




Loss of metabolic flexibility as a result of overexpression of pyruvate dehydrogenase kinases in muscle, liver and the immune system: Therapeutic targets in metabolic diseases

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ABSTRACT

Good health depends on the maintenance of metabolic flexibility, which in turn is dependent on the maintenance of regulatory flexibility of a large number of regulatory enzymes, but especially the pyruvate dehydrogenase complex (PDC), because of its central role in carbohydrate metabolism. Flexibility in regulation of PDC is dependent on rapid changes in the phosphorylation state of PDC determined by the relative activities of the pyruvate dehydrogenase kinases (PDKs) and the pyruvate dehydrogenase phosphatases. Inactivation of the PDC by overexpression of PDK4 contributes to hyperglycemia, and therefore the serious health problems associated with diabetes. Loss of regulatory flexibility of PDC occurs in other disease states and pathological conditions that have received less attention than diabetes. These include cancers, non-alcoholic fatty liver disease, cancer-induced cachexia, diabetes-induced nephropathy, sepsis and amyotrophic lateral sclerosis. Overexpression of PDK4, and in some situations, the other PDKs, as well as under expression of the pyruvate dehydrogenase phosphatases, leads to inactivation of the PDC, mitochondrial dysfunction and deleterious effects with health consequences. The possible basis for this phenomenon, along with evidence that overexpression of PDK4 results in phosphorylation of “off-target” proteins and promotes excessive transport of Ca^{2+} into mitochondria through mitochondria-associated endoplasmic reticulum membranes are discussed. Recent efforts to find small molecule PDK inhibitors with therapeutic potential are also reviewed.

INTRODUCTION

Complete oxidation of glucose, a fuel that provides much of the energy used by all cells and all of the energy used by some cells, requires the reaction catalyzed by the pyruvate dehydrogenase complex (PDC). Despite the importance of its role as a fuel, glucose is a reactive aldehyde and a toxic compound as a result of several mechanisms, as evidenced by the serious health problems associated with diabetes. The maintenance of good health clearly depends on activity of the PDC, not only to

capture the energy during glucose oxidation, but also to prevent the accumulation of excess glucose. In contrast, the absolute requirement for glucose by some cells; for example, red blood cells and neurons, renders glucose essential for life, making tight control of the activity of the PDC essential for good health. In essence, this means that the activity state of the PDC must be flexible; that is, dynamically regulated, partially active when a person is in the fed state and more active when overfed, but less active when a person is fasting and virtually inactive when starving. As every day involves a variable number of feeding and fasting cycles, good health is dependent on the flexibility and plasticity of the activity state of the PDC.

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Pyruvate dehydrogenase complex consists of three components; namely, pyruvate dehydrogenase (E1), dihydrolipoyl acetyltransferase (E2) and dihydrolipoyl dehydrogenase (E3). Dynamic changes in the activity state are achieved by the opposing actions of the four distinct pyruvate dehydrogenase kinases 1–4 (PDK1 to PDK4) and pyruvate dehydrogenase phosphatases 1 and 2 (PDP1 and PDP2), both of which are subject to regulation by allosteric effectors, covalent modification and relatively rapid changes in the amounts of the expressed proteins, either by altered gene expression or proteolytic degradation^{1–3}.

Evidence that has accumulated slowly over the past 50 years has established that the loss of dynamic regulation of the PDC contributes to mitochondrial dysfunction in diabetes^{4–7}. Mitochondria are known as the powerhouses of the cell for playing a major role in metabolic processes and cellular respiration. Their optimal function is crucial for metabolizing energy-rich substrates to efficiently produce adenosine triphosphate (ATP), whereas deterioration of its function directly affects ATP availability in the cell. A lack of insulin in type 1 diabetes and insulin resistance in type 2 result in overexpression of PDK4, inactivation of the PDC throughout the feeding fasting cycle, resistance of reactivation by the PDPs, conservation of substrates for gluconeogenesis, conservation of glucose, hyperglycemia and glucose toxicity.

PDKs show unique tissue expression patterns, kinetic properties and sensitivities to regulatory molecules. General expression patterns of all four PDK proteins in mouse tissues by western blot analysis show that PDK4 protein is relatively scarce in the fed condition⁸. Despite its low basal expression, PDK4 is drastically increased in tissues challenged with stressful stimuli; for example, in cisplatin-exposed renal tissue⁹, high inorganic phosphate-treated vascular smooth muscle cells¹⁰, and diabetes in liver, heart and skeletal muscle^{11–14}. Given that these pathological conditions give rise to mitochondrial dysfunction, as evidenced by mitochondrial reactive oxygen species formation, decreased mitochondrial membrane potential and decreased ATP production^{9,10}, it is plausible to assume PDK4 is mechanistically linked with mitochondrial dysfunction. Among the PDK isoenzymes, PDK4 is almost invariably upregulated in mitochondrial dysfunction-related metabolic diseases.

This review focuses on recent work that shows loss of dynamic regulation of PDC occurs in response to a number of metabolic diseases and pathological states other than diabetes, including obesity, artery and tissue calcification, cancer, cachexia, and cytokine storm. The evidence that overexpression of PDK4 in metabolic diseases can result in “off-target” effects of PDK4; that is, phosphorylation of cytoplasmic and mitochondrial proteins other than the PDC that are deleterious, is also reviewed. Finally, the effort to find small molecule PDK inhibitors with therapeutic potential is reviewed.

ROLE OF PDKS IN MUSCLE INSULIN RESISTANCE AND SKELETAL MUSCLE HOMEOSTASIS

Among the four PDK isoenzymes, PDK4 is most abundantly expressed in all types of skeletal muscle tissues (fast-twitch

glycolytic, fast-twitch oxidative and slow-twitch oxidative), and its expression is further induced on fasting in all skeletal muscle types, but not PDK2¹⁵. In skeletal muscle, PDK4 expression is regulated by transcription factor forkhead box protein O1 (FOXO1)¹⁶, peroxisome proliferator-activated receptor- α ¹⁷, peroxisome proliferator-activated receptor- δ , estrogen-related receptor- α and transcriptional co-activator peroxisome proliferator-activated receptor gamma co-activator-1 α ¹⁸. In the fed condition, an increase in the serum insulin level activates AKT, leading to suppression of FOXO1-mediated PDK4 expression, whereas during starvation, a decline in insulin level reactivates FOXO1 to enhance PDK4 expression¹⁹. This dynamic change in PDK4 level is critical for the metabolic switch from glucose to fatty acid oxidation^{20,21}. PDK4 expression is highly elevated in skeletal muscle tissue of genetically obese, diet-induced obese and insulin-resistant mice, as well as in diabetic rats, whereas the other PDKs remain unchanged^{1,22,23}. Deficiency of PDK4 imposed genetically or by small molecule inhibition promotes complete oxidation of pyruvate, thereby reducing the availability of precursors (lactate, pyruvate and alanine) for gluconeogenesis by the liver²⁴.

PDK4 is a very short-lived protein (half-life ~1 h) relative to other mitochondrial proteins due to rapid degradation by the mitochondrial Lon protease²⁵. Furthermore, loss of the fatty acid translocase, cluster of differentiation 36, dampens starvation-induced FOXO1 and PDK4 expression²⁶, showing that fatty acid uptake is a major factor for induction of PDK4. Increased expression coupled with enhanced stability by fatty acids prolongs upregulation of PDK4 in insulin resistance states¹, aging²⁷ and atrophic muscle^{28,29}, promoting metabolic instability (Figure 1).

The roles of PDK1 and PDK3 in skeletal muscle are less known due to limited study relative to PDK2 and PDK4. Genetic ablation of *Pdk4* protects mice from HFD-induced glucose intolerance and insulin resistance^{30,31}. Recent studies suggest actions of PDK2³² and PDK4^{10,33,34} that are independent of PDC regulation; that is, off-target effects. In this context, PDK2 was shown to suppress mitophagy by phosphorylating presenilin-associated rhomboid-like protein to mediate mutations in phosphatase and tensin homolog-induced kinase 1 degradation³². The relative importance of non-canonical roles of PDK2 and PDK4 in obesity-induced insulin resistance requires additional study.

Calcium plays an essential role in regulating mitochondrial function and skeletal muscle contraction³⁵. In mitochondria, Ca²⁺ stimulates PDP1, which activates PDC through dephosphorylation to promote pyruvate metabolism. Ca²⁺ also stimulates mitochondrial tricarboxylic acid cycle enzymes (isocitrate dehydrogenase and α -ketoglutarate dehydrogenase) and ATP synthase (electron transport chain complex V) activity to drive ATP synthesis³⁶. Calcium moves from the lumen of the endoplasmic reticulum (ER) into mitochondria through an IP3R1, glucose-regulated protein 75 (GRP75) and voltage-dependent anion-selective channel 1 (VDAC1) complex that resides at the

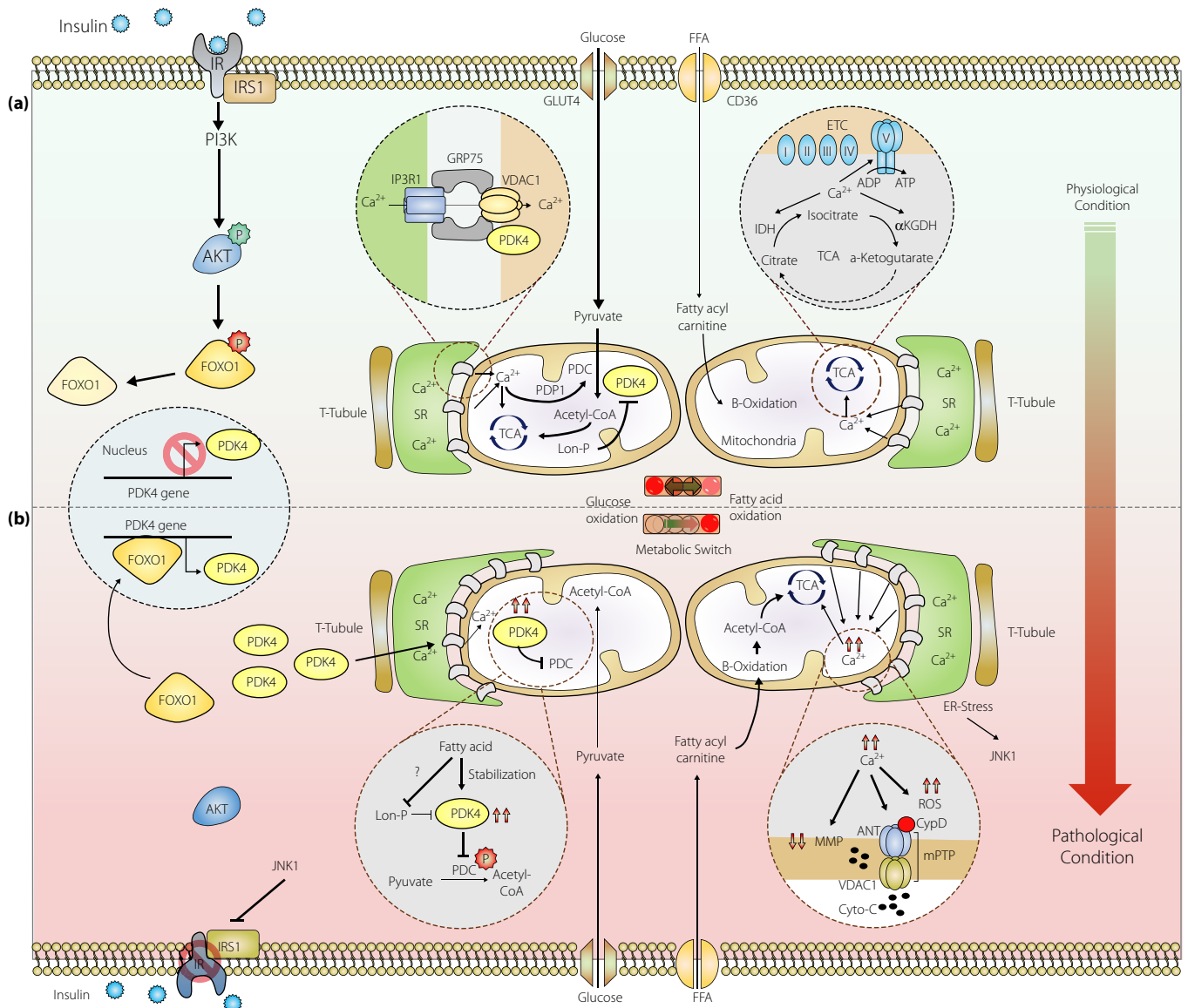


Figure 1 | Pivotal role of pyruvate dehydrogenase kinase 4 (PDK4) in regulating the metabolic switch between glucose and fatty acid oxidation in skeletal muscle in physiological and pathological conditions. (a) In the physiological condition, insulin signaling-mediated protein kinase B (AKT) and forkhead box protein O1 (FOXO1) pathway regulates PDK4 expression. Low expression of PDK4 and optimal mitochondrial Ca^{2+} entry through the inositol 1,4,5 trisphosphate receptor type 1 (IP3R1)–glucose-regulated protein 75 (GRP75)–voltage-dependent anion-selective channel 1 (VDAC1) complex at mitochondria-associated endoplasmic reticulum (ER) membrane (MAM) stimulates pyruvate dehydrogenase phosphatase 1 (PDP1), isocitrate dehydrogenase (IDH), α -ketoglutarate dehydrogenase (α KGDH) and adenosine triphosphate (ATP) synthase activity to enhance glucose oxidation, whereas a decline in insulin level induces PDK4 to activate fatty acid oxidation through inhibition of pyruvate dehydrogenase complex (PDC). This signaling feedback loop plays a crucial role in controlling the metabolic switch and stability. (b) The pathological condition caused by defective insulin signaling or increasing fatty acid level upregulates PDK4 expression and stability. Prolonged induction of PDK4 suppresses pyruvate oxidation and enhances mitochondrial Ca^{2+} flux through MAM to drive fatty acid oxidation, leading to metabolic instability. A constant increase of mitochondrial Ca^{2+} level promotes mitochondrial membrane potential (MMP) loss and reactive oxygen species (ROS) generation results in opening of permeability transition pore (mPTP). In contrast, excessive mitochondrial ROS production induces ER-stress and activates c-Jun N-terminal kinase 1 (JNK1) which negatively affects insulin signaling through inhibition of insulin receptor substrate 1 (IRS1). ANT, adenine nucleotide translocator; CD36, cluster of differentiation 36; CypD, cyclophilin D; Cyto-C, cytochrome C; ETC, electron transport chain; FFA, free fatty acid; GLUT4, glucose transporter 4; GRP75, glucose-regulated protein 75; IR, insulin receptor; Lon-P, mitochondrial Lon proteases; mPTP, mitochondrial permeability transition pore; PDC, pyruvate dehydrogenase complex; PI3K, Phosphoinositide 3-kinases; TCA, tricarboxylic acid cycle; VDAC1, voltage-dependent anion-selective channel 1.

mitochondria-associated ER membrane (MAM)³⁶. The existence of this Ca²⁺ channeling complex between sarcoplasmic reticulum to mitochondria in skeletal muscle is well established^{35,37}. Mitochondrial Ca²⁺ signaling through MAM plays an important role during obesity-induced insulin resistance in hepatic tissue and skeletal muscle³⁸. However, whether passage of Ca²⁺ into mitochondria through MAM induces or prevents insulin resistance in general is unclear due to contradictory findings^{22,37,39,40}. A sub-pool of PDK4 protein is localized in the MAM fraction where it interacts and stabilizes the IP3R1–GRP75–VDAC1 complex and promotes mitochondrial Ca²⁺ uptake (Figure 1). Feeding mice a high-fat diet enhances the enrichment of PDK4 in MAM, and increases formation of the IP3R1–GRP75–VDAC1 complex. Genetic deletion of PDK4 knockout suppresses formation of the complex, reducing mitochondrial Ca²⁺ uptake²². The subpopulations of PDK4 in the mitochondrial matrix space and MAM might have a synergistic effect to regulate the metabolic shift from glucose to fatty acid oxidation. PDK4 in MAM contributes to mitochondrial Ca²⁺ influx to drive the tricarboxylic acid cycle and ATP synthase activity, whereas PDK4 in the matrix inhibits PDC to promote fatty acid oxidation. However, prolonged induction of MAM causes accumulation of too much Ca²⁺ in the mitochondria, which enhances ROS production and causes mitochondrial dysfunction⁴¹. The saturated fatty acid, palmitate, reduces mitochondrial Ca²⁺ retention, resulting in opening of mitochondrial permeability transition pore⁴². Furthermore, excessive beta-oxidation also causes mitochondrial work overload and incomplete fatty acid oxidation⁵. Dysregulated MAM function can lead to ER stress and activation of c-Jun N-terminal kinase and phosphorylation of IRS1, resulting in insulin resistance^{38,43}.

Increased PDK4 expression correlates with severe muscle loss in cancer cachexia²⁸, diabetic nephropathy⁴⁴, sepsis⁴⁵ and amyotrophic lateral sclerosis²⁹. Treatment of C2C12 myotubes with culture medium collected from mouse colorectal cancer cells (C26) induces PDK4 expression and markedly reduces the size of C2C12 muscle fibers. Knockdown of PDK4 prevents tumor media-induced shrinkage of muscle fiber. Furthermore, overexpression of PDK4 induces mitochondrial dysfunction and muscle fiber shrinkage²⁸. These findings suggest that PDK4 plays a central role in mediating metabolic instability-induced skeletal muscle dysfunction through deterioration of mitochondrial function.

ROLE OF HEPATIC PDKS IN NON-ALCOHOLIC FATTY LIVER DISEASE AND DIABETES

Severe hepatic insulin resistance is induced by insulin receptor substrates (Irs) 1/2 double-knockout mice. *Pdk4* gene expression is induced in the liver of the insulin receptor substrates hepatic double-knockout mice as a result of impaired insulin signaling⁴⁶. Deletion of *Pdk4* is more effective than deletion of *Pdk2* in ameliorating hyperglycemia and improving glucose tolerance⁴⁷. In contrast, *Pdk2* deletion is more effective than *Pdk4* deletion in improving insulin sensitivity, suggesting a differential role of PDK isotypes on glucose homeostasis⁴⁷.

Hepatic insulin resistance-mediated reduction of PDC activity and enhanced pyruvate carboxylation contribute to increased hepatic gluconeogenesis in obese mice with hepatic steatosis^{48,49}. PDK2 is greatly increased by feeding mice a high-fat diet¹³, correlating with reduced PDC activity, increased tricarboxylic acid cycle intermediates, and decreased fatty acid oxidation and ketogenesis genes.

PDK2 deficiency in the liver restores PDC flux and causes relative oxaloacetate (OAA) insufficiency as a result of greater PDC flux at the expense of pyruvate carboxylation. Greater abundance of acetyl coenzyme A (acetyl-CoA) relative to OAA led to increased beta oxidation and increased ketogenesis, leading to improvement in hepatic steatosis. At the same time, glucose tolerance was improved as a result of decreased gluconeogenesis due to the decrease in OAA¹³.

In the fasting condition, the expression of estrogen-related receptor- γ , its coactivator peroxisome proliferator-activated receptor gamma co-activator-1 α , and their targets, PEPCK and PDK4, are increased⁵⁰. Furthermore, in the liver of *db/db* diabetic mice, estrogen-related receptor- γ is increased to facilitate gluconeogenesis⁵¹. In agreement with these findings, PDK4 is increased by cyclic adenosine monophosphate (cAMP)–protein kinase A–cAMP response element-binding protein signaling in both the fasting condition and diabetes¹⁴. PDK4 deficiency in the liver improves glucose tolerance in diet-induced obese mice by antagonizing glucagon signaling. Mechanistically, PDK4 deficiency blunts the fatty oxidation rate and decreases ATP synthesis in hepatocytes¹⁴, resulting in activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK), the master regulator of energy homeostasis. AMPK in turn activates phosphodiesterase 4B, which converts cAMP to AMP, thereby decreasing signaling by glucagon. The importance of this mechanism in blocking stimulation of cAMP accumulation by glucagon was shown in a previous study⁵² with a small-molecule activator of AMPK.

PDK4 deficiency also prevents steatohepatitis in the methionine- and choline-deficient diet model⁵³. PDK4 knockout mice consuming a high saturated fat diet also show less hepatic steatosis than wild-type mice³¹. Furthermore, genetic ablation of PDK2/4 decreases hepatic steatosis by an AMPK-dependent decrease in the nuclear carbohydrate-responsive element binding protein, which is responsible for induction of lipogenic enzymes acetyl-CoA carboxylase 1, fatty acid synthase and stearoyl coenzyme A desaturase-1⁵⁴. Collectively, this accumulating evidence highlights a liver-centric role of PDK2 and PDK4 in the pathogenesis of diabetes and non-alcoholic fatty liver disease (Figure 2a,b).

ROLE OF PDKS IN METAINFLAMMATION AND ITS ASSOCIATED DISEASES

Immune cells undergo distinct metabolic reprogramming processes, and their modulation directly integrates with their immunological function^{55–57}. Macrophages and dendritic cells, which comprise a major part of the innate immune system, are

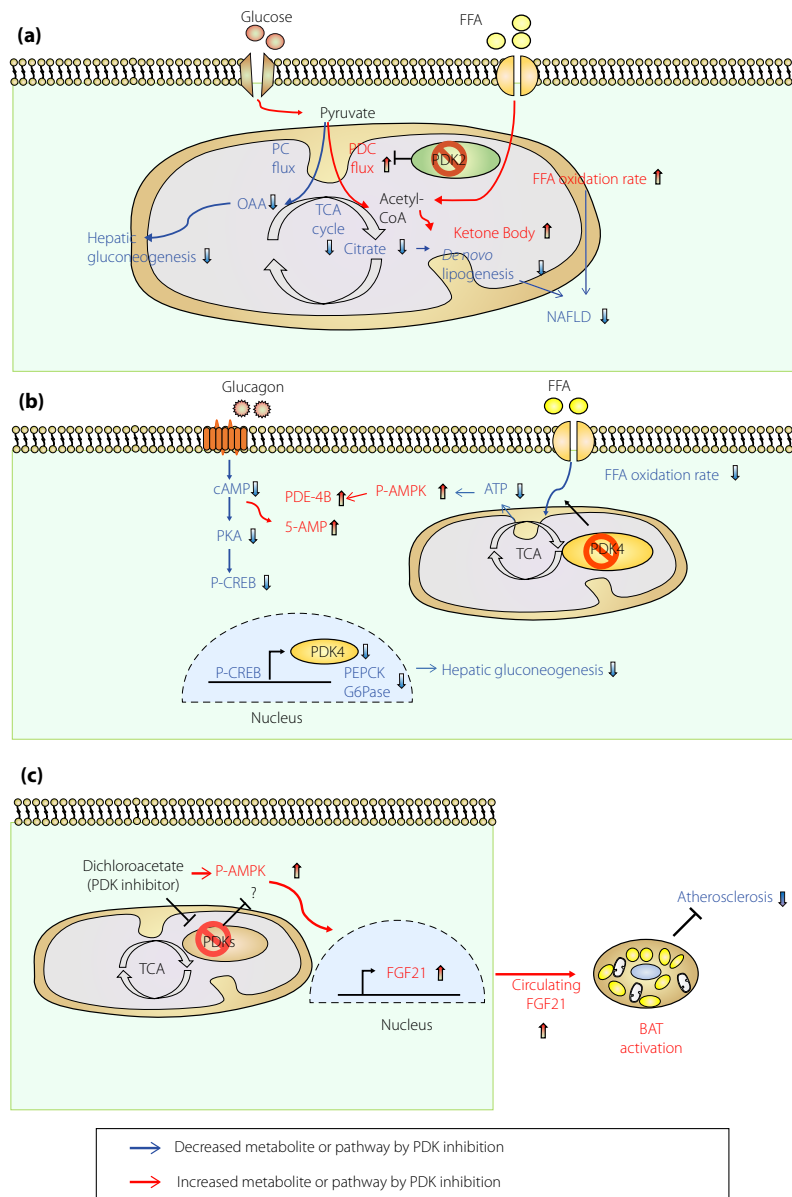


Figure 2 | Liver-centric role of pyruvate dehydrogenase kinase (PDK) inhibition in various metabolic diseases. (a) Under long-term high-fat diet (HFD) feeding-induced insulin resistance and non-alcoholic fatty liver disease (NAFLD), PDK2 is dominantly increased in the liver. Inhibition of PDK2 suppresses pyruvate carboxylase (PC) flux is decreased, whereas pyruvate dehydrogenase complex (PDC) flux is increased, causing a relative oxaloacetate (OAA) insufficiency. As a result, citrate synthesis is decreased and tricarboxylic acid cycle (TCA) cycle intermediates are diminished. The decrease in the concentration of the gluconeogenic precursor OAA reduces hepatic gluconeogenesis, whereas the decrease in citrate is causally linked with decreased de novo lipogenesis. Acetyl coenzyme A (acetyl-CoA) fails to be incorporated into citrate as a result of OAA insufficiency, and therefore is converted to ketone bodies. The resulting increase in fatty acid oxidation to meet the energy demand for ATP contributes to the amelioration of NAFLD. (b) In diet-induced obesity or *db/db* diabetic mice, glucagon signaling (cyclic adenosine monophosphate [cAMP]–protein kinase A [PKA]–cAMP-response element-binding protein [CREB]) is aberrantly activated in the liver. PDK4, but not other isoforms, is also increased by glucagon or cAMP. PDK4 deficiency blunts the signaling by cAMP reduction. This is achieved by less fatty acid oxidation and a subsequent decrease of adenosine triphosphate (ATP). Increased AMP-activated protein kinase (AMPK) phosphorylation activates phosphodiesterase 4B, which is responsible for cAMP degradation. Collectively, this results in less CREB-dependent G6Pase and phosphoenolpyruvate carboxykinase (PEPCK) transcription and attenuated glucagon signaling. (c) In a Western diet-fed, apolipoprotein E (*ApoE*) knockout murine atherosclerosis model, systemic administration of Pan-PDK inhibitor, dichloroacetate, increases AMPK activity in the liver, which boosts *fgf21* transcription. This activates distant brown adipose tissue (BAT), which contributes to reduced adiposity and increased energy expenditure, and prevention of atherosclerosis.

responsible for initiating and directing the first line of defense in response to infection or tissue damage. On exposure to external stimuli, naïve macrophages rapidly polarize to the M1 (classically activated macrophages) phenotype by activating pro-inflammatory processes, including upregulation of pro-inflammatory genes and nitric oxide production^{55,58}. In contrast, during the resolution phase, M2 (alternatively activated macrophages) polarization become more dominant, cells of which are characterized by anti-inflammatory features with Arg-1 induction^{55,58}. Although M1-polarized macrophages rapidly shift toward glycolysis to support their increased energy demand, M2-polarized macrophages rely mostly on mitochondria-driven oxidative phosphorylation to maintain long-term energy supply⁵⁸.

Pyruvate dehydrogenase kinases play a crucial role in regulating metabolic reprogramming in macrophages^{59–61}. Upregulation of PDK, which readily inactivates PDC by phosphorylating the complex, inhibits PDC-catalyzed conversion of cytosolic pyruvate to mitochondrial acetyl-CoA and facilitates glucose conversion to lactate⁶². Thus, enhancement of PDK activity during macrophage activation would inhibit M1 polarization machinery by facilitating pyruvate oxidation, which opposes metabolic shift from oxidative phosphorylation to glycolysis. As expected, expression of PDK1, PDK3 and PDK4 in macrophages is upregulated during M1 polarization, and PDK1 or PDK2/4 deletion or inhibition blocks M1 polarization and skews its phenotype toward M2^{60,61}. In addition, PDK1 plays a role in hypoxia-inducible factor-1 α -induced glycolysis and the regulation of macrophage migratory activity⁵⁹. Although PDK1 is upregulated by hypoxia-inducible factor-1 α , the exact mechanism that enhances expression of the other PDK isoforms during M1 polarization are not been identified^{59–61,63}.

Recent studies have shown that PDKs are involved in metabolic reprogramming of adaptive immunity as well as innate immunity^{64–66}. To adapt to increased energy demands during differentiation and proliferation, T cells immediately switch their metabolic state from oxidative phosphorylation to glycolysis on activation^{57,67}. However, each differentiated T-cell subset has unique features of their metabolic profile, and PDKs finely tune their energy dependency^{64–67}. Relative messenger ribonucleic acid expression of PDK1 was the most abundant isoform, but differentially expressed in the CD4⁺ T-cell subsets; PDK1 showed robust expression in T-helper cell 17 (Th17), intermediate in regulatory T cells (Tregs) and little expression in T-helper cell 1 (Th1)⁶⁴. Inhibition of PDK1 selectively suppresses Th17, enhancing Treg, whereas Th1 is unchanged when measured in the polarizing condition of each subset⁶⁴.

However, T cell-modulating results of PDK1-deficient or inhibition status are varied depending on their experimental conditions, including polarizing effect, glucose availability or interferon- γ secreting cell-types, representatively, Th1 and Tc (cytotoxic CD8⁺ T cells)^{64,65,68}. For example, when T cells are activated without the presence of polarizing cytokine, such as interleukin-12, PDK inhibition by dichloroacetate (DCA)

suppresses interferon- γ production and Th1 proliferation⁶⁸. In CD8⁺ T cells, DCA dampens interferon- γ production in the presence of glucose over the concentration of 2 mmol/L, the phenomenon of which is linked by lactate dehydrogenase-mediated cytokine repression⁶⁵. On activation, PDK1-mediated inactivation of PDC directs lactate dehydrogenase to mediate pyruvate to lactate conversion, which enhances cytokine production by releasing lactate dehydrogenase from adenylate-uridylylate-rich elements⁶⁵.

An increase in pyruvate oxidation by DCA generates mitochondria reactive oxygen species in immune cells, as well as cancer cells^{64,69}. High levels of reduced glutathione level or anti-oxidant thioredoxin-1 seem to support Tregs to have a greater capacity to handle reactive oxygen species, thereby PDK inhibition favors Treg subsets rather than Th1 or Th17^{64,70}.

Although PDK1 is a dominant isoform in T cell, our unpublished data show that T cells in human pathological conditions exhibit different patterns, implying that PDK expression is not only determined by T-cell activation and polarization status. As a metabolic change of T cells is dynamic, PDK expression or activity during the course of immune activation needs to be delineated. Further study is required to examine the factors that modulate PDK activities in T cells during clinical disease progression. PDP, which dephosphorylates phospho-PDC to activate PDC, is also involved in T-cell metabolic reprogramming⁶⁶. Inducible cAMP early repressor directly modulates PDP2 expression, showing that reduced PDC activity leads to enhancement of Th17 differentiation⁶⁶.

So far, several *in vivo* inflammatory disease models have been successful in showing PDK as a promising therapeutic target^{59,60}. DCA exerts a therapeutic effect on sepsis, experimental allergic encephalomyelitis, asthma and arthritis^{63,64,68,71}. PDK2/4-deficient bone marrow transplantation or pan-PDK inhibitor, KPLH1130, administration ameliorates high-fat diet-induced insulin resistance and adipose tissue inflammation⁶⁰. Overall, these studies highlight the importance of PDK activities in the regulation of immune cell function (Figure 3).

PDK INHIBITOR AS A THERAPEUTIC OPTION IN METABOLIC SYNDROME

Evidence that PDK inhibition has therapeutic potential continues to grow^{2,3,72}. The aforementioned pleiotropic effects of PDK inhibition, including targeting macrophage polarization, restoration of muscle glucose tolerance and suppression of hepatic glucose production, as well as prevention of non-alcoholic fatty liver disease or non-alcoholic steatohepatitis, have raised the possibility of a PDK inhibitor as a therapeutic option for metabolic syndrome. Furthermore, we recently observed that DCA increases brown adipose tissue (BAT)⁷³. In Western diet-fed apolipoprotein E (*ApoE*)^{-/-} mice, DCA stimulates hepatic fibroblast growth factor 21 (*fgf21*) messenger ribonucleic acid expression in an AMP-activated protein kinase-dependent manner. The increase was sufficient to increase serum FGF21 level and subsequent activity of BAT⁷³ (Figure 2c). Furthermore, an

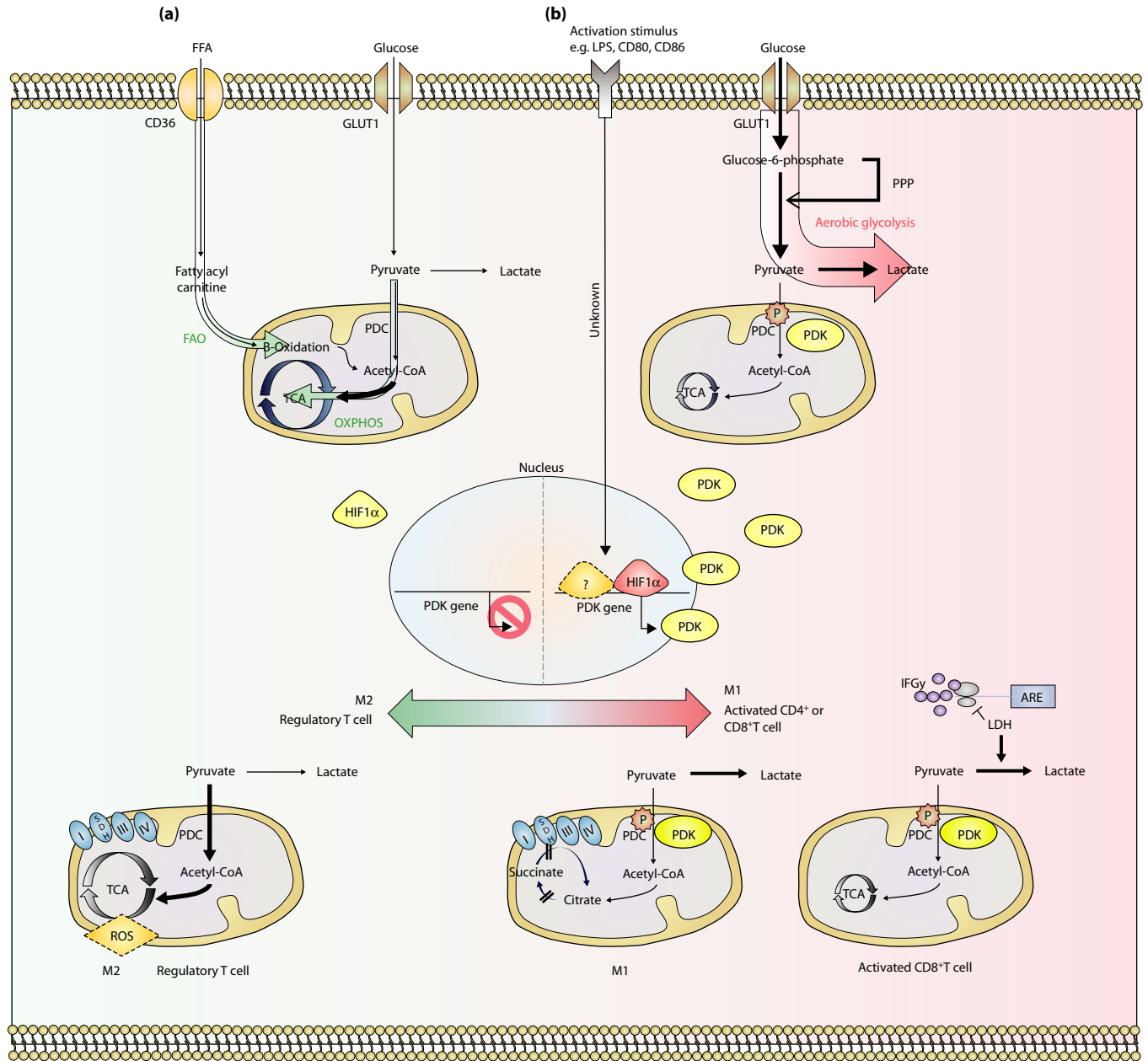


Figure 3 | The role of pyruvate dehydrogenase kinase (PDK) in immune cell metabolism. (a) Enhancement in PDK activity increases aerobic M1 (M1 macrophage), CD8⁺ T cells and CD4⁺ T cells (T-helper 1 or T-helper 17 cells), which facilitates pyruvate conversion to lactate during activation. (b) In contrast, PDK activities in M2 (M2 macrophage) and regulatory T cells are relatively suppressed, which favors oxidative phosphorylation over the glycolysis pathway. Acetyl-CoA, acetyl coenzyme A; ARE, adenylate-uridylylate-rich elements; CD36, cluster of differentiation 36; GLUT4, glucose transporter 4; HIF1 α , hypoxia-inducible factor-1 α ; IFN γ , interferon-gamma; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; PDC, pyruvate dehydrogenase complex; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle.

unbiased [U-¹³C]-glucose tracer-based metabolomics study showed that PDC flux activity is decreased, whereas Ser293 p-PDC level is decreased on β 3-adrenergic activation in brown adipocyte⁷⁴. Although whether PDK deletion in brown adipocyte further activates BAT activation remains unknown, this finding raises the possibility of a BAT-autonomous role of

PDC and PDK in the regulation of activity, apart from liver-BAT cross-talk mediated by FGF21.

As in PDK knockout mice, DCA is effective in promoting glucose oxidation and lowering blood glucose in diabetes⁷⁵, which is supported by recent evidence that DCA can exert a metformin-like effect by promoting AMPK activation^{14,73}. DCA

also lowers cAMP accumulation, which was increased in *db/db* mice liver compared with *db/+* mice¹⁴. Importantly, DCA was effective in treating patients with diabetes^{76,77}, as well as reducing lactate levels in patients with lactic acidosis of various causes⁷⁸. Nevertheless, the potential for adverse effects, such as toxic neuropathy⁷⁹, or controversy surrounding its efficacy on the neurological outcome in children with congenital lactic acidosis⁸⁰ has limited its wide application in the real world.

A recently developed PDK inhibitor, PS10, preferentially targets PDK2 and PDK4. The half maximal inhibitory concentrations are 0.80 $\mu\text{mol/L}$ for PDK2, 0.77 for PDK4, 2.1 for PDK1 and 21.3 for PDK3⁸¹. Four-week intraperitoneal injection of PS10 (70 mg/kg) improved glucose tolerance and hepatic steatosis in diet-induced obesity mice⁸¹. PS10 also improves hepatic insulin sensitivity (as evidenced by restoration of the phosphorylated protein kinase B/total Akt ratio) and decreases expression of lipogenic genes (acetyl coenzyme carboxylase 1, fatty acid synthase and stearoyl coenzyme A desaturase-1) in the liver⁵⁴.

Very recently, we and collaborators developed PDK4 inhibitor compound **8c** that binds to an allosteric site of the PDKs with greater inhibitory potency than PS10⁸². One-week oral administration (100 mg/kg) improved glucose tolerance in high-fat diet-fed mice. A novel PDK inhibitor, JTT-251 by Japan Tobacco and Akros, has finished a phase I clinical trial⁸³.

FUTURE PERSPECTIVES AND CONCLUSION

Accumulating evidence has shown that the pathophysiology of diabetes is complex and intertwined. “Ominous Octet”⁸⁴ or “Egregious Eleven”⁸⁵ perhaps best describes the complexity and uncertainty of the pathophysiology of the disease. Taking into account insulin resistance is a common denominator of type 2 diabetes and obesity, it is likely that mitochondrial dysfunction is the utmost underlying pathophysiology of the disease^{7,86,87}. The ER–mitochondrial contact site is increased in the livers of obese individuals⁴⁰, which accounts for mitochondrial dysfunction and ER stress, as observed in our previous finding²². We also observed that increased PDK4 is responsible for mitochondrial dysfunction in vascular smooth muscle cells, which is recovered by PDK4 deficiency or DCA treatment¹⁰. It is plausible that mitochondrial dynamics proteins play a role in mitochondrial dysfunction in obesity or in type 2 diabetes, given that muscle from obese or type 2 diabetes patients shows a reduced expression of mitofusin-2 (*Mfn2*)⁸⁸, and bariatric surgery is associated with an increased muscle *Mfn2* expression⁸⁸. In this regard, beyond promoting aerobic glycolysis (Warburg effect), the new role of PDKs, especially PDK4, needs to be elucidated and established in terms of a mitochondrial dynamics insulin-resistant milieu.

In summary, in addition to inducing complete oxidation of three carbon gluconeogenic substrates in the muscle and liver, inhibition of PDK4 improves muscle insulin sensitivity by perturbing ER–mitochondrial contact, and suppresses glucagon-induced gluconeogenesis by decreasing liver cAMP in obese rodent models. Furthermore, suppression of PDKs in myeloid

cells attenuates obesity and hyperglycemia by inhibition of M1 macrophage polarization. Activation of PDK can activate BAT through promoting FGF21 production in the liver. Small molecules specifically targeting PDK4 and/or PDK2 are being developed that might find application in metabolic diseases. Whether these small molecule inhibitors will decrease MAM formation in the muscle, suppress M1 polarization in adipose tissue macrophage, attenuate hepatic gluconeogenesis, and enhance BAT activity remain to be determined.

Taking into consideration that mitochondrial dysfunction is the culprit in insulin resistance, a mitochondria-centric approach including targeting PDKs might advance our understanding and provide a novel therapeutic approach for metabolic diseases.

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DISCLOSURE

The authors declare no conflict of interest.

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