



REVIEW

The pyruvate dehydrogenase complex as a therapeutic target for age-related diseases

Peter W. Stacpoole

Departments of Medicine (Division of Endocrinology and Metabolism) and Biochemistry and Molecular Biology, College of Medicine, University of Florida, Gainesville, FL 32611, USA

Summary

Considerable research has been conducted on mitochondrial biology as it pertains to aging. However, relatively little attention has been accorded the pyruvate dehydrogenase complex (PDC) relative to how we grow old and acquire age-related diseases. The purpose of this review is threefold: first, to describe the physiological chemistry of the PDC and define its place in normal cellular bioenergetics; second, to compare and contrast the pathogenesis and clinical features of congenital PDC deficiency with discrete examples of age-associated dysfunction of the complex; and third, to summarize recent findings in *Caenorhabditis elegans* that shed additional new light on the significance of the PDC to the aging process.

Key words: aging; Alzheimer's disease; *Caenorhabditis elegans*; cancer; dichloroacetate; glucose intolerance; mitochondria; pyruvate dehydrogenase complex.

Introduction

Interest in the association between mitochondrial dysfunction and the pathobiology of aging and age-related disorders has only waxed since the free radical theory of aging was posited over half a century ago (Harman, 1956). Modern interpretations of this theory emphasize the importance of genetically or environmentally induced disruption of mitochondrial electron transport and inadequate antioxidant defense mechanisms in causing abnormal accumulation of damaging reactive oxygen species (ROS) (Balaban *et al.*, 2005). Indeed, the term 'mitochondrial disease' is most commonly applied to congenital or acquired defects in the terminal steps of oxidative phosphorylation (OXPHOS) embodied in the five complexes of the respiratory chain of enzymes (DiMauro *et al.*, 2006). These complexes, comprised of nuclear DNA and mitochondrial DNA-encoded proteins, enable the stepwise transfer of electrons from reducing equivalents (NADH and FADH₂) to molecular oxygen and result ultimately in the synthesis of ATP. Less frequently considered is the impact of more proximal steps in mitochondrial fuel metabolism that can dramatically influence the generation of free radicals and energy. Prominent among these early mitochondrial reactions is that catalyzed by the pyruvate dehydrogenase complex (PDC), which functionally links glycolysis in the cytoplasm to OXPHOS in the mitochondrion.

Correspondence

Peter W. Stacpoole, UF College of Medicine, 1600 SW Archer Road M2-238, P.O. Box 100226, Gainesville, FL 32610, USA. Tel.: 352 273 9023; fax: 352 273 9013; e-mail: pws@ufl.edu

Accepted for publication 18 January 2012

The pyruvate dehydrogenase complex

The PDC is distributed heterogeneously within the mitochondrial matrix (Margineantu *et al.*, 2002) and catalyzes the irreversible oxidation of pyruvate to acetyl CoA (Fig. 1). The reaction is rate-limiting under aerobic conditions for the oxidative removal of glucose and pyruvate and for other 3-carbon metabolites (alanine and lactate) in equilibrium with pyruvate. As befitting such a critical gatekeeper enzyme, the PDC is highly regulated. Rapid changes in catalytic activity are achieved primarily by reversible phosphorylation by PDC kinases (PDKs) and phosphatases (PDPs). Humans possess at least four PDK and two PDP isoforms that are expressed differentially among tissues (Linn *et al.*, 1969; Bowker-Kinley *et al.*, 1998). End products of the PDC-catalyzed reaction, acetyl CoA and NADH, each increase the activity of PDKs, leading to phosphorylation and inactivation of the PDC, as does a rise in intramitochondrial ATP. In contrast, pyruvate inhibits PDK activity as do several halogenated xenobiotics that are structurally similar to pyruvate. One of these analogs, dichloroacetate (DCA), has been used extensively in the laboratory and clinically to modulate PDC activity (reviewed in Stacpoole, 2011). In turn, the activity of PDPs may be positively regulated by insulin and by magnesium and calcium ions.

Congenital PDC deficiency

It may not appear intuitively obvious why a treatise on the PDC's role in aging should include a discussion of the fate of children born with loss-of-function mutations in the complex. However, the natural history of congenital PDC deficiency (reviewed in Patel *et al.*, 2011) and the biochemical concomitants of this rare disease provide insight into the clinical presentation and underlying mechanisms of many age-related disorders. Several cardinal clinical features of PDC deficiency are remarkably similar to those associated with aging. For example, neurocognitive and neuromuscular complications, such as mental retardation, hypotonia, ataxia, peripheral neuropathy and exercise intolerance, are common findings in PDC-deficient children and neuroimaging frequently reveals structural brain abnormalities, including cerebral atrophy and ventriculomegaly. No proven therapies exist for congenital PDC deficiency, and most affected patients die within the first two decades of life.

Studies using cultures of skin fibroblasts from patients harboring various mutations in the PDC E1 α subunit have shown that these cells exhibit high rates of glycolysis and lactate production compared to similarly treated fibroblasts from healthy donors (Simpson *et al.*, 2006). In PDC deficiency, glucose carbon is diverted from acetyl CoA synthesis to oxaloacetate formation via pyruvate carboxylase, although overall flux through the tricarboxylic (TCA) cycle is reduced. The metabolic abnormalities are reversed on treatment with the prototypic PDK inhibitor DCA. These data indicate that aerobic glycolysis is a prominent feature of PDC deficiency but that, in at least some cases, dephosphorylation of the mutated enzyme can increase residual enzyme activity and shift glucose metabolism from glycolysis toward increased OXPHOS. E1 α -deficient cells also accumulate superoxide (O₂⁻) from the Qo site of complex III of the

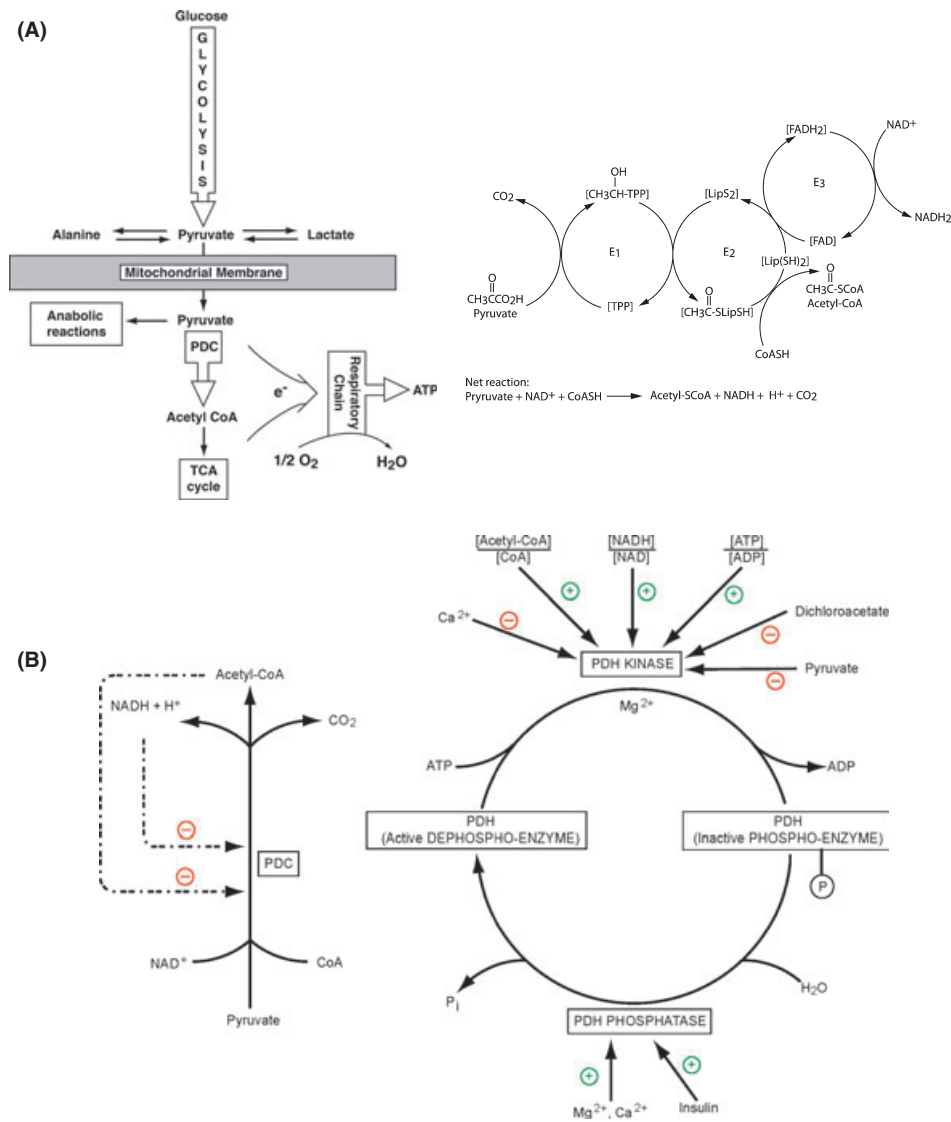


Fig. 1 Panel A – The 9.5M Da eukaryotic complex is organized into multiple copies of 3 enzymatic components (Zhou *et al.*, 2001; Smolle *et al.*, 2006). The heterotetrameric ($\alpha 2\beta 2$) pyruvate dehydrogenase (E1) decarboxylates pyruvate in the presence of thiamine pyrophosphate (TPP). Dihydropyridine acetyltransferase (E2) transfers the acetyl group to a lipoyl moiety that synthesizes up to 60 molecules of acetyl CoA from reduced coenzyme A per macromolecular complex. Reduced lipoate is reoxidized by dihydropyridine dehydrogenase (E3) in a coupled redox reaction in which NADH is generated. The pyruvate dehydrogenase complex (PDC) also utilizes an E3 binding protein (E3BP) to tether the E3 component to the complex's core (Brautigam *et al.*, 2006). The net reaction thus provides glucose-derived acetyl CoA for the tricarboxylic cycle and reducing equivalents (NADH) for the respiratory chain or for anabolic reactions, such as lipid synthesis. Five requisite cofactors enable pyruvate oxidation (thiamine; B1) and the synthesis of coenzyme A (pantothenic acid; B5), acetyl CoA (lipoic acid), and NADH (riboflavin; B2 and niacin; B3). The gene for the E1 α subunit is located on the X chromosome, and all components of the complex are nuclear encoded. Panel B – Rapid regulation of the PDC is mediated primarily by reversible phosphorylation of up to three serine residues on the E1 α subunit, rendering the complex inactive. Phosphorylation of E1 α is facilitated by a family of four pyruvate dehydrogenase kinase isoforms (PDK 1–4), whereas two pyruvate dehydrogenase phosphatase isoforms (PDP 1 and 2) dephosphorylate, and activate, the PDC. PDKs themselves are activated by a rise in the intramitochondrial ratio of acetyl CoA:CoA and NADH:NAD⁺, as well as by an increase in cellular energy charge (ATP:ADP). Pyruvate and certain structurally related halogenated analogs, such as dichloroacetate (DCA), inhibit PDK activity. PDKs are positively regulated by insulin or magnesium ions and PDP1 can be activated by calcium ions. PDK and PDP isoforms are differentially expressed in tissues and PDK isoforms exhibit variable sensitivity to pyruvate and DCA (Bowker-Kinley *et al.*, 1998).

respiratory chain. Pathological accumulation of O₂⁻ may be due mainly to its underutilization, because the activity of mitochondrial manganese superoxide dismutase (MnSOD) is also reduced in PDC-deficient cells (Glu-shakova *et al.*, 2011). A particularly intriguing and unexpected finding in PDC-deficient fibroblasts was the overexpression of hypoxia inhibitory factor 1 α (HIF1 α). HIF1 α transactivates numerous genes involved in critical pathways of cell metabolism, growth and survival (Gordan *et al.*, 2007; Semenza, 2011), including those encoding glucose transporters and most glycolytic enzymes. It also transactivates PDK (Kim *et al.*, 2006), thereby

down-regulating the PDC and OXPHOS. Together, HIF1 α 's effects on glucose metabolism provide crucial insight into the molecular mechanisms of the Warburg effect, a phenomenon first described in tumor cells almost a century ago (Warburg, 1930). The mechanism for HIF1 α overexpression in PDC deficiency is unknown, although ROS generated by complex III are thought to be required for activation of HIF1 α under conditions of hypoxia (Klimova & Chandel, 2008). In addition, glycolytic metabolites, such as pyruvate, stabilize HIF1 α by inhibiting its degradation by cytoplasmic prolyl hydroxylases (Lu *et al.*, 2005), creating a positive feedback loop

whereby HIF1 α activity in PDC deficiency is maintained by increased glycolytic flux. Regardless of the precise mechanism, HIF1 α overexpression may contribute to the Warburg effect operative in PDC-deficient cells and to further inhibition of residual PDC activity.

Congenital defects in the PDC and respiratory chain enzymes share many common clinical features (Patel *et al.*, 2011; Scaglia *et al.*, 2004). Thus, it could be argued that acquired PDC deficiency is no more relevant to the aging process than acquired pathological changes in other OXPHOS enzymes. However, what distinguishes the PDC is evidence that mitigation or reversal of the phenotype in several age-related disorders, such as glucose intolerance (Stacpoole *et al.*, 1978), ischemia–reperfusion injury or heart failure (Bersin & Stacpoole, 1997), pulmonary arterial hypertension (Bonnet *et al.*, 2006), neurodegeneration (Stacpoole, 1997) and cancer (Archer *et al.*, 2008), is achievable by therapeutic targeting of the complex. Each of these conditions is pathologically distinct, yet, together, find common ground as disorders of metabolic integration in which PDC dysregulation figures prominently, as described more fully by the following examples.

Glucose intolerance

Blood glucose concentration is maintained within well-defined limits by mechanisms regulating its uptake, storage, and utilization. Remodeling of glucose metabolism, leading to progressive tissue resistance to insulin action and glucose utilization, is a well-recognized concomitant of aging and contributing environmental factors, such as diet and physical activity. In 1963, Randle *et al.* (1963) proposed a ‘glucose–fatty acid cycle’ that described fuel selection in mammals and posited a reciprocal relationship between the utilization of glucose and long-chain fatty acids derived from

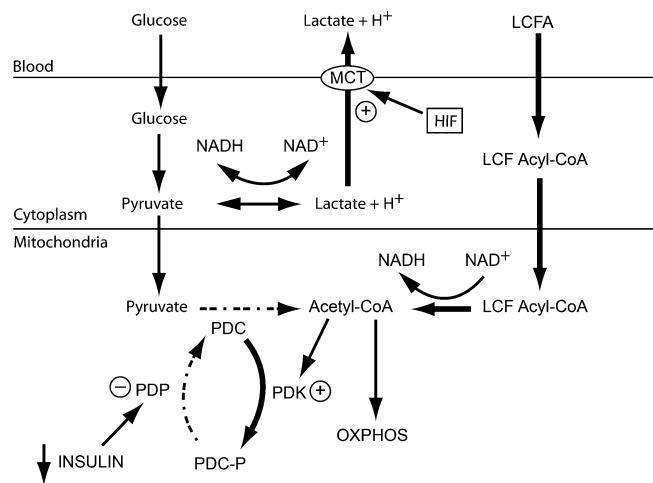


Fig. 2 Metabolic remodeling in glucose intolerance. In aging, an increase in muscle long-chain fatty acid (LCFA) beta-oxidation (β -ox) raises intramitochondrial concentrations of acetyl CoA and reduced nicotinamide dinucleotide (NADH) and contributes disproportionately to tricarboxylic acid cycle flux and oxidative phosphorylation (OXPHOS). The resulting increased ratios of acetyl CoA:CoA and NADH/NAD⁺ increase the activity of PDK isoforms, particularly in insulin-sensitive tissues, such as skeletal and cardiac muscle, thereby inhibiting the pyruvate dehydrogenase complex (PDC) and contributing to the age-associated decline in peripheral insulin sensitivity. PDC inhibition also increases production of lactic acid, which exits cells mainly by two H⁺/lactate monocarboxylate transporters (MCT) 1 and 4, the latter being regulated by HIF1 α (Le Floch *et al.*, 2011; Ullah *et al.*, 2006). In addition, decreased insulin secretion by the pancreas and/or insulin action at peripheral tissues may contribute to inhibition of the PDC by decreasing tonic inhibition on PDK and tonic stimulation of PDP, which helps maintain the PDC in its unphosphorylated, catalytically active, form.

the diet or tissue stores (Fig. 2). Multiple enzyme control points were identified through which the metabolism of glucose or fat imposed inhibitory effects on the oxidation of the other substrate. One such crucial control point is the PDC, whereby NADH, acetyl CoA, and ATP generated by fatty acid oxidation inhibit the complex and contribute to the suppression of mitochondrial glucose oxidation. Since this seminal work, the glucose–fatty acid cycle has undergone extensive scrutiny and revision (reviewed in Hue & Taegtmeyer, 2009), while maintaining the relevance of the PDC as an important factor in the processes of fuel selection and fuel flux.

In vivo and *in vitro* studies in mammalian skeletal muscle and heart, two tissues in which the glucose–fatty acid cycle is particularly robust (Nuutila *et al.*, 1992), have shown that an age-related decline occurs in the activity of the PDC that may contribute to the metabolic ‘inertia’ in stimulating aerobic glucose oxidation during the transition from rest to exercise (Gurd *et al.*, 2008), but this transition can be accelerated by pharmacological inhibition of muscle PDK with DCA (Howlett *et al.*, 1999). This finding is consistent with the drug’s glucose and lactate-lowering effects in patients with type 2 diabetes mellitus (Stacpoole *et al.*, 1978). Euglycemic insulin clamp experiments in 21- and 71-year-old healthy adults demonstrate increased plasma free fatty acid concentration, lipid turnover, and lipid oxidation but decreased glucose oxidation in elderly subjects at all insulin levels administered (Bonadonna *et al.*, 1994). Not surprisingly, knockout of skeletal muscle PDK4 in mice is associated with improved peripheral glucose tolerance and insulin sensitivity and with increased glucose but decreased fatty acid oxidation in muscle (Jeoung & Harris, 2008). Moreover, the age-related increase in circulating glucose and free fatty acid levels observed in rats parallels the age-related decline in total PDC activity in heart and sarcopenic skeletal muscle (Nakai *et al.*, 1997; Martin *et al.*, 2007). These changes are also associated with an increase in mitochondrial ROS and a decrease in MnSOD activity (Martin *et al.*, 2007), reminiscent of the biochemical abnormalities associated with congenital PDC deficiency. Insulin secretion also requires a coupled PDC–OXPHOS system (Krus *et al.*, 2010), and genetically induced inhibition of beta-cell PDC leads to decreased glucose-stimulated insulin secretion and hyperglycemia (Srinivasan *et al.*, 2010).

Cancer

Cancer is the archetypal disease of aging, with 60% of newly diagnosed cancers found in people over 65 years of age and 70% of cancer-related deaths occurring in this same population (Cancer Trends Progress Report, 2009/2010). Yet, despite its complex biology and protean manifestations, a striking property shared by most cancers is a reliance on aerobic glycolysis to provide the energy required to survive and grow (Gatenby & Gillies, 2004). Warburg originally postulated that mitochondrial defects in fuel metabolism were central to carcinogenesis and tumor progression (Warburg, 1930). Our modern understanding of the molecular mechanisms underlying the relationships between aerobic glycolysis and cancer informs that mitochondrial dysfunction in cancer is potentially reversible. Once again, the PDC is relevant both to the pathogenesis of the Warburg effect in tumors and to its reversal.

Normoxic overexpression of HIF1 α in most, if not all, human cancers stimulates glucose uptake, glycolysis, and also PDK, which inhibits the PDC. Consequently, OXPHOS is suppressed and pyruvate accumulates, which stabilizes HIF1 α , creating a positive feedback loop between HIF1 α and glycolysis (Lu *et al.*, 2002, 2005) that can be reversed by knockdown of PDK (McFate *et al.*, 2008). In a landmark study, Bonnet *et al.* (2007) investigated downstream metabolic consequences of the Warburg effect in various human tumors in cell culture or implanted into nude athymic rats. Compared to normal cells, cancer cells exhibited the expected

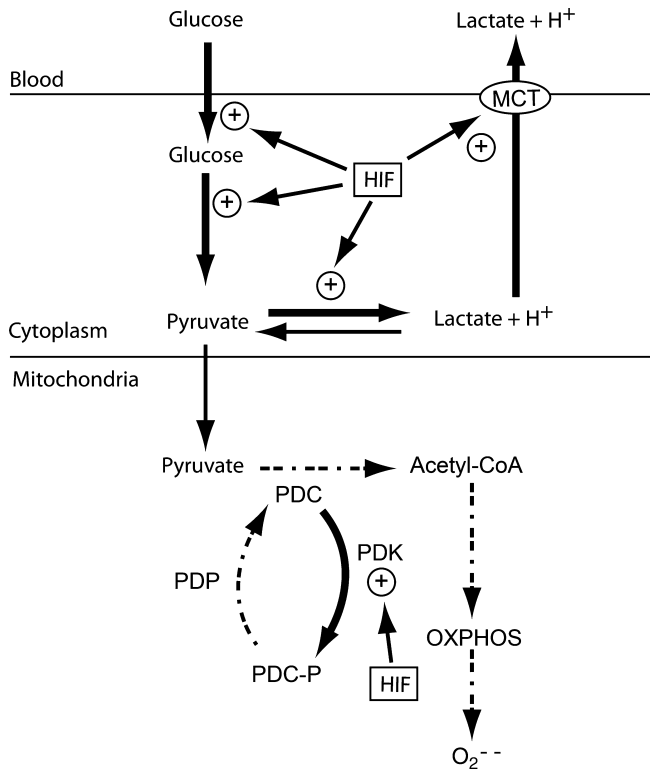


Fig. 3 Metabolic remodeling in cancer. Up-regulation and stabilization of the transcription factor hypoxia inducible factor-1 α (HIF1 α) provides the molecular mechanism for the Warburg effect in cancer. Up-regulation of HIF-1 α increases glucose uptake, glycolysis, and lactate in cytoplasm and activates PDK, inhibiting the pyruvate dehydrogenase complex (PDC) and causing cancer cells to rely on glycolysis for energy, rather than on oxidative metabolism. The reduction in OXPHOS decreases superoxide (O_2^-) production by the respiratory chain, resulting in perturbation of redox signaling mechanisms and inhibition of apoptosis (Bonnet *et al.*, 2007; Archer *et al.*, 2008). In addition, MCT 1 and 4 allow cellular egress of lactate and protons, thereby reducing tumor acidosis and promoting tumor survival. MCT4 is regulated by HIF1 α (Le Floch *et al.*, 2011; Ullah *et al.*, 2006).

up-regulation of PDK and inhibition of OXPHOS, leading to reduced production of O_2^- (Fig. 3). In addition, cancer cells had high mitochondrial membrane potential and low expression of the oxygen-sensitive potassium channel Kv1.5, both of which contributed to apoptosis resistance. DCA inhibited PDK in tumor cells, reactivated the PDC, and reversed the Warburg effect, thus decreasing glycolysis and increasing OXPHOS and O_2^- . The drug also selectively up-regulated Kv1.5 channels and induced apoptosis only in tumor cells by a mechanism involving inhibition of nuclear factor of activated T cells. Qualitatively similar findings have been obtained in several other human tumor types exposed to DCA (reviewed in Papandreou *et al.*, 2011). DCA appears to inhibit PDK by two mechanisms: by directly inhibiting the kinase (Roche & Hiromasa, 2007; Li *et al.*, 2009) and by decreasing the expression and stability of HIF1 α (Archer *et al.*, 2008; Sun *et al.*, 2011). Proof-of-concept validation of these preclinical reports was recently demonstrated in five patients with glioblastoma multiforme who received oral DCA for up to 15 months (Michelakis *et al.*, 2010). Together, these data indicate that the PDC and its regulatory kinases are not only fundamental to the pathology of cancer cell metabolism but are also exciting new targets for therapy.

The magnitude of ROS accumulation in cancer and their pathophysiological role in the disease are controversial. Bonnet's findings of

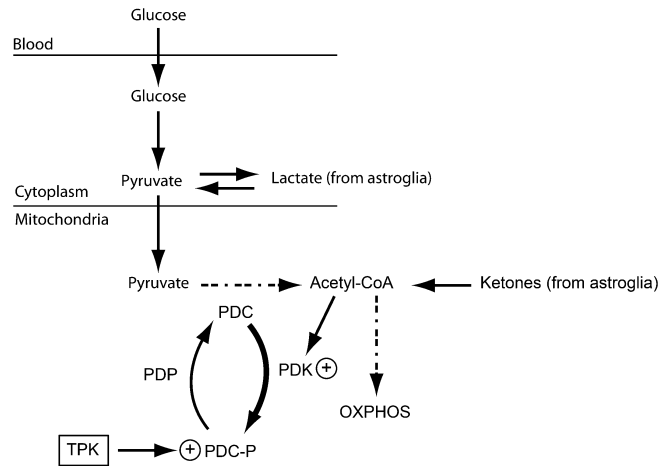


Fig. 4 Metabolic remodeling in Alzheimer's disease (AD) neurons. Neuronal pyruvate dehydrogenase complex (PDC) activity may be inhibited by at least two mechanisms. First, oxidation of ketones provided by astroglial cells as an energy substrate for neurons may increase the mitochondrial ratio of acetyl CoA:CoA, which activates PDK and inhibits the PDC. Second, accumulation of beta-amyloid in AD cells causes pathological activation of tau protein kinase I/glycogen synthase kinase β (TPK), which is also capable of phosphorylating the PDC. Consequently, glucose oxidation is inhibited in AD neurons, resulting in diminished OXPHOS and energy failure.

decreased O_2^- accumulation are at variance with some results reported for other cancers (reviewed in Gogvadze *et al.*, 2010) and for congenital PDC deficiency (Glushakova *et al.*, 2011), despite concordance in demonstrating aerobic glycolysis and suppression of MnSOD in these diseases. An important consideration is the degree to which ROS accumulates in these disorders, because O_2^- levels in PDC-deficient fibroblasts were not sufficient to cause evidence of oxidative changes in cellular lipids or proteins or to DNA (Glushakova *et al.*, 2011), whereas ROS-associated damage to cellular components has been reported in cancer (reviewed in Anastasiou *et al.*, 2011; Hamanaka & Chandel, 2011).

Alzheimer's disease

Brain glucose oxidation and oxygen consumption decline with aging (Martin *et al.*, 1991; Kalpouzos *et al.*, 2009). Studies employing magnetic resonance spectroscopy and infusions of stable isotopes of glucose and acetate in healthy young and elderly subjects suggest that brain neuronal glucose oxidation is decreased and nonoxidative glucose removal is increased in the elderly, whereas aging appears to have comparatively less impact on astrocytic energetics (Boumezbeur *et al.*, 2010). These findings are consistent with the observed age-related metabolic shift toward aerobic glycolysis and ketone body oxidation in rodent brain (Ross *et al.*, 2010; Yao *et al.*, 2010) and with a decrease in unphosphorylated (active) PDC (Zhou *et al.*, 2009) (Fig. 4). Although the latter experiments were conducted using whole brains of rats, it is likely that the major effect of aging on PDC activity is exerted on neurons. This is because PDK is normally highly expressed and the PDC is strongly inhibited in astrocytes from rat brain, whereas neuronal PDC activity is close to maximum levels because of lower PDK expression (Halim *et al.*, 2010). This metabolic dichotomy makes sense from a teleological standpoint, considering that glia are considered to be an important provider of lactate used as oxidative fuel by neurons (reviewed in Magistretti, 2006) and that mammalian neuronal PDC activity normally operates at near-maximum levels to maintain vital energetic functions (reviewed in Robinson, 2001). In addition,

many neurodegenerative diseases occurring in mid- to late-life are associated with abnormal brain oxidative metabolism. Although the etiology of mitochondrial dysfunction in neurodegenerative diseases is undoubtedly multifactorial (reviewed in Coskun *et al.*, 2011; Green *et al.*, 2011) intriguing new data implicate reduced PDC activity and up-regulation of aerobic glycolysis in several of these disorders, particularly Alzheimer's disease (AD).

It has long been known that diminished brain glucose metabolism in patients with AD precedes the appearance of overt clinical manifestations of the disease. Cognitively normal subjects who are homozygous for the *APOE* ϵ 4 allele that confers heightened risk of developing AD show decreased glucose metabolism in the same brain regions as do patients with AD (Small *et al.*, 1995; Reiman *et al.*, 1996). Postmortem analysis of brains from patients with AD have repeatedly demonstrated significant decreases in the activities of the PDC and α -ketoglutarate dehydrogenase, a TCA cycle enzyme structurally and functionally similar to the PDC (Blass *et al.*, 2000; Bubber *et al.*, 2005). Reduced PDC activity and intramitochondrial respiration have been recapitulated in a transgenic mouse model of AD (Yao *et al.*, 2009). The brains of these animals contained extracellular aggregates of β -amyloid peptide (A β) and intraneuronal neurofibrillary tangles composed of hyperphosphorylated tau protein, both of which are considered important in the pathogenesis of AD (Sato *et al.*, 2002). A β exposure activates tau protein kinase I/glycogen synthase kinase 3 β (TPKI/GSK-3 β), which phosphorylates tau protein into forms typically found in AD brains. Although the E1 α subunit of the PDC is reported to be a substrate for TPKI/GSK-3 β (Hoshi *et al.*, 1996), it is difficult to understand how this cytoplasmic enzyme could exert regulatory control over the PDC. Nevertheless, regional reductions in cerebral glucose metabolism correlate with the magnitude of deposition of neurofibrillary tangles and, hence, of abnormal tau protein (Planel *et al.*, 2004). Furthermore, studies in human cancer cells have revealed that the PDC exists not only within the mitochondrial matrix but also on the outer mitochondrial membrane (Hitosugi *et al.*, 2011). Thus, it is possible that TPKI/GSK-3 β could exert regulatory influence on PDC molecules 'exposed' to the cytoplasmic environment. Precise localization of the PDC in AD brains has not been reported.

Recent findings using positron emission tomography applied to healthy young adults provide new insight into regional brain metabolism that might first appear inconsistent with some of the earlier results obtained in aged or AD individuals. Aerobic glycolysis and mitochondrial oxidative metabolism appear to be differentially located in the normal resting brain (Vaishnavi *et al.*, 2010) and can account for up to 15% of glucose metabolism in adults (Powers *et al.*, 1998). Moreover, regional variations in aerobic glycolysis are not tightly correlated with brain metabolic activity, implying that such regions rely on a comparatively inefficient pathway of energy generation to maintain normal resting function. However, the spacial distribution of aerobic glycolysis in normal young adults also correlates with A β deposition both in subjects with overt AD and in cognitively normal individuals with elevated levels of A β (Vlassenko *et al.*, 2010). Although the significance of brain A β accumulation to the Warburg effect remains controversial (c.f. Newington *et al.*, 2011), most evidence strongly supports an age- and disease-dependent decline in brain mitochondrial function, in which reduced activity of the PDC plays a major role.

On aging worms and the PDC

Caenorhabditis elegans has been a useful model by which to study mechanisms of aging, including studies of the relationship between aging and energy metabolism. Induced loss-of-function mutations of complex I

cause lactic acidosis, oxidative stress and decreased OXPHOS, fecundity, and survival. This pathology can be mitigated by dietary supplementation with certain water-soluble vitamins or with DCA, which decreases lactate concentrations and increases fecundity and survival (Grad & Lemire, 2004). Although the effect of vitamins might be rationalized on the basis of their actions as cofactors for OXPHOS enzymes or as antioxidants, the effect of DCA in animals with complex I mutations is harder to explain. The drug has no known direct effect on any respiratory chain complex. Moreover, it might be assumed that increasing the mitochondrial NADH/NAD⁺ ratio by stimulating flux through the PDC and the TCA cycle might worsen a condition in which reoxidation of NADH is already diminished. However, anecdotal reports of biochemical and clinical benefit of DCA in children with congenital deficiency of complex I or other respiratory chain components (Stacpoole *et al.*, 1997) suggest that increasing PDC activity might improve mitochondrial energetics, providing there was sufficient residual activity of the downstream mutated enzyme to accommodate the increased provision of reducing equivalents. So might it be for the lowly nematode. Indeed, exposing normal worms to DCA preserves locomotive activity and improves their lifespan (Schaffer *et al.*, 2011). These salutary effects are probably mainly due to inhibition of PDK and subsequent stimulation of the PDC, because *C. elegans* survival is reduced by knockout of PDC but is enhanced by knockout of PDK (Mouchiroud *et al.*, 2011).

Summary

The PDC is central to mitochondrial fuel metabolism and, thus, to organismal health and survival. Deficiency of the complex, either through normal aging or by the imposition of congenital or acquired diseases, displays a strikingly similar pathology and affords stature to the PDC and its regulatory kinases as potential therapeutic targets for multiple rare and common disorders. Such age-associated conditions as sarcopenia, glucose intolerance, neurodegenerative diseases, and cancer represent fertile areas for continued research into the pathophysiological significance of how this fascinating macromolecular complex is perturbed and how it can be therapeutically manipulated.

References

- Anastasiou D, Pouligiannis G, Asara JM, Boxer MB, Jiang JK, Shen M, Bellinger G, Sasaki AT, Locasale JW, Auld DS, Thomas CJ, Vander Heiden MG, Cantley LC (2011) Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. *Science* **334**, 1278–1283.
- Archer SL, Gomberg-Maitland M, Maitland ML, Rich S, Garcia JG, Weir EK (2008) Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1 α -Kv1.5 O₂-sensing pathway at the intersection of pulmonary hypertension and cancer. *Am J Physiol Heart Circ Physiol* **294**, H570–H578.
- Balaban RS, Nemoto S, Finkel T (2005) Mitochondria, oxidants, and aging. *Cell* **120**, 483–495.
- Bersin RM, Stacpoole PW (1997) Dichloroacetate as metabolic therapy for myocardial ischemia and failure. *Am. Heart J.* **134**, 841–855.
- Blass JP, Sheu RK, Gibson GE (2000) Inherent abnormalities in energy metabolism in Alzheimer disease. Interaction with cerebrovascular compromise. *Ann. N. Y. Acad. Sci.* **903**, 204–221.
- Bonadonna RC, Groop LC, Simonson DC, DeFronzo RA (1994) Free fatty acid and glucose metabolism in human aging: evidence for operation of the Randle cycle. *Am. J. Physiol.* **266**, E501–E509.
- Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Bonnet S, Harry G, Hashimoto K, Porter CJ, Andrade MA, Thebaud B, Michelakis ED (2007) A mitochondria-K⁺ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. *Cancer Cell* **11**, 37–51.
- Bonnet S, Michelakis ED, Porter CJ, Andrade-Navarro MA, Th baud B, Bonnet S, Haromy A, Harry G, Moudgil R, McMurtry MS, Weir EK, Archer SL (2006) An

- abnormal mitochondrial-hypoxia inducible factor-1 α -Kv channel pathway disrupts oxygen sensing and triggers pulmonary arterial hypertension in fawn hooded rats: similarities to human pulmonary arterial hypertension. *Circulation* **113**, 2630–2641.
- Boumezbeur F, Mason GF, de Graaf RA, Behar KL, Cline GW, Shulman GI, Rothman DL, Petersen KF (2010) Altered brain mitochondrial metabolism in healthy aging as assessed by in vivo magnetic resonance spectroscopy. *J. Cereb. Blood Flow Metab.* **30**, 211–221.
- Bowker-Kinley MM, Davis WI, Wu P, Harris RA, Popov KM (1998) Evidence for existence of tissue-specific regulation of the mammalian pyruvate dehydrogenase complex. *Biochem. J.* **329**, 191–196.
- Brautigam CA, Wynn RM, Chuang JL, Machius M, Tomchick DR, Chuang DT (2006) Structural insight into interactions between dihydrolipoamide dehydrogenase (E3) and E3 binding protein of human pyruvate dehydrogenase complex. *Structure* **14**, 611–621.
- Bubber P, Haroutunian V, Fisch G, Blass JP, Gibson GE (2005) Mitochondrial abnormalities in Alzheimer brain: mechanistic implications. *Ann. Neurol.* **57**, 695–703.
- Coskun P, Wyrembak J, Schriener S, Chen HW, Marciniack C, Laferla F, Wallace DC (2011) A mitochondrial etiology of Alzheimer and Parkinson disease. *Biochim. Biophys. Acta*. PMID 21871538, in press.
- DiMauro S, Hirano M, Schon EA (2006) Approaches to the treatment of mitochondrial diseases. *Muscle Nerve* **34**, 265–283.
- Gatenby RA, Gillies RJ (2004) Why do cancers have high aerobic glycolysis? *Nat. Rev. Cancer* **4**, 891–899.
- Glushakova LG, Judge S, Cruz A, Pourang D, Mathews CE, Stacpoole PW (2011) Increased superoxide accumulation in pyruvate dehydrogenase complex deficient fibroblasts. *Mol. Genet. Metab.* **104**, 255–260.
- Gogvadze V, Zhivotovskiy B, Orrenius S (2010) The Warburg effect and mitochondrial stability in cancer cells. *Mol. Aspects Med.* **31**, 60–74.
- Gordan JD, Thompson CB, Simon MC (2007) HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. *Cancer Cell* **12**, 108–113.
- Grad LI, Lemire BD (2004) Mitochondrial complex I mutations in *Caenorhabditis elegans* produce cytochrome c oxidase deficiency, oxidative stress and vitamin-responsive lactic acidosis. *Hum. Mol. Genet.* **13**, 303–314.
- Green DR, Galluzzi L, Kroemer G (2011) Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science* **333**, 1109–1112.
- Gurd BJ, Peters SJ, Heigenhauser GJ, LeBlanc PJ, Doherty TJ, Paterson DH, Kowalchuk JM (2008) O₂ uptake kinetics, pyruvate dehydrogenase activity, and muscle deoxygenation in young and older adults during the transition to moderate-intensity exercise. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, R577–R584.
- Halim ND, Mcfate T, Mohyeldin A, Okagaki P, Korotchikina LG, Patel MS, Jeoung NH, Harris RA, Schell MJ, Verma A (2010) Phosphorylation status of pyruvate dehydrogenase distinguishes metabolic phenotypes of cultured rat brain astrocytes and neurons. *Glia* **58**, 1168–1176.
- Hamanaka RB, Chandel NS (2011) Cell biology. Warburg effect and redox balance. *Science* **334**, 1219–1220.
- Harman D (1956) Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**, 298–300.
- Hitosugi T, Fan J, Chung TW, Lythgoe K, Wang X, Xie J, Ge Q, Gu TL, Polakiewicz RD, Roesel JL, Chen GZ, Boggon TJ, Lonial S, Fu H, Khuri FR, Kang S, Chen J (2011) Tyrosine phosphorylation of mitochondrial pyruvate dehydrogenase kinase 1 is important for cancer metabolism. *Mol. Cell* **44**, 864–877.
- Hoshi M, Takashima A, Noguchi K, Murayama M, Sato M, Kondo S, Saitoh Y, Ishiguro K, Hoshino T, Imahori K (1996) Regulation of mitochondrial pyruvate dehydrogenase activity by tau protein kinase I/glycogen synthase kinase β in brain. *Proc. Natl Acad. Sci. U.S.A.* **93**, 2719–2723.
- Howlett RA, Heigenhauser GJ, Hultman E, Hollidge-Horvat MG, Spriet LL (1999) Effects of dichloroacetate infusion on human skeletal muscle metabolism at the onset of exercise. *Am. J. Physiol.* **277**(1 Pt 1), E18–E25.
- Hue L, Taegtmeyer H (2009) The Randle cycle revisited: a new head for an old hat. *Am. J. Physiol. Endocrinol. Metab.* **297**, E578–E591.
- Jeoung NH, Harris RA (2008) Pyruvate dehydrogenase kinase-4 deficiency lowers blood glucose and improves glucose tolerance in diet-induced obese mice. *Am. J. Physiol. Endocrinol. Metab.* **295**, E46–E54.
- Kalopoulos G, Chetelat G, Baron JC, Landeau B, Mevel K, Godeau C, Barre L, Constans JM, Viader F, Eustache F, Desgranges B (2009) Voxel-based mapping of brain gray matter volume and glucose metabolism profiles in normal aging. *Neurobiol. Aging* **30**, 112–124.
- Kim JW, Tcheryshyov I, Semenza GL, Dang CV (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab.* **3**, 177–185.
- Klimova T, Chandel NS (2008) Mitochondrial complex III regulates hypoxic activation of HIF. *Cell Death Differ.* **15**, 660–666.
- Krus U, Kotova O, Spégel P, Hallgard E, Sharoyko VV, Vedin A, Moritz T, Sugden MC, Koeck T, Mulder H (2010) Pyruvate dehydrogenase kinase 1 controls mitochondrial metabolism and insulin secretion in INS-1 832/13 clonal beta-cells. *Biochem. J.* **429**, 205–213.
- Le Floch R, Chiche J, Marchiq I, Naiken T, Ilk K, Murray CM, Critchlow SE, Roux D, Simon MP, Pouyssegur J (2011) CD147 subunit of lactate/H⁺ symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. *Proc. Natl Acad. Sci. U.S.A.* **108**, 16663–16668.
- Li J, Kato M, Chuang DT (2009) Pivotal role of the C-terminal DW-motif in mediating inhibition of pyruvate dehydrogenase kinase 2 by dichloroacetate. *J. Biol. Chem.* **284**, 34458–34467.
- Linn TC, Pettit FH, Reed LJ (1969) Alpha-keto acid dehydrogenase complexes. X. Regulation of the activity of the pyruvate dehydrogenase complex from beef kidney mitochondria by phosphorylation and dephosphorylation. *Proc. Natl Acad. Sci. U.S.A.* **62**, 234–241.
- Lu H, Forbes RA, Verma A (2002) Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. *J. Biol. Chem.* **277**, 23111–23115.
- Lu H, Dalgard CL, Mohyeldin A, McFate T, Tait AS, Verma A (2005) Reversible inactivation of HIF-1 prolyl hydroxylases allows cell metabolism to control basal HIF-1. *J. Biol. Chem.* **280**, 41928–41939.
- Magistretti PJ (2006) Neuron-glia metabolic coupling and plasticity. *J. Exp. Biol.* **209**, 2304–2311.
- Margineantu DH, Brown RM, Brown GK, Marcus AH, Capaldi RA (2002) Heterogeneous distribution of pyruvate dehydrogenase in the matrix of mitochondria. *Mitochondrion* **1**, 327–338.
- Martin AJ, Friston KJ, Colebatch JG, Frackowiak RS (1991) Decreases in regional cerebral blood flow with normal aging. *J. Cereb. Blood Flow Metab.* **11**, 684–689.
- Martin C, Dubouchaud H, Mosoni L, Chardigny JM, Oudot A, Fontaine E, Vergely C, Keriel C, Rochette L, Leverve X, Demaison L (2007) Abnormalities of mitochondrial functioning can partly explain the metabolic disorders encountered in sarcopenic gastrocnemius. *Aging Cell* **6**, 165–177.
- McFate T, Mohyeldin A, Lu H, Thakar J, Henriques J, Halim ND, Wu H, Schell MJ, Tsang TM, Teahan O, Zhou S, Califano JA, Jeoung NH, Harris RA, Verma A (2008) Pyruvate dehydrogenase complex activity controls metabolic and malignant phenotype in cancer cells. *J. Biol. Chem.* **283**, 22700–22708.
- Michelakis ED, Sutendra G, Dromparis P, Webster L, Haromy A, Niven E, Maguire C, Gammer TL, Mackey JR, Fulton D, Abdulkarim B, McMurtry MS, Petruk KC (2010) Metabolic modulation of glioblastoma with dichloroacetate. *Sci. Transl. Med.* **2**, 31ra34.
- Mouchiroud L, Molin L, Kasturi P, Triba MN, Dumas ME, Wilson MC, Halestrap AP, Roussel D, Masse I, Dallièrè N, Ségalat L, Billaud M, Solari F (2011) Pyruvate imbalance mediates metabolic reprogramming and mimics lifespan extension by dietary restriction in *Caenorhabditis elegans*. *Aging Cell* **10**, 39–54.
- Nakai N, Sato Y, Oshida Y, Yoshimura A, Fujitsuka N, Sugiyama S, Shimomura Y (1997) Activities of liver pyruvate dehydrogenase complex and 3-hydroxyacyl-CoA dehydrogenase in sand rat (*Psammomys obesus*). *Life Sci.* **60**, 2309–2314.
- National Cancer Institute (2010) Cancer trends progress report (2009/2010) update. *Bethesda*, MD: NIH, DHHS. URL <http://progressreport.cancer.gov> [accessed on 21 September 2011].
- Newington JT, Pitts A, Chien A, Arseneault R, Schubert D, Cumming RC (2011) Amyloid beta resistance in nerve cell lines is mediated by the Warburg Effect. *PLoS One* **6**, e19191–e19202.
- Nuutila P, Koivisto VA, Knuuti J, Ruotsalainen U, Teräs M, Haaparanta M, Bergman J, Solin O, Voipio-Pulkki LM, Wegelius U, Yki-Jarvinen H (1992) Glucose-free fatty acid cycle operates in human heart and skeletal muscle in vivo. *J. Clin. Invest.* **89**, 1767–1774.
- Papandreou I, Goliasova T, Denko NC (2011) Anticancer drugs that target metabolism: is dichloroacetate the new paradigm? *Int. J. Cancer* **128**, 1001–1008.
- Patel KP, O'Brien TW, Subramony SH, Shuster J, Stacpoole PW (2011) The spectrum of pyruvate dehydrogenase complex deficiency: clinical, biochemical and genetic features in 371 patients. *Mol. Genet. Metab.* **105**, 34–43.
- Panel E, Miyasaka T, Launey T, Chui D-H, Tanemura K, Sato S, Murayama O, Ishiguro K, Tatebayashi Y, Takashima A (2004) Alterations in glucose metabolism induce hypothermia leading to tau hyperphosphorylation through differential inhibition of kinase and phosphatase activities: implications for Alzheimer's Disease. *J. Neurosci.* **24**, 2401–2411.
- Powers WJ, Rosenbaum JL, Dence CS, Markham J, Videen TO (1998) Cerebral glucose transport and metabolism in preterm human infants. *J. Cereb. Blood Flow Metab.* **18**, 632–638.

- Randle PJ, Garland PB, Hales CN, Newsholme EA (1963) The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitu. *Lancet* **1**, 785–789.
- Reiman EM, Caselli RJ, Yun LS, Chen K, Bandy D, Minoshima S, Thibodeau SN, Osborne D (1996) Preclinical evidence of Alzheimer's Disease in persons homozygous for the $\epsilon 4$ allele for apolipoprotein E. *N. Engl. J. Med.*, **334**, 752–758.
- Robinson BH (2001) Lactic acidemia (disorders of pyruvate carboxylase, pyruvate dehydrogenase). In *The Metabolic and Molecular Bases of Inherited Disease* CR Scriver, AL Beaudet, WS Sly, D Valle, eds). New York: McGraw-Hill, Part 10, Chapter 100.
- Roche TE, Hiromasa Y (2007) Pyruvate dehydrogenase kinase regulatory mechanisms and inhibition in treating diabetes, heart ischemia, and cancer. *Cell. Mol. Life Sci.* **64**, 830–849.
- Ross JM, Öberg J, Brené S, Coppotelli G, Terzioglu M, Pernold K, Gojny M, Sitnikov R, Kehr J, Trifunovic A, Larsson NG, Hoffer BJ, Olson L (2010) High brain lactate is a hallmark of aging and caused by a shift in the lactate dehydrogenase A/B ratio. *Proc. Natl Acad. Sci. U.S.A.* **107**, 20087–20092.
- Sato S, Tatebayashi Y, Akagi T, Chui DH, Murayama M, Miyasaka T, Planel E, Tanemura K, Sun X, Hashikawa T, Yoshioka K, Ishiguro K, Takashima A (2002) Aberrant tau phosphorylation by glycogen synthase kinase-3beta and JNK3 induces oligomeric tau fibrils in COS-7 cells. *J. Biol. Chem.* **277**, 42060–42065.
- Scaglia F, Towbin JA, Craigen WJ, Belmont JW, Smith EO, Neish SR, Ware SM, Hunter JV, Fernbach SD, Vladutiu GD, Wong LJ, Vogel H (2004) Clinical spectrum, morbidity, and mortality in 113 pediatric patients with mitochondrial disease. *Pediatrics* **114**, 925–931.
- Schaffer S, Gruber J, Ng LF, Fong S, Wong YT, Tang SY, Halliwell B (2011) The effect of dichloroacetate on health- and lifespan in *C. elegans*. *Biogerontology* **12**, 195–209.
- Semenza GL (2011) Hypoxia-inducible factor 1: regulator of mitochondrial metabolism and mediator of ischemic preconditioning. *Biochim. Biophys. Acta* **1813**, 1263–1268.
- Simpson NE, Han Z, Berendzen KM, Sweeney CA, Oca-Cossio JA, Constantinidis I, Stacpoole PW (2006) Magnetic resonance spectroscopic investigation of mitochondrial fuel metabolism and energetics in cultured human fibroblasts: effects of pyruvate dehydrogenase complex deficiency and dichloroacetate. *Mol. Genet. Metab.* **89**, 97–105.
- Small GW, Mazziotta JC, Collins MT, Baxter LR, Phelps ME, Mandelkern MA, Kaplan A, La Rue A, Adamson CF, Chang L, Guze BH, Corder EH, Saunders AM, Haines JL, Pericak-Vance MA, Roses AD (1995) Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer Disease. *JAMA* **273**, 942–947.
- Smolle M, Prior AE, Brown AE, Cooper A, Byron O, Lindsay JG (2006) A new level of architectural complexity in the human pyruvate dehydrogenase complex. *J. Biol. Chem.* **281**, 19772–19780.
- Srinivasan M, Choi CS, Ghoshal P, Pliss L, Pandya JD, Hill D, Cline G, Patel MS (2010) β -Cell-specific pyruvate dehydrogenase deficiency impairs glucose-stimulated insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* **299**, E910–E917.
- Stacpoole PW (1997) Lactic acidosis and other mitochondrial disorders. *Metabolism* **46**, 306–321.
- Stacpoole PW (2011) The dichloroacetate dilemma: environmental hazard versus therapeutic goldmine – both or neither? *Environ. Health Perspect.* **119**, 155–158.
- Stacpoole PW, Moore GW, Kornhauser DM (1978) Metabolic effects of dichloroacetate in patients with diabetes mellitus and hyperlipoproteinemia. *N. Engl. J. Med.* **298**, 526–530.
- Stacpoole PW, Barnes CL, Hurbanis MD, Cannon SL, Kerr DS (1997) Treatment of congenital lactic acidosis with dichloroacetate. *Arch. Dis. Child.* **77**, 535–541.
- Sun W, Chang SS, Fu Y, Liu Y, Califano JA (2011) Chronic CSE treatment induces the growth of normal oral keratinocytes via PDK2 upregulation, increased glycolysis and HIF1 α stabilization. *PLoS One* **6**, e16207–e16214.
- Ullah MS, Davies AJ, Halestrap AP (2006) The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1 α -dependent mechanism. *J. Biol. Chem.* **281**, 9030–9037.
- Vaishnavi SN, Vlassenko AG, Rundle MM, Snyder AZ, Mintun MA, Raichle ME (2010) Regional aerobic glycolysis in the human brain. *Proc. Natl Acad. Sci. U.S.A.* **107**, 17757–17762.
- Vlassenko AG, Vaishnavi SN, Couture L, Sacco D, Shannon BJ, Mach RH, Morris JC, Raichle ME, Mintun MA (2010) Spatial correlation between brain aerobic glycolysis and amyloid- β (A β) deposition. *Proc. Natl Acad. Sci. U.S.A.* **107**, 17763–17767.
- Warburg O (1930) *Über der Stoffwechsel der Tumoren*. London: Constable.
- Yao J, Irwin RW, Zhao L, Nilsen J, Hamilton RT, Diaz Brinton R (2009) Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's Disease. *Proc. Natl Acad. Sci. U.S.A.* **106**, 14670–14675.
- Yao J, Hamilton RT, Cadenas E, Brinton RD (2010) Decline in mitochondrial bioenergetics and shift to ketogenic profile in brain during reproductive senescence. *Biochim. Biophys. Acta* **1800**, 1121–1126.
- Zhou ZH, McCarthy DB, O'Connor CM, Reed LJ, Stoops JK (2001) The remarkable structural and functional organization of the eukaryotic pyruvate dehydrogenase complexes. *Proc. Natl Acad. Sci. U.S.A.* **98**, 14802–14807.
- Zhou Q, Lam PY, Han D, Cadenas E (2009) Activation of c-Jun-N-terminal kinase and decline of mitochondrial pyruvate dehydrogenase activity during brain aging. *FEBS Lett.* **583**, 1132–1140.