily diet may help to prevent the developing of diabetes type 2 in western

diet.

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