Journal of Cellular Biochemistry 55S:1–11 (1994)

ARTICLES

Glucose Fatty Acid Interactions and the Regulation of Glucose Disposal

Philip J. Randle, David A. Priestman, Sharad C. Mistry, and Antony Halsall

Nuffield Department of Clinical Biochemistry, John Radcliffe Hospital, Oxford OX3 9DU, England

Abstract Glucose is essential for the energy metabolism of some cells and conservation of glucose is obligatory for survival during starvation. The principal site of this glucose conservation is the mitochondrial pyruvate dehydrogenase (PDH) complex, which is regulated by reversible phosphorylation (phosphorylation is inactivating). In cells in which glucose oxidation is switched off during starvation, fatty acids are used as fuel, and acetyl CoA and NADH formed by β-oxidation promote phosphorylation of PDH complex by activation of PDH kinase. A longer-term mechanism further increases PDH kinase activity in response to cAMP and products of β-oxidation of fatty acids. Coordinated inhibition of glycolytic flux mediated by effects of citrate on PFK1 and PFK2 in muscles and liver results in an associated inhibition of glucose uptake. Similar mechanisms lead to impaired glucose oxidation in diabetes.

Key words: pyruvate dehydrogenase complex, pyruvate dehydrogenase kinase, pyruvate dehydrogenase phosphatase, hexokinase, phosphofructokinase 1, phosphofructokinase 2, glucose 6-phosphatase, Cori cycle starvation, diabetes

HISTORICAL AND GENERAL

This subject had its origin in the realisation that although glucose is needed as a fuel during starvation, the major fuel reserve in man and laboratory animals is triacylglycerol. Because it was known that fatty acids (FFA) cannot give rise to glucose, it was pertinent to enquire whether intermediates in FFA oxidation may inhibit glucose oxidation. This led to the further consideration that such intermediates might also inhibit glucose oxidation in diabetes.

Some 30 years ago, these considerations led to the formulation of the idea of the glucose fatty acid cycle [Randle et al., 1963]. The essential components of this regulatory cycle, which owed much to parallel work by H.E. Morgan, J.R. Williamson, J.C. Shipp, and L.H. Opie were as follows: (1) the relationship between glucose and FFA metabolism is reciprocal and not dependent; (2) in vivo, oxidation of FFA and ketone bodies released into the circulation in diabetes and starvation may inhibit catabolism of glucose in muscle; (3) in vitro, the oxidation of FFA released from muscle triacylglycerol may have similar effects; (4) these effects of FFA and ketone body oxidation are mediated by inhibition of the pyruvate dehydrogenase (PDH) complex, phosphofructo 1-kinase (PFK1), and hexokinase (HK); (5) the essential mechanism is an increase in the mitochondrial ratio of [acetyl CoA]/[CoA], which inhibits the PDH complex directly, and which indirectly leads to inhibition of PFK1 by citrate and of HK by glucose 6-phosphate; and (6) the effect of physiological concentrations of insulin to activate glucose transport in heart muscle is inhibited by FFA and ketone bodies. The mechanism of citrate accumulation (unspanning of the citrate cycle) was detailed subsequently [Randle et al., 1970]. A full review of historical aspects of substrate competition is given in Randle et al. [1966].

Although received favourably at the time, the concept went out of favour as a result of some negative findings with in vitro skeletal muscle preparations in the late 1960s to early 1970s. The process of revival began in the mid-1970s with unequivocal demonstrations of effects of FFA and ketone bodies on in vitro glucose utilization by rat soleus [Cuendet et al., 1975; Maizels et al., 1977] and perfused rat hindquarter [Rennie and Holloszy, 1977]. Inhibitory effects of FFA on glucose utilization by liver in vitro were demonstrated in 1988 [Hue et al., 1988]. Inhibitory effects of FFA oxidation on glucose disposal and oxidation in man have been shown

Received October 19, 1993; accepted December 3, 1993.

Address reprint requests to Philip J. Randle, Nuffield Department of Clinical Biochemistry, Radcliffe Infirmary, Oxford OX3 6HE, United Kingdom.

unequivocally and repeatedly by (1) indirect calorimetry in conjunction with glucose and insulin/glucose clamp [Thiébaud et al., 1982b; Ferrannini et al., 1983; Boden et al., 1991; Johnson et al., 1992; Wolfe et al., 1988]; (2) positron emission tomography in heart, and in hindlimb and forelimb skeletal muscles [Nuutila et al., 1992]; and (3) forearm and hindlimb perfusion studies [Piatti et al., 1991; Walker et al., 1990, 1991; Yki-Jarvinen et al., 1991].

Since these concepts were formulated in the 1960s, two further major discoveries have been made with respect to mechanism. These are the regulation of the PDH complex by reversible phosphorylation [Linn et al., 1969] and the discovery of fructose 2,6-bisphosphate and the bifunctional PFK2/fructose 2,6-bisphosphatase [reviewed in Hers and Van Schaftingen, 1982; Pilkis et al., 1987]. The following discussion details the mechanism of the regulation of the PDH complex by FFA oxidation and places it in context in relationship to overall glucose disposal and its regulation.

REGULATION OF THE PYRUVATE DEHYDROGENASE COMPLEX BY REVERSIBLE PHOSPHORYLATION

Reactions and Chemistry of the Complex

The PDH complex is a mitochondrial multienzyme complex catalyzing the reaction of pyruvate with CoA and NAD to form acetyl CoA, $NADH_2$, and CO_2. It utilises intramitochondrial substrates and coenzymes and provides acetyl CoA for the citrate cycle and (in liver/adipose tissue) for fatty acid synthesis. The complex contains multiple copies of three component enzymes and also a binding protein (protein X), which links E3 to the complex. The subunit composition, M_r, and number of copies of the individual enzymes per purified complex are shown in Table I. The complex is assembled on a core of 60 copies of E2. The number of copies of E1 varies from 30 in bovine kidney complex and 40 in bovine heart complex to 60 in pig heart complex [Barrera et al., 1972; Sugden and Randle, 1978; Roche et al., 1993]. The elementary reactions catalysed by the individual enzymes are shown in Figure 1. The decarboxylation reaction catalysed by E1 is essentially irreversible and this confers irreversibility on the holocomplex reaction. The other reactions are freely reversible. The enzymes interact through lipoate residues attached to the mobile arm of E2, which functions to transfer acetyl (to CoA) and H via the flavoprotein E3 to NAD. End-product inhibition of the PDH complex by acetyl CoA (competitive with CoA) and $NADH_2$ (competitive with NAD) is based on reversal of reactions involving E3 and E2 leading to formation of hydroxyethyl-TPP-E1 thus blocking the decarboxylation of pyruvate. For review and/or

TABLE I. Component Enzymes of the Mammalian Pyruvate Dehydrogenase Complex

Enzyme		M_r (kDa)[a]	Moles per complex[b]
E1	Pyruvate dehydrogenase holoenzyme (α-subunit 42; β-subunit 36)	156	30–60 60–120
E2	Dihydrolipoate acetyl transferase (subunit)	52	60
E3	Dihydrolipoyl dehydrogenase holoenzyme subunits	110 55	12 24
X	E3 binding protein	52	6
	Pyruvate dehydrogenase kinase holoenzyme (α-subunit 46; β-subunit 43)	93	5 5
PDH	Phosphatase holoenzyme (subunits 97, 50)		

[a]Data for M_r are from our own observations with pig heart PDH complex, except for (1) PDH kinase, based on our own observations with the bovine kidney E2–protein X-PDH kinase subcomplex (kindly supplied by Dr. T.E. Roche); and (2) bovine kidney PDH phosphatase, from Stepp et al. [1983]. [b]Moles per complex are based on Barrera et al. [1972], Sugden and Randle [1978], Teague et al. [1982], Stepp et al. [1983], and Roche et al. [1993].

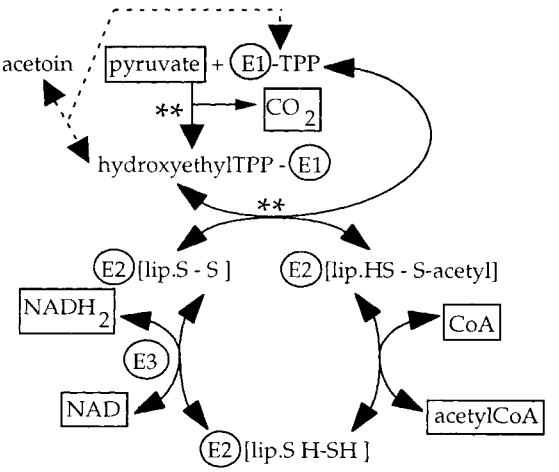

Fig. 1. Elementary reactions of the PDH complex. Substrates and products are boxed; enzymes are circled; lip, lipoate; TPP, thiamin pyrophosphate. **Reactions inhibited by phosphorylation of E1α.

further references see Reed [1981], Randle [1986], and Roche et al. [1993].

Reversible Phosphorylation in the PDH Complex: Component Enzymes

As purified, the PDH complex contains an intrinsic PDH kinase. The addition of ATPMg to the complex results in phosphorylation and inactivation. A PDH phosphatase, which separates from the complex during its purification catalyses dephosphorylation and reactivation. There are no known allosteric activators of the phosphorylated form of the complex. The two regulatory enzymes, PDH kinase and PDH phosphatase, are mitochondrial, they utilise intramitochondrial substrates and effectors. Phosphorylation is on three seryl residues in the α-chain of the E1 component of the PDH complex. It results in inactivation of the two reactions catalysed by E1, i.e., decarboxylation of pyruvate and reductive acetylation of E2-lipoate by hydroxyethylTPP carbanion (see Fig. 1). This blocks the holocomplex reaction; the other elementary reactions of the holocomplex are unaffected [for bibliography, see Reed, 1981; Randle, 1986].

Inactivation of the purified complex and of PDH complex in mitochondria is due almost entirely (> 80% purified complex; > 90% in mitochondria) to phosphorylation of site 1 (phosphorylation of site 2 is also inactivating but phosphorylation of site 3 is not). This is because relative rates of phosphorylation are site 1 ≫ site 2 > site 3 and (in mitochondria) because relative rates of dephosphorylation are site 2 > site 1 = site 3 (PDH kinase and PDH phosphatase are simultaneously active in mitochondria and in vivo). Phosphorylation of sites 2 and 3 inhibits dephosphorylation of site 1 and reactivation by the phosphatase [for bibliography, see Reed, 1981; Randle, 1986; Sale and Randle, 1982a,b]. The amino acid sequences around the phosphorylation sites (identified originally in tryptic phosphopeptides from bovine and pig complexes) are highly conserved. Reversible phosphorylation of PDH complex is widespread in animal tissues containing the complex, including those of humans [Stansbie, 1976].

PDH kinase has been purified and contains two subunits α (M_r = 46kDa) and β (M_r = 43 kDa). The α-subunit is the catalytic subunit. The kinase is much more active toward its substrate (E1α) when E2 is present. PDH phosphatase I has two subunits α (M_r = 97 kDa) and β (M_r = 50 kDa; catalytic subunit). A second phosphatase has been described in bovine kidney mitochondria, but its function is uncertain.

Reversible phosphorylation is now accepted as the major mechanism regulating the PDH complex in animal tissues though end product inhibition is likely to be important in rapid adjustments of flux. For reasons discussed in more detail elsewhere reversible phosphorylation is also accepted as the major determinant of the rate of glucose oxidation in animal tissues.

Regulation of the PDH Complex by Reversible Phosphorylation in Tissues In Vivo and In Vitro

It is convenient to follow the usual *convention* and to refer to the active (dephosphorylated) and inactive (phosphorylated) forms of PDH complex as PDHa and PDHb. Except in adipose tissue of rats fed a high fat diet for a prolonged period, the total amount of PDH complex in rat tissues (PDHa + PDHb) is not altered by the physiological and pathophysiological variations to be detailed. Except where individual references are given, full bibliographies may be found in Randle et al. [1978, 1988] and Randle [1986].

Effects of diet, obesity, diabetes, and exercise. In the rat tissues examined (heart, skeletal muscles, liver, kidney, adipose tissue and intestine) percent of PDHa is decreased by starvation, high fat/low carbohydrate diet and insulin deficient diabetes (alloxan or streptozotocin). The maximum changes require 24–48 hr and reversal by carbohydrate refeeding or insulin treatment also requires 24–48 hr. Percentage PDHa is also decreased in heart muscle of gold-thioglucose obese hyperinsulinaemic rats in which glucose oxidation is impaired in muscles and adipocytes.

Effects of starvation, diabetes, high-fat diet or obesity to decrease per cent PDHa persist into isolated tissues incubated in vitro and effects of contraction in skeletal muscles and of increased work in heart is also demonstrable with in vitro preparations. The effect of exercise to increase per cent PDHa is decreased in hearts of starved or diabetic rats as compared with controls.

Short term (rapid) in vitro effects of hormones are as follows. Insulin increases percentage of PDHa in rat adipocytes by 1.8–2-fold in 5–8 min and a smaller effect (approximately 1.2-fold) is seen in rat hepatocytes. Consistent in vitro effects of insulin in heart or skeletal muscle have yet to be demonstrated. Rapid effects of other hormones comprise mainly increases in percent-

age of PDHa induced in liver by α-adrenergic agonists, glucagon and vasopressin and in heart muscle by β-adrenergic agonists. The role of hormones in longer-term regulation is discussed in a later section.

In vitro effects of short chain (C2–C8) and of long chain FFA (C16) and of ketone bodies to decrease per cent PDHa have been shown in perfused rat heart (approximately 70% decrease) and in perfused rat liver (FFA only). Effects of ketone bodies to decrease percentage PDHa have also been demonstrated in skeletal muscles (perfused rat hindquarter). The effects of long-chain FFA (but not of ketone bodies) are blocked by inhibitors of β-oxidation such as sodium 2-tetradecylglycidate.

Short-Term Regulation of PDH Kinase and PDH Phosphatases

PDH kinase. Some of the principle metabolite effectors of PDH kinase are paired such that the activity of PDH kinase is enhanced by increasing mitochondrial concentration ratios of acetyl CoA/CoA, $NADH_2$/NAD, and ATP/ADP, (i.e., the activating effects of acetyl CoA and NADH are reversed by CoA and NAD, respectively; ATP is a substrate inhibited competitively by ADP). Pyruvate is an inhibitor of PDH kinase; the degree of inhibition is enhanced by increasing ADP concentration. These regulatory interactions have been shown with purified PDH complexes and in studies with isolated rat heart and skeletal muscle mitochondria [for full bibliography, see Randle et al., 1978, 1988; Randle, 1986]. It has been claimed that PDH kinase may be activated directly by acetyl CoA and $NADH_2$ in assays with peptide substrates [Reed, 1981]. However, there is now overwhelming evidence that the effects of acetyl CoA and $NADH_2$ to activate PDH kinase with the PDH complex as substrate involve reductive acetylation or reduction of lipoate in the complex. This was suggested initially by the observations that pyruvate and acetoin can activate PDH kinase in the presence of TPP (thiamin pyrophosphate), whereas they are inhibitors of the kinase in the absence of TPP; and that activation of PDH kinase by acetyl CoA requires a nonstimulatory concentration of $NADH_2$ or of some other reducing agent such as dithiothreitol but is not dependent on TPP [Cooper et al., 1974, 1975; Kerbey et al., 1976, 1979; Roche and Cate, 1976; Cate and Roche, 1978, 1979]. For the elementary reactions involved in reduction and acetylation of lipoate, see Figure 1. Other evidence is given by Rahmatullah and Roche [1985] and Rahmatullah et al. [1985]. These short term regulators of PDH kinase modulate phosphorylation of all three sites in PDH complex [Kerbey et al., 1979].

PDH phosphatases. PDH phosphatase I requires Mg^{2+} for activity (K_m, 0.5 mM) [Hucho et al., 1972; Randle et al., 1974]; in the presence of Mg^{2+}, the enzyme is activated by Ca^{2+} in the physiological range (0.1–10 μM; K_m 0.5 μM) [Denton et al., 1972; Randle et al., 1974]. The effects of Ca^{2+}, at concentrations in the physiological range, to effect conversion of PDHa to PDHb have been readily demonstrable in isolated mitochondria [Midgley et al., 1987]. Ca^{2+} may act by effecting binding of the phosphatase to E2. PDH phosphatase I may also be inhibited by $NADH_2$ (reversed by NAD) [Reed, 1981]. PDH phosphatase II is divalent cation independent but is activated by spermine and is inhibited by adenine and guanine nucleotides (Ki, 0.1–0.2 mM) [Damuni and Reed, 1987].

Mechanism of exercise and short-term actions of hormones. The major factor responsible for the increase in percentage PDHa in muscle during contraction is activation of PDH phosphatase I by the associated increase in mitochondrial [Ca^{2+}]—a passive consequence of the increase in cytosolic [Ca^{2+}], which initiates contraction. The increases in percentage PDHa induced in liver by α-adrenergic agonists, glucagon and vasopressin, and in heart by β-adrenergic agonists are likewise mediated by Ca^{2+} [for review of evidence, see Denton and McCormack, 1990].

The action of insulin to effect conversion of PDHb into PDHa in rat adipocytes is effected through activation of PDH phosphatase by a mechanism that is not mediated by known effectors of PDH phosphatase I (Mg^{2+} or Ca^{2+}) or II (spermine). The effect of insulin is stable, and increased activity of PDH phosphatase is demonstrable in mitochondria isolated from adipose tissue exposed to the hormone. The insulin effect is rapidly lost when mitochondria are disrupted by freezing and thawing [Hughes and Denton, 1976; Thomas and Denton, 1986; Rutter et al., 1992].

Longer-Term Regulation of PDH Kinase

Discovery. Longer-term regulation of reversible phosphorylation of the PDH complex was first suspected from the observation that per-

centage PDHa in perfused hearts of 48-hr starved or alloxan-diabetic rats is lower than in hearts of normal rats perfused with FFA. This suspicion was confirmed when it was observed that the effect of starvation or diabetes to lower percentage PDHa in heart muscle persists into mitochondria prepared from the tissue and incubated in vitro with 2-oxoglutarate/malate or succinate [Kerbey et al., 1976]. It was shown later by direct analysis that this was not due to differences in mitochondrial [ATP]/[ADP] or [acetyl CoA]/[CoA] or [$NADH_2$]/[NAD] [Kerbey et al., 1977]. This led on to the demonstration that 48-hr starvation or alloxan-diabetes increase the activity of PDH kinase 2–3-fold in extracts of heart, mammary gland, skeletal muscle, or liver mitochondria [Hutson and Randle, 1978; Baxter and Coore, 1978; Fuller and Randle, 1984; Denyer et al., 1986]. This was a stable form of activation because it persisted through isolation and purification of mitochondria, incubation of mitochondria at 30°C with uncoupler to effect conversion of PDHb to PDHa, and extraction. The effects of starvation or diabetes to increase PDH kinase and decrease percentage PDHa were initially demonstrated over 24–48 hr, and reversal by refeeding of starved rats also took 24–48 hr to complete [Kerbey and Randle, 1982]. More recently, detailed time courses have shown that the major decreases in percentage PDHa in response to starvation occur at 12–18 hr in skeletal muscles and at 4–8 hr in heart and liver [Holness et al., 1989; Holness and Sugden, 1989]. After refeeding (48-hr starvation) restoration of percentage PDHa in heart began at 1–2 hr, but most of the restoration (70%) occurred after 4 hr. In liver, restoration was complete by 4 hr [Holness and Sugden, 1989]. Restoration of normal PDH kinase activity in liver was 50% complete after 4 hr and 90% complete after 8 hr [Jones et al., 1992].

Factors that may mediate longer-term effects of starvation and diabetes on PDH kinase. The question as to which hormones or metabolites may mediate longer-term effects of starvation on the activity of PDH kinase has been investigated with rat hepatocytes, cardiac myocytes, and soleus muscle strips in tissue culture [Fatania et al., 1986; Marchington et al., 1989, 1990; Stace et al., 1992]. These studies have shown uniformly that culture of cells from fed rats with agents that increase cAMP (glucagon or dibutyryl-cAMP or 8-bromo-cAMP), or with FFA (n-octanoate or albumin-bound palmitate) increases PDH kinase activity 2–3-fold. The effect of glucagon was detectable within 1 hr of culture but took 21 hr to reach 2-fold. Culture of hepatocytes from fed rats had no effect on PDH kinase in the absence of agonists. Culture of hepatocytes from starved rats reversed the effect of starvation on PDH kinase activity by about 60 percent in 21 hr; this reversal was blocked by octanoate or dibutyryl cAMP or a combination. Representative data for hepatocytes are shown in Figure 2.

PDH kinase activator protein (KAP). It was possible to separate from PDH complex in mitochondrial extracts a fraction that enhanced the PDH kinase activity of highly purified pig heart PDH complex. This separation was achieved by ultracentrifugation or, more effectively, by gel filtration on Sephacryl S300. The activity thus separated was thermolabile, nondialysable, and inactivated by trypsin and was termed kinase activator protein [Kerbey and Randle, 1981, 1982; Kerbey et al., 1984] because it increased the PDH kinase activity of purified pig heart PDH complex, which contains intrinsic PDH kinase. It could therefore be either free PDH kinase or a protein activator of the intrinsic PDH kinase.

There is now substantial evidence that rat liver KAP is a free PDH kinase. KAP phosphorylates and inactivates pig heart PDHE1 that is devoid of PDH kinase activity. It also phosphorylates and inactivates E1 in *S. cerevisiae* PDH

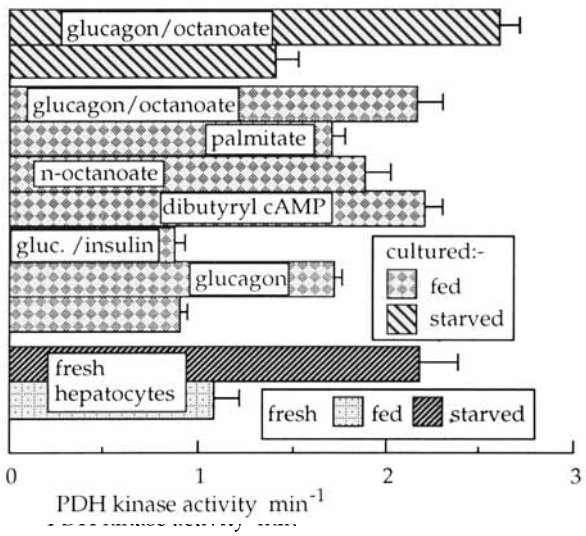

Fig. 2. Effects of 24-hr culture ± glucagon, insulin, dibutyryl cAMP, and fatty acids on PDH kinase activity measured in mitochondrial extracts of rat hepatocytes. Results are mean ± SEM. (Data from Marchington et al., 1989.)

complex, which, although devoid of PDH kinase, is nevertheless a substrate for mammalian PDH kinases. The fed/starved difference is retained in assays with these substrates. Like PDH kinase, KAP is inactivated by thiol-reactive reagents such as N-ethylmaleimide and p-chloromercuribenzoate (the latter is reversed by dithiothreitol). KAP was also shown to undergo pseudo first order inactivation by fluorosulphonylbenzoyladenosine which is known to block the ATP binding sites of protein kinases [Mistry et al., 1991]. More recent and as yet unpublished studies have shown that antibodies prepared against highly purified KAP cross-react with the α-chain of bovine kidney PDH kinase (shown by Western blots with E2-X–kinase subcomplex kindly provided by Dr. T.E. Roche). It has also been shown [Jones and Yeaman, 1991] that KAP phosphorylates synthetic peptide substrates for PDH kinase.

Longer-term regulation of enzymatic activity usually involves a change in enzyme concentration, but this does not appear to be the case with the increase in PDH kinase activity effected by starvation. Initial evidence was provided by the effect of varying concentrations of KAP, prepared from liver mitochondria of fed and starved rats, on PDH kinase activity of purified pig heart PDH complex. The effect of starvation was to increase the V_{max} of PDH kinase at saturating ATP from 7.3 (fed) to 17.3 min^{-1} (apparent first order rate constants) (the rate constant in the absence of KAP was 0.2). The KAP concentration required for 0.5 V_{max} was unchanged [Denyer et al., 1986]. This conclusion was confirmed by purifying KAP from fed and starved rats to apparent homogeneity as established by SDS–PAGE and N-terminal sequence analysis. The specific activity of purified KAP from starved rats was 4.5-fold greater than that from fed rats [Priestman et al., 1992]. SDS–PAGE gave a single band of M_r = 45 kDa, suggesting that KAP is the free α-subunit of PDH kinase (the Mr of KAP on SDS–PAGE was lower by about 1kDa than the 46kDa given by the α-subunit of PDH kinase in the bovine kidney PDHE1-X-kinase subcomplex provided by Dr. T.E. Roche).

Further support for this conclusion has been obtained in more recent unpublished studies employing purified polyclonal antibodies to KAP in an enzyme-linked immunosorbent assay (ELISA) to measure the concentration of KAP protein in mitochondrial extracts. This showed no significant difference in KAP protein concentration between extracts of liver mitochondria from fed and starved rats.

Two alternative hypotheses consistent with these findings are under consideration viz. that starvation increases the specific activity of KAP by covalent modification; or that there are two isozymes of PDH kinase which differ in specific activity and that starvation increases the relative concentration of the more active isozyme. It has not been possible to ascertain in vivo whether protein synthesis is required for the effect of starvation on PDH kinase activity because rats undergoing starvation are intolerant of protein synthesis inhibitors such as cycloheximide. Studies with hepatocytes in tissue culture show that cycloheximide completely blocked the effect of dibutyryl cAMP to increase PDH kinase activity, but that it did not block the effect of palmitate (Fig. 3). Etomoxir, an inhibitor of β-oxidation of FFA blocked the effect of palmitate (Fig. 3), indicating that a product of β-oxidation is likely to mediate the effect.

Mechanisms by Which Starvation and Diabetes Decrease Activity of the Pyruvate Dehydrogenase Complex and Glucose Oxidation

Figure 4 summarises the current state of knowledge of the mechanisms by which starvation and diabetes leads to phosphorylation and

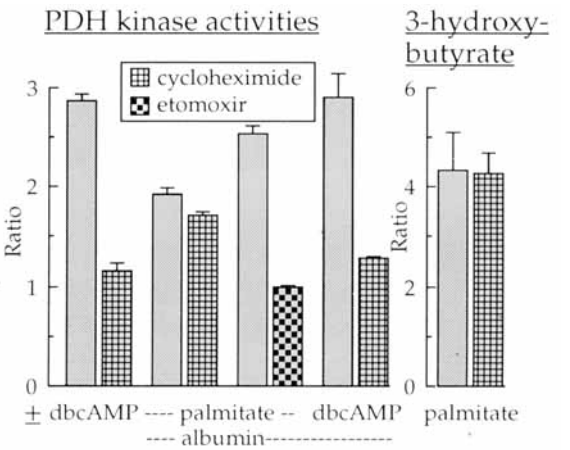

Fig. 3. Effects of cycloheximide and of the β-oxidation inhibitor Etomoxir on longer-term regulation of PDH kinase in hepatocytes. **Left:** Effect of 30 μM-cycloheximide or 1 mM-Etomoxir on the increase in PDH kinase activity induced in cultured rat hepatocytes by 50 μM-dibutyryl cAMP or 0.6 mM-palmitate/0.8% bovine serum albumin. **Right:** 3-Hydroxybutyrate formation as an index of the rate of β-oxidation of fatty acids. Results are means ± SEM of ratios ± dibutyryl cAMP or ± palmitate in the absence or presence of cycloheximide or of Etomoxir; i.e., a ratio > 1 indicates an effect of dibutyryl cAMP or of palmitate.

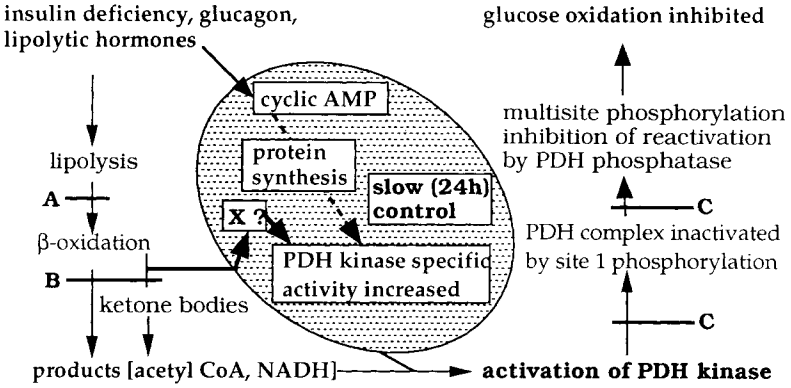

Fig. 4. Mechanisms leading to inactivation of PDH complex in starvation and in insulin-deficient diabetes in rat tissues. A, B, C inhibitors of lipolysis (e.g., Acipimox; 5-methypyrazole 3-carboxylate), β-oxidation of fatty acids (e.g., 2-tetradecylglycidate, Etomoxir), and of PDH kinase (e.g., dichloroacetate) respectively. X, unidentified metabolite of the β-oxidation pathway.

inactivation of PDH complex in rat heart, skeletal muscle, and liver (the only tissues studied in all aspects). In Figure 4, the short-term mechanisms occupy the periphery, and the longer-term mechanisms the central shaded area.

That shorter-term mechanisms based on oxidation of lipid fuels are of continuing importance in starvation and diabetes can be deduced from rapid (minutes) restoration of per cent PDHa to normal in rat heart muscle following in vivo and in vitro administration of the β-oxidation inhibitor sodium tetradecylglycidate (TDG) [Caterson et al., 1982]. TDG was not effective in skeletal muscle [Caterson et al., 1982; Holness et al., 1989] but the lipolysis inhibitor 5-methylpyrazole 3-carboxylic acid inhibited the decrease in percentage PDHa in skeletal muscles effected by starvation, whereas elevation of plasma FFA with corn oil/heparin accelerated it [Holness et al., 1989]. There is some evidence to suggest that the effects of FFA to decrease percentage PDHa, and glucose oxidation can be inhibited by hyperinsulinaemia [Jenkins et al., 1988; Bonadonna et al., 1989; Holness and Sugden, 1990]. The likely role of cAMP in mediating longer-term effects of starvation and diabetes on PDH kinase is assumed from the known increases in tissue cAMP in starved and/or diabetic rats [Exton et al., 1972, 1973; Selawry et al., 1973; Stace et al., 1992].

Holness and Sugden [1990] have used incorporation of [^{3}H]-2-deoxyglucose into [^{3}H]-2-deoxyglucose 6-phosphate as an index of glucose utilization by individual muscles in vivo and have thus compared effects of elevation of FFA with corn oil/heparin on glucose utilization indices and percentage PDHa. Their findings indicated that in skeletal muscles FFA decreased percentage PDHa before there was any significant change in glucose utilization index; i.e., the effects of FFA on glucose utilization either require longer exposure to FFA (7–9 versus 3–5 hr) or longer exposure in conjunction with a longer period of starvation (the animals received no food during these studies). In humans, Boden et al. [1991] find that the effect of raising plasma FFA to inhibit glucose oxidation is more rapid than the effects to inhibit glucose uptake (and presumably glycogen synthesis).

In humans, it is known that starvation, type I diabetes and type II diabetes, and elevation of plasma FFA in normal people decrease glucose oxidation and increase FFA oxidation and that elevation of plasma FFA increases muscle [acetyl CoA]/[CoA] [e.g., see Boden et al., 1991; Cahill and Owen, 1968; Caprio et al., 1990; Felber et al., 1988; Groop et al., 1989, 1990]. It is not known whether percentage active PDHa in tissues is changed in humans by starvation, diabetes or elevation of plasma FFA. In humans, inhibitors of lipolysis (Acipimox), FFA oxidation (Etomoxir, tetradecylglycidate), and PDH kinase (dichloroacetate) increase glucose oxidation [Fulcher et al., 1992; Hubinger et al., 1992; Rousselle et al., 1982; Tutweiler, 1989; Vaag et al., 1991].

FATTY ACIDS AND THE REGULATION OF GLUCOSE DISPOSAL

Glucose Oxidation, Glucose Storage, Cori Cycle

Virtually all the glucose utilised by animal tissues is either stored as glycogen or triacylglycerol or oxidised by a combination of glycolysis,

the PDH complex reaction, and the citrate cycle. Oxidation is limited by O_2 consumption and physical activity is the major determinant of O_2 consumption. Glucose oxidation is enhanced by intake of the sugar, but the maximum rate at rest is about 16 g/hr for a 70-kg man, whereas the maximum rate of nonoxidative disposal (= glycogen synthesis) is >118 g/hr [Thiébaud et al., 1982b]. Protein oxidation is relatively constant over a wide range of circulating insulin and glucose concentrations, whereas FFA oxidation is readily suppressed by a combination of hyperglycaemia and associated hyperinsulinaemia [Thiébaud et al., 1982a]. The relationships between glucose utilization, glucose production glucose oxidation, and PDH complex activity are displayed in Figure 5. In tissues, glycolytic flux generally exceeds pyruvate oxidation, so lactate is present in blood and pyruvate in cells; for this reason, it is assumed that PDH complex activity is rate-limiting for glucose oxidation. Conversion of PDHb to PDHa with dichloroacetate suffices to increase glucose oxidation in tissues of starved or diabetic animals [reviewed in Randle et al., 1988]. The continuing production of lactate by extrahepatic tissues with reconversion to glucose in liver (the Cori cycle) is about 10–15% of glucose disposal rate in humans. It has been shown that after oral glucose when there is hyperlactataemia, and stimulation of glycogen synthesis by insulin, limb muscles may actually remove circulating lactate (presumably by oxidation) [Jackson et al., 1987]. The source of this lactate is possibly gut and liver, thus suggesting the possibility of "reverse lactate cycling" postprandially.

FFA and Glucose Utilisation and Production

FFA and ketone bodies inhibit glucose utilisation in heart and skeletal muscles, especially when insulin is present and the mechanisms include inhibition of PFK1 by citrate leading to accumulation of glucose 6-phosphate and inhibition of HK; and inhibition of glucose transport at physiological insulin concentrations (shown only for heart) [see Randle et al., 1966; Rennie and Holloszy, 1977; Maizels et al., 1977]. FFA inhibit glucose utilization and glycolysis in liver and the mechanisms involve inhibition of PFK2 by citrate; inhibition of PFK1 by citrate and by decreased [fructose 2,6-bisphosphate]; and stimulation of glucose recycling (i.e., increased flux through glucose 6-phosphatase with unchanged flux through glucokinase [Hue et al., 1988; Berry et al., 1993]). In humans, glucose recycling is increased in acromegaly, but acute elevation of plasma FFA by growth hormone administration did not increase glucose recycling [Neely et al., 1992]. Stimulation of gluconeogenesis by FFA may involve activation of pyruvate carboxylase by acetyl CoA; inhibition of the PFK2 activity of the bifunctional enzyme with consequential decrease in hepatic fructose 2,6-bisphosphate, inhibition of PFK1 and activation of fructose 1,6-bisphosphatase; and activation of glucose 6-phosphatase.

Some studies in humans have suggested that increased FFA oxidation induced by increasing plasma FFA may inhibit glycogen synthesis [Felber et al., 1988; Bonadonna et al., 1989]. The mechanism proposed by Felber et al. [1988] is muscle oxidation of FFA, leading to accumula-

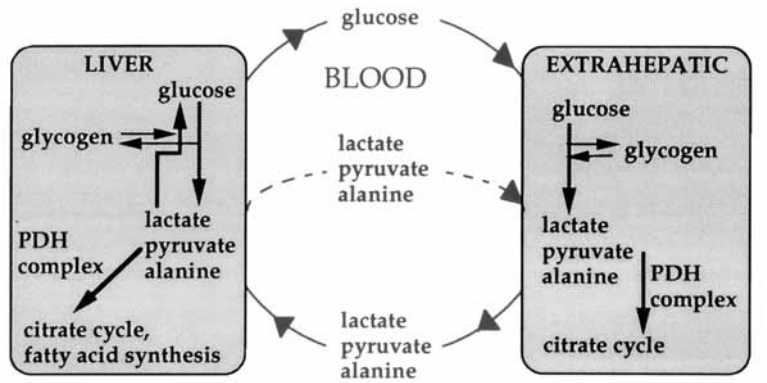

Fig. 5. Glucose recycling (the Cori cycle), "reverse lactate cycling," and relationships with glucose uptake (or formation), glycogen synthesis, and breakdown in liver and in muscles, as well as the role of the pyruvate dehydrogenase complex.

tion of glycogen, inhibition of GS phosphatase (protein phosphatase I), and phosphorylation of glycogen synthase (GS). Bonadonna et al. [1989] observed a decrease in percentage GSa. Other studies have shown no effect of acute elevation of plasma FFA on glycogen synthesis [Johnson et al., 1992].

CONCLUDING REMARKS

The glucose fatty acid cycle is 30 years old this year; the past decade in particular has witnessed a substantial resurgence of interest in FFA glucose interactions and the regulation of glucose disposal. Part of this renewed interest is due to technical developments that have made it possible to study FFA glucose interactions in vivo in humans under controlled conditions of circulating glucose and insulin and with much greater precision. The discovery of longer-term regulation of PDH kinase by FFA in liver and muscles is a novel departure which should pave the way for more general studies of longer-term regulation of glucose metabolism by FFA. Obvious targets for such study are regulatory enzymes in glucose production, as well as glycogen synthesis and glucose transporters.

REFERENCES

Barrera CR, Namihira G, Hamilton L, Munk P, Eley MH, Linn TC, Reed LJ (1972): Keto acid dehydrogenase complexes. XVI. Studies on the subunit structure of the pyruvate dehydrogenase complexes from bovine kidney and heart. Arch Biochem Biophys 148:343–358.

Baxter MA, Coore HG (1978): The mode of regulation of pyruvate dehydrogenase of lactating mammary gland. Biochem J 174:553–561.

Berry MN, Phillips JW, Henly DC, Clark DG (1993): Effects of fatty acid oxidation on glucose utilization by isolated hepatocytes. FEBS Lett 319:26–30.

Boden G, Jadell F, White J, Liang Y, Mozzoli M, Chen X, Coleman E, Smith C (1991): Effect of fat on insulin-stimulated carbohydrate metabolism in normal man. J Clin Invest 88:960–966.

Bonadonna RC, Zych K, Boni C, Ferrannini E, DeFronzo RA (1989): Time dependence of the interaction between lipid and glucose in humans. Am J Physiol 257:E49–E56.

Cahill GF Jr, Owen OE (1968): Some observations on carbohydrate metabolism in man. In Dickens F, Randle PJ, Whelan WJ (eds): "Carbohydrate Metabolism and Its Disorders." Vol. I. New York: Academic Press, pp. 497–522.

Caprio S, Amiel S, Tamborlane WV, Gelfand RA, Sherwin RS (1990): Defective free fatty acid and oxidative glucose metabolism in IDDM during hypoglycemia—Influence of glycemic control. Diabetes 39:134–141.

Cate RL, Roche TE (1978): A unifying mechanism for stimulation of mammalian pyruvate dehydrogenase kinase by reduced nicotinamide adenine dinucleotide, dihydrolipoamide, acetyl coenzyme A, or pyruvate. J Biol Chem 253:496–503.

Cate RL, Roche TE (1979): Function and regulation of mammalian pyruvate dehydrogenase complex. Acetylation, interlipoyl acetyl transfer, and migration of the pyruvate dehydrogenase component. J Biol Chem 254:1659–1665.

Caterson ID, Fuller SJ, Randle PJ (1982): Effect of the fatty acid oxidation inhibitor 2-tetradecylglycidic acid on pyruvate dehydrogenase complex activity in starved and diabetic rats. Biochem J 208:53–60.

Cooper RH, Randle PJ, Denton RM (1974): Regulation of heart muscle pyruvate dehydrogenase kinase. Biochem J 143:625–641.

Cooper RH, Randle PJ, Denton RM (1975): Stimulation of phosphorylation and inactivation of pyruvate dehydrogenase by physiological inhibitors of the pyruvate dehydrogenase reaction. Nature 257:808–809.

Cuendet GS, Loten EG, Renold AE (1975): Evidence *that* the glucose fatty acid cycle is operative in isolated skeletal (soleus) muscle. Diabetologia 12:336.

Damuni Z, Reed LJ (1987): Purification and characterization of a divalent cation-independent, spermine-stimulated protein phosphatase from bovine kidney mitochondria. J Biol Chem 262:5135–5138.

Denton RM, Randle PJ, Martin BR (1972): Stimulation by calcium ions of pyruvate dehydrogenase phosphate phosphatase. Biochem J 128:161–163.

Denyer GS, Kerbey AL, Randle PJ (1986): Kinase activator protein mediates longer-term effects of starvation on activity of pyruvate dehydrogenase kinase in rat liver mitochondria. Biochem J 239:347–354.

Exton JH, Friedmann N, Wong EH-A, Brineaux JP, Corbin JD, Park CR (1972): Interaction of glucocorticoids with glucagon and epinephrine in the control of gluconeogenesis and glycogenolysis in liver and of lipolysis in adipose tissue. J Biol Chem 247:3579–3588.

Exton JH, Harper SC, Tucker AL, Ho R-J (1973): Effects of insulin on gluconeogenesis and cyclic AMP levels in perfused livers from diabetic rats. Biochim Biophys Acta 329:23–40.

Fatania HR, Vary TC, Randle PJ (1986): Modulation of pyruvate dehydrogenase kinase activity in cultured hepatocytes by glucagon and n-octanoate. Biochem J 234:233–236.

Felber JP, Golay A, Felley C, Jéquier E (1988): Regulation of glucose storage in obesity and diabetes: Metabolic aspects. Diabetes Metab Rev 4:691–700.

Ferrannini E, Barrett EJ, Bevilacqua A, DeFronzo RA (1983): Effect of fatty acids on glucose production and utilization in man. J Clin Invest 72:1737–1747.

Fulcher GR, Walker M, Catalano C, Farrer M, Alberti KGMM (1992): Acute metabolic and hormonal responses to the inhibition of lipolysis in non-obese patients with non-insulin-dependent (type-2) diabetes mellitus—Effects of Acipimox. Clin Sci 82:565–571.

Fuller SJ, Randle PJ (1984): Reversible phosphorylation *of* pyruvate dehydrogenase in rat skeletal-muscle mitochondria. Biochem J 219:635–646.

Groop LC, Bonadonna RC, Delprato S, Ratheiser K, Zyck K, Ferrannini E, DeFronzo RA (1989): Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus—Evidence for multiple sites of insulin resistance. J Clin Invest 84:205–213.

Groop LC, Bonadonna RC, Delprato S, Ratheiser K, DeFronzo RA (1990): Effect of prolonged overnight fasting on energy metabolism in non-insulin-dependent diabetic and non-diabetic subjects. Acta Endocrinol (Copenh) 123: 30–36.

Hers H-G, Van Schaftingen E (1982): Fructose 2,6-bisphosphate 2 years after its discovery. Biochem J 206:1–12.

Holness MJ, Sugden MC (1989): Pyruvate dehydrogenase activities during the fed-to-starved transition and on refeeding after acute or prolonged starvation. Biochem J 258:529–533.

Holness MJ, Sugden MC (1990): Glucose utilization in heart, diaphragm and skeletal muscle during the fed-to-starved transition. Biochem J 270:245–249.

Holness MJ, Li Y-L, Sugden MC (1989): Time courses of the responses of pyruvate dehydrogenase activities to short-term starvation in diaphragm and selected skeletal muscles of the rat. Biochem J 265:771–776.

Hubinger A, Weikert G, Wolf HPO, Gries FA (1992): The effect of Etomoxir on insulin sensitivity in type-2 diabetic patients. Horm Metab Res 24:115–118.

Hucho F, Randall DD, Roche TE, Burgett MW, Pelley JW, Reed LJ (1972): Keto acid dehydrogenase complexes. XVII. Kinetic and regulatory properties of pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase from bovine kidney and heart. Arch Biochem Biophys 151:328–340.

Hue L, Maisin L, Rider MH (1988): Palmitate inhibits liver glycolysis: Involvement of fructose 2,6-bisphosphate in the glucose/fatty acid cycle. Biochem J 251:541–545.

Hughes WA, Denton RM (1976): Incorporation of ^{32}Pi into pyruvate dehydrogenase phosphate in mitochondria from control and insulin-treated adipose tissue. Nature 264: 471–473.

Hutson NJ, Randle PJ (1978): Enhanced activity of pyruvate dehydrogenase kinase in rat heart mitochondria in alloxan-diabetes or starvation. FEBS Lett 92:73–76.

Jackson RA, Hamling JB, Sim BM, Hawa MI, Blix PM, Nabarro JDN (1987): Peripheral lactate and oxygen metabolism in man: The influence of oral glucose loading. Metabolism 36:144–150.

Jenkins AB, Storlein LH, Chisholm DJ, Kraegen EW (1988): Effects of non-esterified fatty acid availability on tissue-specific glucose utilization in rats in vivo. J Clin Invest 82:293–299.

Johnson AB, Argyraki M, Thow JC, Cooper BG, Fulcher G, Taylor R (1992): Effect of increased free fatty acid supply on glucose metabolism and skeletal muscle glycogen synthase activity in normal man. Clin Sci 82:219–226.

Jones BS, Yeaman SJ (1991): Longer term regulation of pyruvate dehydrogenase complex. Evidence that kinase-activator protein (KAP) is free pyruvate dehydrogenase kinase. Biochem J 275:781–783.

Jones BS, Yeaman SJ, Sugden MC, Holness MJ (1992): Hepatic pyruvate dehydrogenase kinase activities during the starved-to-fed transition. Biochim Biophys Acta 1134: 164–168.

Kerbey AL, Randle PJ (1981): Thermolabile factor accelerates pyruvate dehydrogenase kinase reaction in heart mitochondria of starved or alloxan diabetic rats. FEBS Lett 127:188–192.

Kerbey AL, Randle PJ (1982): Pyruvate dehydrogenase kinase/activator in rat heart mitochondria. Biochem J 206: 103–111.

Kerbey AL, Randle PJ, Cooper RH, Whitehouse S, Pask HT, Denton RM (1976): Regulation of pyruvate dehydrogenase in rat heart. Mechanism of regulation of proportions of dephosphorylated and phosphorylated enzyme by oxidation of fatty acids and ketone bodies and of effects of diabetes; role of coenzyme A, acetyl coenzyme A and reduced and oxidized nicotinamide-adenine dinucleotide. Biochem J 154:327–348.

Kerbey AL, Radcliffe PM, Randle PJ (1977): Diabetes and the control of pyruvate dehydrogenase in rat heart mitochondria by concentration ratios of ATP/ADP, NADH/NAD^+ and acetyl CoA/CoA. Biochem J 164:509–519.

Kerbey AL, Radcliffe PM, Randle PJ, Sugden PH (1979): Regulation of kinase reactions in pig heart pyruvate dehydrogenase complex. Biochem J 181:427–433.

Kerbey AL, Richardson LJ, Randle PJ (1984): The role of intrinsic kinase and of kinase/activator protein in the enhanced phosphorylation of pyruvate dehydrogenase complex in starvation. FEBS Lett 176:115–119.

Linn TC, Pettit FH, Reed LJ (1969): Regulation of the activity of the pyruvate dehydrogenase complex by phosphorylation and dephosphorylation. Proc Natl Acad Sci USA 62:234–241.

Maizels EZ, Ruderman NB, Goodman MN, Lau D (1977): Effect of acetoacetate on glucose metabolism in the soleus and extensor digitorum longus muscles of the rat. Biochem J 162:234–241.

Marchington DR, Kerbey AL, Giardina MG, Jones AE, Randle PJ (1989): Longer term regulation of pyruvate dehydrogenase in cultured rat hepatocytes. Biochem J 257:487–491.

Marchington DR, Kerby AL, Randle PJ (1990): Longer term regulation of pyruvate dehydrogenase in cultured rat cardiac myocytes. Biochem J 267:245–247.

Midgley PJW, Rutter GA, Thomas AP, Denton RM (1987): Effects of Ca^{2+} and Mg^{2+} on the activity of pyruvate dehydrogenase phosphate phosphatase within toluene-permeabilized mitochondria. Biochem J 241:371–377.

Mistry SC, Priestman DA, Kerbey AL, Randle PJ (1991): Evidence that rat liver pyruvate dehydrogenase kinase activator protein is a pyruvate dehydrogenase kinase. Biochem J 275:775–779.

Neely RD, Rooney DP, Bell PM, Bell NP, Sheridan B, Atkinson AB, Trimble ER (1992): Influence of growth hormone on glucose-glucose 6-phosphate cycle and insulin action in normal humans. Am J Physiol 263:E980–987.

Nuutila P, Koivisto VA, Knuuti J, Ruotsalainen U, Teras M, Haaparanta M, Bergman J, Solin O, Voipiopulkki LM, Wegelius U, Ykijarvinen H (1992): Glucose-free fatty acid cycle operates in human heart and skeletal muscle in vivo. J Clin Invest 89:1767–1774.

Piatti PM, Monti LD, Pacchioni M, Pontiroli AE, Pozza G (1991): Forearm Insulin-mediated and non-insulin-mediated glucose uptake and muscle metabolism in man—Role of free fatty acids and blood glucose levels. Metab Clin Exp 40:926–933.

Pilkis SJ, Claus TH, Kountz PD, El-Maghrabi MR (1987): Enzymes of the fructose 6 phosphate-fructose 1,6 bisphosphate substrate cycle. In Boyer PD, Krebs EG (eds): "The Enzymes." Vol. XVIII. Orlando, Florida: Academic Press, pp 4–46.

Priestman DA, Mistry SC, Kerbey AL, Randle PJ (1992): Purification and partial characterization of rat liver pyruvate dehydrogenase kinase activator protein (free pyruvate dehydrogenase kinase). FEBS Lett 308:83–86.

Rahmatullah M, Roche TE (1985): Modification of bovine kidney pyruvate dehydrogenase kinase activity by CoA esters and their mechanism of action. J Biol Chem 260: 10146–10152.

Rahmatullah M, Roche TE, Jilka JM, Kazemi M (1985): Mechanism of activation of bovine kidney pyruvate dehydrogenase kinase by malonyl-CoA and enzyme-catalyzed decarboxylation of malonyl-CoA. Eur J Biochem 150:181–187.

Randle PJ (1986): Fuel selection in animals. Nineteenth Ciba Medal Lecture. Biochem Soc Trans 14:799–806.

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 mellitus. Lancet 1:785–789.

Randle PJ, Garland PB, Hales CN, Newsholme EA, Denton RM, Pogson CI (1966): Interactions of metabolism and the physiological role of insulin. Recent Prog Horm Res 22:1–44.

Randle PJ, England PJ, Denton RM (1970): Control of the tricarboxylate cycle and its interactions with glycolysis during acetate utilisation in rat heart. Biochem J 117:677–695.

Randle PJ, Denton RM, Pask HT, Severson DL (1974): Calcium ions and the regulation of pyruvate dehydrogenase. Biochem Soc Symp 39:75–88.

Randle PJ, Sugden PH, Kerbey AL, Radcliffe PM, Hutson NJ (1978): Regulation of pyruvate oxidation and conservation of glucose. Biochem Soc Symp 43:47–67.

Randle PJ, Kerbey AL, Espinal J (1988): Mechanisms decreasing glucose oxidation in diabetes and starvation: Role of lipid fuels and hormones. Diabetes Metab Rev 4:623–628.

Reed LJ (1981): Regulation of mammalian pyruvate dehydrogenase complex by a phosphorylation–dephosphorylation cycle. Curr Top Cell Regul 18:95–106.

Rennie MJ, Holloszy JO (1977): Inhibition of glucose uptake and glycogenolysis by availability of oleate in well oxygenated perfused skeletal muscle. Biochem J 168:161–170.

Roche TE, Cate RL (1976): Evidence for lipoic acid mediated NADH and acetyl-CoA stimulation of liver and kidney pyruvate dehydrogenase kinase. Biochem Biophys Res Commun 72:1375–1383.

Roche TE, Powers-Greenwood SL, Shi WF, Zhang WB, Ren SZ, Roche ED, Cox DJ, Sorenson CM (1993): Sizing of bovine heart and kidney pyruvate dehydrogenase complex and dihydrolipoyl transacetylase core by quasielastic light scattering. Biochemistry 32:5629–5637.

Rousselle J, Buckert A, Pahud P, Jequier E, Felber JP (1982): Relationship between glucose oxidation and glucose tolerance in man. Metabolism 31:866–870.

Rutter GA, Diggle TA, Denton RM (1992): Regulation of pyruvate dehydrogenase by insulin and polyamines within electropermeabilized fat-cells and isolated mitochondria. Biochem J 285:435–439.

Sale GJ, Randle PJ (1982a): Role of individual phosphorylation sites in inactivation of pyruvate dehydrogenase complex in rat heart mitochondria. Biochem J 203:99–108.

Sale GJ, Randle PJ (1982b): Occupancy of phosphorylation sites in pyruvate dehydrogenase phosphate complex in rat heart in vivo. Relation to proportion of inactive complex and rate of reactivation by phosphatase. Biochem J 206: 221–229.

Selawry H, Gutman R, Fink G, Recant L (1973): The effects of starvation on tissue adenosine 3′-5′ monophosphate levels. Biochem Biophys Res Commun 51:198–204.

Stace PB, Fatania HR, Jackson A, Kerbey AL, Randle PJ (1992): Cyclic AMP and free fatty acids in the longer-term regulation of pyruvate dehydrogenase kinase in rat soleus muscle. Biochim Biophys Acta 1135:201–206.

Stansbie D (1976): Regulation of the human pyruvate dehydrogenase complex. Clin Sci Mol Med 51:445–452.

Stepp LR, Pettit FH, Yeaman SJ, Reed LJ (1983): Purification and properties of pyruvate dehydrogenase kinase from bovine kidney. J Biol Chem 258:9454–9458.

Sugden PH, Randle PJ (1978): Regulation of pig heart pyruvate dehydrogenase by phosphorylation. Studies on the subunit and phosphorylation stoichiometries. Biochem J 173:659–668.

Teague WM, Pettit FH, Wu T-L, Silberman SR, Reed LJ (1982): Purification and properties of pyruvate dehydrogenase phosphatase from bovine heart and kidney. Biochemistry 21:5585–5592.

Thiébaud D, DeFronzo RA, Maeder E, Jequier E, Felber J-P (1982a): The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. Diabetes 31:957–963.

Thiébaud D, DeFronzo RA, Jacot E, Golay A, Acheson K, Maeder E, Jequier E, Felber J-P (1982b): Effect of long chain triglyceride infusion on glucose metabolism in man. Metabolism 31:1128–1136.

Thomas AP, Denton RM (1986): Use of toluene-permeabilized mitochondria to study the regulation of adipose tissue pyruvate dehydrogenase in situ. Biochem J 238:93–101.

Tutwiler GF (1989): Glucose fatty acid cycle—Possible therapeutic implications. In Larkins R, Zimmet P, Chisholm D (eds): "Diabetes, 1988." Amsterdam: Elsevier, pp 175–179.

Vaag A, Skott P, Damsbo P, Gall MA, Richter EA, Beck-Nielsen H (1991): Effect of the antilipolytic nicotinic acid analogue Acipimox on whole-body and skeletal muscle glucose metabolism in patients with non-insulin-dependent diabetes-mellitus. J Clin Invest 88:1282–1290.

Walker M, Fulcher GR, Catalano C, Petranyi G, Orskov H, Alberti KGMM (1990): Physiological levels of plasma non-esterified fatty acids impair forearm glucose uptake in normal man. Clin Sci 79:167–174.

Walker M, Fulcher GR, Sum CF, Orskov H, Alberti KGMM (1991): Effect of glycemia and non-esterified fatty acids on forearm glucose uptake in normal humans. Am J Physiol 261:E304–E311.

Wolfe BM, Kllein S, Peter EJ, Schmidt BF, Wolfe RR (1988): Effect of elevated free fatty acids on glucose oxidation in normal humans. Metabolism 37:323–329.

Yki-Jarvinen H, Puhakainen I, Koivisto VA (1991): Effect of free fatty acids on glucose uptake and nonoxidative glycolysis across human forearm tissues in the basal state and during insulin stimulation. J Clin Endocrinol Metab 72: 1268–1277.