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Glucose and glycogen utilisation in myocardial ischemia – Changes in metabolism and consequences for the myocyte

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Abstract

Experimentally, enhanced glycolytic flux has been shown to confer many benefits to the ischemic heart, including maintenance of membrane activity, inhibition of contracture, reduced arrhythmias, and improved functional recovery. While at moderate low coronary flows, the benefits of glycolysis appear extensive, the controversy arises at very low flow rates, in the absence of flow; or when glycolytic substrate may be present in excess, such that high glucose concentrations with or without insulin overload the cell with deleterious metabolites. Under conditions of total global ischemia, glycogen is the only substrate for glycolytic flux. Glycogenolysis may only be protective until the accumulation of metabolites (lactate, H^+, NADH, sugar phosphates and Pi) outweighs the benefit of the ATP produced.

The possible deleterious effects associated with increased glycolysis cannot be ignored, and may explain some of the controversial findings reported in the literature. However, an optimal balance between the rate of ATP production and rate of accumulation of metabolites (determined by the glycolytic flux rate and the rate of coronary washout), may ensure optimal recovery. In addition, the effects of glucose utilisation must be distinguished from those of glycogen, differences which may be explained by functional compartmentation within the cell. (Moll Cell Biochem **180:** 3–26, 1998)

Key words: glycolysis, glycogenolysis, high energy phosphate stores, glycolytic flux, cardiomyocytes, myocardial ischemia

Introduction

Increased provision of glucose is usually beneficial to the ischemic myocardium, as anaerobic glycolysis may be the sole source of energy. The 'glucose hypothesis' [1] suggests that the benefits of enhanced glucose provision include increased energy production, reduced loss of K^+ ions and attenuated arrhythmias, inhibition of changes in the trans-membrane action potential, altered extracellular volume, and decreased circulating free fatty acids whose intermediates may be toxic to the ischemic heart. These mechanisms result in reduced incidence of arrhythmias, reduced ischemic contracture, and improved recovery of function.

Despite many studies showing benefits of enhanced glucose utilisation [2–11], there are several important exceptions. These include (1) a trial published by the British Medical Research Council in the Lancet, which did not find a beneficial effect associated with glucose-insulin-potassium (GIK) treatment of patients with infarction [12], (2) a report showing that addition of glucose in cardioplegic solutions was detrimental to the myocardium [13], (3) a finding that reduction in glycogen levels may be beneficial to the globally ischemic rat heart [14] and (4) the benefits ascribed to preconditioning (one or more brief episodes of ischemia and reperfusion protect against a subsequent sustained ischemic episode [15]), which are associated with reduced glyco-genolysis [16, 17].

These studies have led to a controversy over the therapeutic use of glucose provision, with the resultant discontinuance of GIK therapy for patients with myocardial infarction. However, the adverse findings described above may largely be attributed to incorrect dosage of glucose and impaired

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removal of metabolites, as well as lack of differentiation between glycogen and glucose utilisation. We suggest that glucose provision is beneficial at an optimal concentration, but that increased glycolysis may indeed be deleterious by increased metabolite accumulation. Removal of the metabolites by increased residual flow should result in increased recovery with higher glucose concentrations. The basis for this relationship relies on an understanding of the utilisation of glucose in ischemia. The regulation of glucose (and glycogen) metabolism is discussed briefly (for fuller discussions see refs e.g. [18–20]). The perturbations in metabolism induced by ischemia are discussed, together with the implications for the ischemic myocardium. Finally, the implications of differences in glycogen vs. glucose metabolism for the ischemic myocardium are reviewed.

Glucose uptake

The primary determinant of glucose utilisation is the rate of uptake into the cell, which is mediated by facilitated diffusion through specific glucose transporters. The rate of uptake is determined by the concentration gradient, the number of pores available in the membrane, and the affinity of the carriers. Once taken up into a cell, glucose is rapidly phosphorylated, preventing efflux and providing a constant gradient for glucose uptake.

Two types of facilitated glucose uptake mechanisms are found in animals. The Na^+-dependent co-transport of glucose, driven by the Na^+ gradient, does not occur in heart cells [21]. Up to six isoforms of the facilitative transporters, designated GLUT, are found, the distributions of which are determined by the glucose requirements of the individual tissue types [21, 22]. GLUT 1 is particularly abundant in red blood cells, and is also found in myocytes, both within the sarcolemmal and intracellular vesicle membranes. The insulin-sensitive glucose transporter, designated GLUT 4 [23], is also distributed between vesicles in the cytosolic pool, and the plasma membrane. Insulin acutely stimulates glucose transport in muscle and fat by recruiting up to about 40% of cytosolic GLUT 4 to the plasma membrane, compared to a normal level of 1%. Glucose uptake can thus be increased by 10–40 fold. GLUT 1 translocation may also be triggered by insulin [24]. A recent finding using a transgenic mouse model is that ablation of the GLUT 4 gene results in cardiac hypertrophy [25], suggesting that this mechanism of metabolic regulation is an important determinant of cell growth.

GLUT 1 has a lower affinity (about 5–10 mM [22]), but a high capacity for glucose, while GLUT 4 has a low capacity but a higher affinity (4.3 mM [26]). However, the relative numbers of transporters in the cell indicate that GLUT 4 is largely responsible for insulin-stimulated glucose uptake, and is far more efficient [26].

Insulin is a major regulatory hormone of carbohydrate metabolism in the heart [27]. Insulin promotes glucose entry and stimulates glycolysis, enhances synthesis of glycogen, fatty acids and proteins, and inhibits glycogen and fat utilisation. Insulin receptor activation promotes an intrinsic tyrosine kinase activity which leads to insulin receptor substrate 1 (IRS-1) phosphorylation. IRS-1 binds with Src homology 2 proteins (SH2-phosphotyrosine binding sites), including phosphoinositide 3-kinase (PI 3-kinase), Ras GTPase-activating protein, phospholipase C and others [28]. PI 3-kinase phosphorylates phosphoinositides, and is involved in growth factor stimulation. In addition, PI 3-kinase may mediate GLUT 4 translocation to the membrane [28] and increase glycogen synthesis. The insulin receptor in turn can be phosphorylated by protein kinase Cα [29]. The subsequent effects are not clear, but this finding suggests that insulin activity is affected by mechanisms which regulate intracellular Ca^{2+} (e.g. inositol polyphosphates [30]), and may thus be altered in ischemia.

In transgenic mice, increased expression of GLUT 1 increases glucose uptake and glycogen storage, implying that glucose transport is the rate-limiting step of glucose utilisation. Most factors which increase glucose utilisation act via increased recruitment of transporters to the membrane, including cAMP following β adrenergic stimulation [31, 32], hypoxia [33],and also possibly ischemia [34, 35]. In addition, the function of GLUT 4 transporters within the sarcolemma may be upregulated, by correct orientation within the membrane [36–39]. For example, adenosine, an important regulator of glucose utilisation, may enhance only insulin-stimulated glucose uptake [35], without any measurable change in sarcolemmal GLUT 4 density. Changes in transporter orientation may also be important in modulating glucose uptake in ischemic tissue. Cyclic guanosine monophosphate (cGMP) may also stimulate glucose transport by a direct action on the transporters [40], although the mechanism is unclear, and has been disputed [41].

The rate of glucose uptake is also determined by the metabolic requirements of the cell, dietary state, oxygen availability, hormones other than insulin (glucagon, catecholamines, thyroid), and the relative availability of the different substrates [27, 42]. In a perfused rat heart, glucose uptake increases linearly in the range of 1.25–5 mM glucose, but is saturated at concentrations above 10–12 mM [43]. Glucose uptake is increased with glycogen depletion [44], increased work rate [45] or exercise [46] in direct response to increased energy requirements. In an isolated heart, the majority of glucose taken up is oxidised, although some may go to glycogen synthesis (about 5–10% [47, 48]) and to lactate formation, depending on the availability of oxygen. In the presence of other substrates and insulin, the distribution to glycogen may increase.

Fatty acids compete with glucose as the substrate of choice [45], and limit entry of glucose when present in high concen-

trations – the 'glucose-sparing' effect [49]. *In vivo*, when blood free fatty acids are elevated, glucose is directed to the liver for storage as glycogen.

Glucose utilisation within the cell

Glucose utilisation involves several integrated major pathways, including glycolysis, glycogen synthesis/breakdown, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation. Intersecting pathways include mechanisms to reduce the redox potential (the malate-aspartate and the α-glycerophosphate shuttles), anaplerotic pathways to replenish the TCA cycle, and the contribution of a number of amino acids, as well as fatty acid synthesis/breakdown. These cycles in turn are regulated by substrate balance, hormones (insulin, adrenaline, glucagon), ions (Mg^{2+}, Ca^{2+}, H^+), and the energy status of the cell (levels of high energy phosphate metabolites and the cytosolic phosphorylation potential).

Glycogen

Glycogen is the storage form of glucose, and is found mainly in large macroparticles, consisting of 10 000–30 000 glucose molecules bound with either α-1,4 (majority of bonds – straight bond) or α-1,6 linkages (branch points). The macromolecules have a molecular mass of 10^4 kDa, and constitute

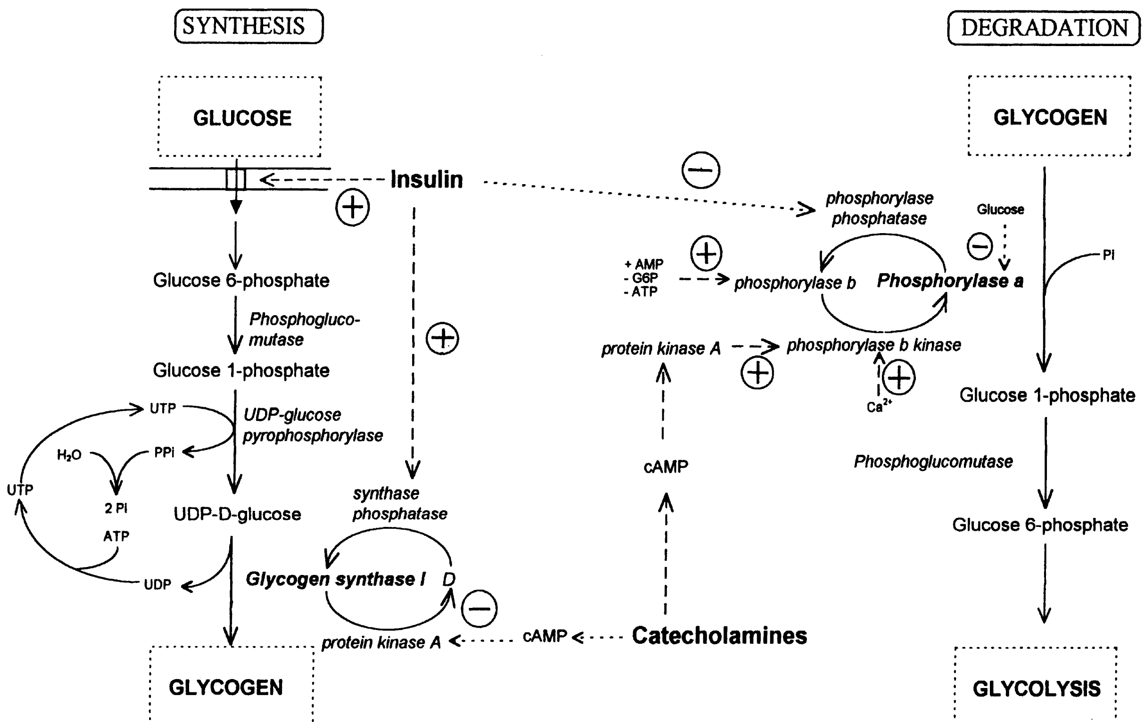

Fig. 1. Glycogen synthesis and degradation. *Glycogen synthesis* – Glycogen is synthesised from uridine diphosphate glucose (UDP-glucose), an activated form of glucose derived from glucose 1-phosphate (G1P) and uridine triphosphate (UTP). Pyrophosphate (PPi) is formed, and then hydrolysed to 2 Pi, an essentially irreversible reaction which drives glycogen synthesis. The UDP is then cleaved from the glucose by glycogen synthase and the glucose moiety is attached to the non-reducing end of a glycogen branch. Synthase phosphatase activates glycogen synthase from an inactive phosphorylated (b or D) to an active dephosphorylated form (a or I) (D – dependent on; I – independent of G6P). PKA phosphorylates and thus inhibits glycogen synthase. G6P is a potent stimulator of glycogen synthase activity (from [19, 235]). *Glycogen degradation* – Phosphorylase is the main enzyme of glycogen breakdown, cleaving glucose moieties at α-1,4 bonds from a non-reducing end of a glycogen branch, and phosphorylating these with Pi. However, phosphorylase stops cleaving when there are 4 terminal residues from the α-1,6 branch point. A transferase is required to transfer the remaining α-1,4 residues to the linear branch. The remaining α-1,6 bond is cleaved by amylo-1,6-glucosidase, which results in a glucose molecule, not G1P. Phosphorylase is upregulated by phosphorylation (from *b* – inactive to *a* – active) by a Ca^{2+}-dependent phosphorylase *b* kinase. Thus a close co-operation between contraction and glycogen breakdown exists, regulated by Ca^{2+}. This kinase is activated by cAMP-dependent protein kinase A following β-stimulation. PKA simultaneously inhibits glycogen synthesis. Phosphorylase phosphatase inhibits phosphorylase *a* by dephosphorylation [20, 51]. Inactive phosphorylase *b* can be directly stimulated by increases in AMP, and decreases in G6P and ATP. Phosphorylase *a* is inhibited by high concentrations of glucose.

1% of muscle mass. Recently, a new acid-precipitable 'form' of glycogen, or proglycogen, has been described [50]. This 400 kDa molecule has a high protein content (10 vs. 0.35% in macromolecular form) accounting for its response to acid. Proglycogen constitutes about 3–50% of total glycogen, depending on the tissue type (as much as 50% in heart muscle [19]). Proglycogen is an efficient receptor of glucose residues from UDP-glucose and may be an intermediate in the synthesis and degradation of glycogen [19]. Glycogenin, a self-glycosylating protein, thought to be the primer, or backbone of glycogen synthesis, has also been described. Glycogenin forms proglycogen and finally becomes part of the macroglycogen molecule (for in-depth discussion, see [19]).

Glycogen synthesis is stimulated by insulin, and increased glucose or glucose 6-phosphate (G6P) levels, while glycogenolysis is enhanced by cyclic adenosine monophosphate (cAMP), increased energy requirements and decreased glucose availability. There are two distinct pathways for glycogen synthesis and breakdown, each regulated by hormones which stimulate one pathway, while inhibiting the other (Fig. 1). Glycogen synthesis and utilisation was thought to follow the 'last on, first off' principle, whereby the last carbon molecule to be attached is the first to be cleaved off, but this has recently been disputed [47].

Glycogen synthase is the most important enzyme in glycogen synthesis (Fig. 1). Two forms of glycogen synthase, one acting on proglycogen and another on macromolecular glycogen, have been identified [19], possibly accounting for different rates of synthesis of the different forms of glycogen. Insulin stimulates glycogen synthesis by enhanced glycogen synthase dephosphorylation via a number of protein kinases (Fig. 1) [51]. Insulin also promotes dephosphorylation, and thus inactivation, of phosphorylase, the primary enzyme in glycogen breakdown (Fig. 1). Insulin also greatly increases glucose uptake, increasing substrate levels for glycogen synthesis. Fasting (short term) increases glycogen deposition by enhanced fatty acid oxidation, and inhibition of glycolysis in the absence of insulin [52]. Transient ischemia also activates glycogen synthase, possibly via G6P-mediated activation of phosphatase [53]. This effect appears contrary to the normal concept of ischemia-induced glycogen breakdown, but may be involved in preconditioning and in ischemia with a residual coronary flow (or hibernation, a chronic moderate reduction in coronary flow [54]).

About 9 protein kinases phosphorylate/dephosphorylate glycogen synthase [51], the most important being cAMP-dependent protein kinase A (PKA) [20]. PKA phosphorylation inhibits glycogen synthase and thus glycogen synthesis (Fig. 1) [55]. Protein kinase C and phosphorylase kinase also inhibit the enzyme. These factors in turn stimulate glycogen breakdown.

Glycogen breakdown follows a simple pathway (Fig. 1). A glucose moiety bound by an α-1,4 (straight) bond is cleaved from the large macromolecule and phosphorylated by phosphorylase *a* with Pi. This occurs without the expenditure of an ATP molecule, and prevents diffusion out of the cell. G1P is converted to G6P by phosphoglucomutase, an enzyme which favours the formation of G6P, unless G6P is in high concentrations. G6P then enters the glycolytic pathway. The complete oxidation of G1P (or G6P) yields about 37 ATP, while storage consumes slightly more than one ATP (although these values are now disputed [56]). The equilibrium favours glycogen breakdown, thus the energy yield is very efficient, at about 97%. Glycogenolysis is stimulated by hypoxia, ischemia, glucagon, and epinephrine.

The rates of breakdown of the different forms of glycogen appear to be very different [57]. Acid-extractable glycogen reflects changes in ischemia more closely, representing a subtraction of glycogen more responsive to degradation i.e. macromolecular glycogen. Glycogen in perchloric precipitate, i.e. proglycogen, remained unchanged during ischemia [57, 58].

The glycolytic pathway

The glycolytic pathway is shown in Fig. 2. For the purposes of the present discussion, glycolysis is defined as the breakdown of G6P to pyruvate. G6P is the entry point for both glucose and glycogen. Glucose is phosphorylated by hexokinase (HK) with the hydrolysis of ATP, an essentially irreversible reaction. An additional ATP is consumed, but 4 ATP are then produced if glycolysis goes through to pyruvate, together with 2 (NADH + H^+). The net ATP production is thus 2 ATP. If glycogen is broken down, the net production is 3 ATP, because Pi is utilised in the initial phosphorylation step, rather than ATP. The final product of glycosis is pyruvate, which can then follow a number of pathways, which determines the total amount of ATP derived from a glucose molecule. Anaeroic glycolysis implies that pyruvate is converted to lactate.

Levels of regulation of glycolysis

Glucose utilisation is regulated at several points along the glycolytic pathway as well as by factors governing glucose transport and glycogenolysis. However, the significance of regulation at each of these points is still controversial (see below). In addition, there is much controversy over whether the control of glycolysis exists at certain defined points [59–61] or regulation is distributed along the entire length of the pathway [62–64]. Recent evidence suggests that, under control conditions with sufficient substrate, the control of glycolysis rests largely at the level of glucose transport and hexokinase (58%), and that only 25% of control is mediated at steps below phosphoglucoismerase (see Fig. 2) [63] (remainder – glycogen synthesis). While these proportions may alter in the presence of insulin and other substrates, as well as ischemia, the fundamental concept is that regulation occurs

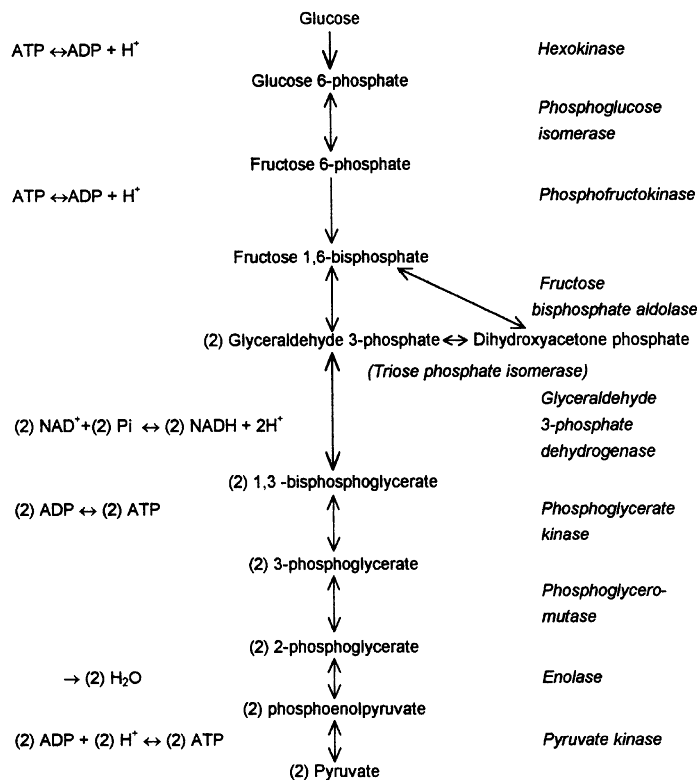

Fig. 2. The glycolytic pathway. Hexokinase phosphorylates glucose on entering the cell. G6P then undergoes a fully reversible conformational change to fructose 6-phosphate (F6P). Concentrations of G6P are generally 10 fold higher than of F6P. F6P is phosphorylated to fructose 1,6-bisphosphate (F1,6-BP) by phosphofructokinase-1 (PFK-1). Because this reaction uses the energy of ATP hydrolysis, the equilibrium greatly favours F1,6-BP. Glucose utilisation has thus consumed two ATP molecules by this stage, whereas glycogenolysis has consumed only one ATP per glucose residue, and a Pi. The above compounds are all hexose sugars. However, with 2 phosphate groups attached, the 6-C chain can be symmetrically broken down to two phosphorylated 3-C chains. Two distinct molecules are formed – glyceraldehyde 3-phosphate (GAP), and dihydroxyacetone phosphate (DHAP). DHAP is either converted to GAP by triose phosphate isomerase, or to α-glycerophosphate (αGP) (see Fig. 3). To continue glycolysis, GAP is simultaneously oxidised and phosphorylated with Pi to 1,3-bisphosphoglycerate by glyceraldehyde 3-phosphate dehydrogenase (GAPDH). One phosphate group on each 3-C molecule is subsequently cleaved off to form ATP, when 1,3-bisphosphoglycerate is converted to 3-phosphoglycerate (3PG) by phosphoglycerate kinase. 3PG undergoes a conformational change to 2-phosphoglycerate, which is dehydrated with the formation of an enol group, to phosphoenolpyruvate (PEP). The high phosphoryl-transfer potential of PEP allows the transfer of the remaining high energy phosphate group to $ADP+H^+$, with the end products of pyruvate and ATP. This reaction, catalysed by pyruvate kinase (PK), is virtually irreversible. The net ATP production from the breakdown of glucose to pyruvate is 2 ATP; from glycogen, 3 ATP are produced.

at multiple steps, and the importance of the classical points of regulation, specifically PFK and GAPDH, is reduced [63].

The concept of 'channelling' [65] implies cellular localisation of enzymes to allow efficient transfer of products of one enzyme reaction to the next [65]. The enzymes of a given pathway thus form a 'metabolon' or efficient set of linked pathways [62, 66], which suggests tight regulation of substrate utilisation. Glycogen metabolism is an example of a metabolon, with discrete particles containing both substrate and enzymes for efficient synthesis and breakdown. Glycolysis (breakdown of glucose) may also be viewed as a 'metabolon', with the enzymes functionally grouped together, in particular near the sarcolemma and sarcoplasmic reticulum (SR). These concepts imply that entry of a substrate into the pathway ensures complete breakdown under normal conditions, and thus that overall regulation at any single point (or enzyme) is unlikely. In addition, in 'normal' conditions when substrate is in excess, the point of regulation may differ greatly from that in ischemia, when reduced substrate supply may be the main limiting factor. Regulation of glycolysis in low flow ishcemia, whether by substrate supply or enzyme inhibition, is particularly controversial.

Within these limitations, the following steps are thought to be the main sites of glycolytic regulation.

Hexokinase (HK) is stimulated by increased cytosolic glucose levels, and inhibited by increased G6P. As extracellular glucose levels increase, the intracellular G6P concentration reaches a plateau, which does not change with glucose concentrations greater than 2 mM. The estimated intracellular glucose concentration at this point is about 300 μM. HK is then saturated with glucose, and any further increase in glycolysis is prevented. If HK is inhibited, the glucose can pass out of the cell again; if the extracellular concentration is high, glucose can accumulate intracellularly, and affect cell osmolarity. If G6P accumulates, glycogen can be synthesised; alternatively, increased glycogen breakdown will increase G6P levels. G6P levels and HK activity are thus major determinants of glucose utilisation in muscle in the presence of physiological concentrations of glucose and insulin i.e. adequate supply of substrate [63, 67].

Phosphofructokinase (PFK-1) is inhibited by a high ATP content, an effect enhanced by citrate (from the TCA cycle – although the mitochondial carrier for citrate may not be very active in myoctes [68]) and reversed by AMP. The reaction catalysed by this enzyme is virtually irreversible because of ATP hydrolysis, which suggests that this is an important step in the control of glycolysis [69]. The rate of glucose breakdown is thus signalled by the need for ATP as determined by the ATP/AMP ratio. The reaction catalysed by adenylate kinase (ATP + AMP ↔ ADP) amplifies the signal. If ATP falls by 15%, ADP levels increase 2 fold, with a greater than 5 fold increase in AMP. Thus a relatively small decrease in ATP levels markedly stimulates gycolysis.

PFK-1 is also strongly inhibited by an increased $[H^+]_i$ [69], an effect which may prevent excessive lactate and H^+ accumulation. However, fructose 2,6-bisphosphate (F2,6-BP) may be the most potent stimulator of PFK-1, with a feed forward effect [70, 71]. F2,6-BP is formed from F6P by hydrolysis of ATP, catalysed by an enzyme called PFK-2 (to distinguish from PFK1) [71]. This reaction is stimulated by F6P. Dephosphorylation of F2,6-BP to F6P allows continued glycolysis from F6P (Fig. 3).

In ischemia, regulation of glycolysis is thought to occur mainly at GAPDH [59] (although this is open to question – see later). However, if cytosolic glycolytic substrate is in excess, e.g. with the addition of insulin, G6P and F6P levels rise substantially [72], suggesting that under these conditions, PFK-1 may be inhibited (by a low pHi), and thus this step becomes more important in overall control of the rate of glycolytic flux.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is inhibited by an accumulation of NADH and lactate [73, 74], and was thought to be the major regulatory step of glycolysis in ischemia [59–61, 73, 74]. The product of the reaction, 1,3-bisphosphoglycerate, is also a potent inhibitor of the enzyme. Breakdown products of ATP (ADP, AMP and Pi) may stimulate the enzyme. GAPDH is especially implicated as the control step in ischemia [59]. However, in hypoxia when glycolysis is stimulated, NADH levels rise substantially, which contradicts the findings in ischemia. Inhibition of glycolysis at this step in ischemia by the end products is thus open to question (see below).

Pyruvate kinase (PK) may also be regulated, with inhibition by ATP, and stimulation by fructose 1,6-bisphosphate (F1,6-BP). However, the contribution of other glycolytic enzymes, and the major role of pyruvate dehydrogenase (see below), render this regulatory step less significant [63].

Alternate fates of metabolites of glycolysis

Glucose 6-phosphate is a main branch point of carbohydrate metabolism (see Fig. 3). G6P is the precursor of glycogen synthesis, as well as the entry point of glycogen breakdown into glycolysis. G6P can also be used to restore levels of NADPH and D-ribose 5-phosphate by the pentose shunt (see Fig. 3).

Glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP) are interconverted by triose phosphate isomerase. GAP can be converted directly to glycerol, and then to glycerol 3-phosphate (also called α-glycerophosphate – αGP) but the more usual reaction is the conversion of DHAP to αGP, which can then be incorporated into triglycerides (Fig. 3). αGP accumulates in ischemia as an end product of glycolysis, and is also involved in regulation of the redox potential (see below).

Pyruvate is the major end-product of glycolysis. From this point a number of options are available, dependent on the energy status of the cell (see Fig. 4). Under normal conditions, pyruvate is converted to acetyl Co A by pyruvate dehydrogenase (PDH), the irreversible step to glucose oxidation. This enzyme is regulated closely by ratios of substrate and product (see Fig. 4), and its activity can be modified by many other factors, including importantly fatty acid oxidation. This step determines whether pyruvate from glycolysis will be converted to acetyl CoA or to lactate, and thus whether the energy from glucose will be efficiently extracted. Acetyl CoA may then enter the TCA cycle by combining with oxaloacetate to form citrate, or can elongate fatty acids. Pyruvate can also be exported from the cell, together with a H^+ [75].

Under anaerobic conditions, or with extreme exercise, the TCA cycle is inhibited by accumulated NADH from inhibition of oxidative phosphorylation. Pyruvate is then converted to lactate by lactate dehydrogenase (LDH), to allow regeneration of NAD^+ and continuation of glycolysis (Fig. 4). Lactate is a 'dead end', its only possible fate is re-conversion to pyruvate, or export from the cell (also with a H^+ [75]). When sufficient

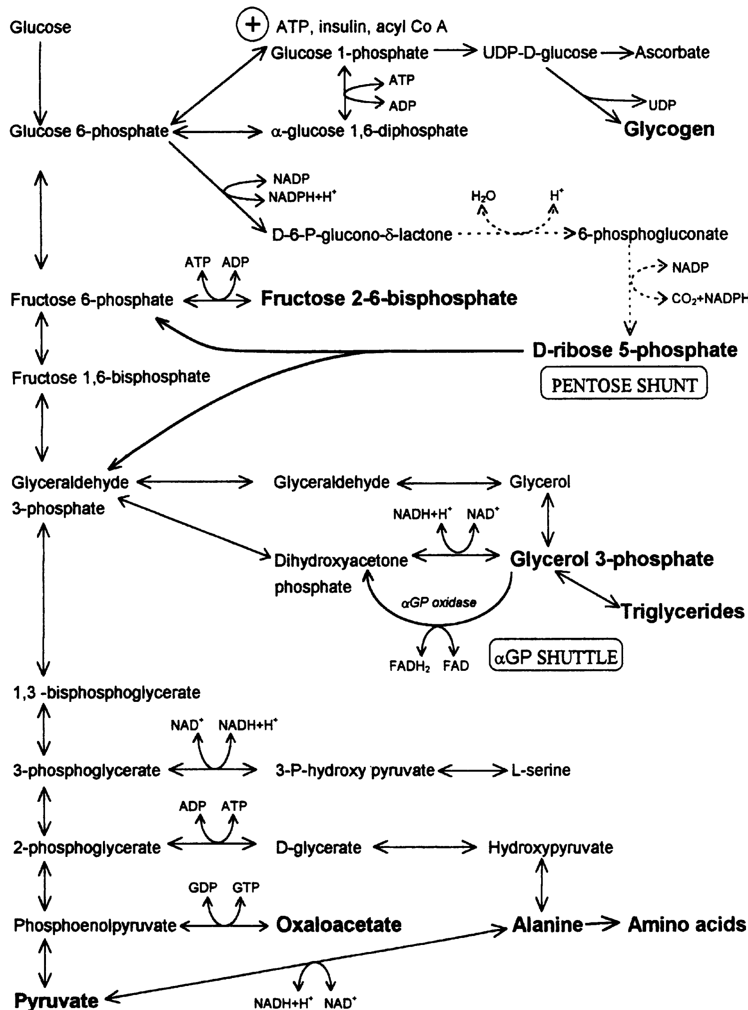

Fig. 3. Branch points from glycolysis. G6P is a junction for the formation and breakdown of glycogen, as well as the pentose shunt. In the pentose shunt, G6P is converted to D-6-P glucono-δ-lactone by glucose 6-phosphate dehydrogenase (G6PDH), which uses NADP. This compound is hydrolysed to 6-phosphogluconate which is converted to D-ribose 5-phosphate, with an additional NADP utilised. 2 molecules of NADPH are thus regenerated. D-ribose 5-phosphate can then be converted to F6P or GAP, re-entering the glycolytic pathway. The proportions which flow along the pathways are dependent on the requirement of the cell for D-ribose 5-phosphate, NADPH, or continued glycolysis. αGP shuttle – conversion of DHAP to αGP is catalysed by α-glycerophosphate dehydrogenase. αGP, readily permeable to the mitochondrial membrane, can subsequently be re-oxidised to DHAP by αGP oxidase, a transmembrane mitochondrial protein linked to FAD, with the consumption of a molecule of O_2 and production of H_2O. This system allows transport of NADH into the mitochondria against a high concentration gradient, although the cost is an ATP molecule, given that only 2 ATP are produced per $FADH_2$, rather than 3 per NADH. However, the level of αGP oxidase is low in muscle, and this mechanism may not be important. αGP is required in the formation of triglycerides, and levels increase significantly during ischemia. Other glycolytic metabolites contribute to various pathways as shown. These are not considered important in ischemia. For fates of pyruvate see Fig. 4.

oxygen is available, lactate can be oxidised to pyruvate and NADH will be regenerated. NADH can then be transported into the mitochondria, and utilised by oxidative phosphorylation (Fig. 5). If glycolysis continues in the absence of oxygen, H^+ accumulate from a reduced turnover of ATP [76] together with lactate. Most of the deleterious effects associated with enhanced glycolysis in ischemia are attributed to these products.

Pyruvate is also a major anaplerotic substrate (mechanisms which 'top up' the TCA cycle intermediates), supplying α-

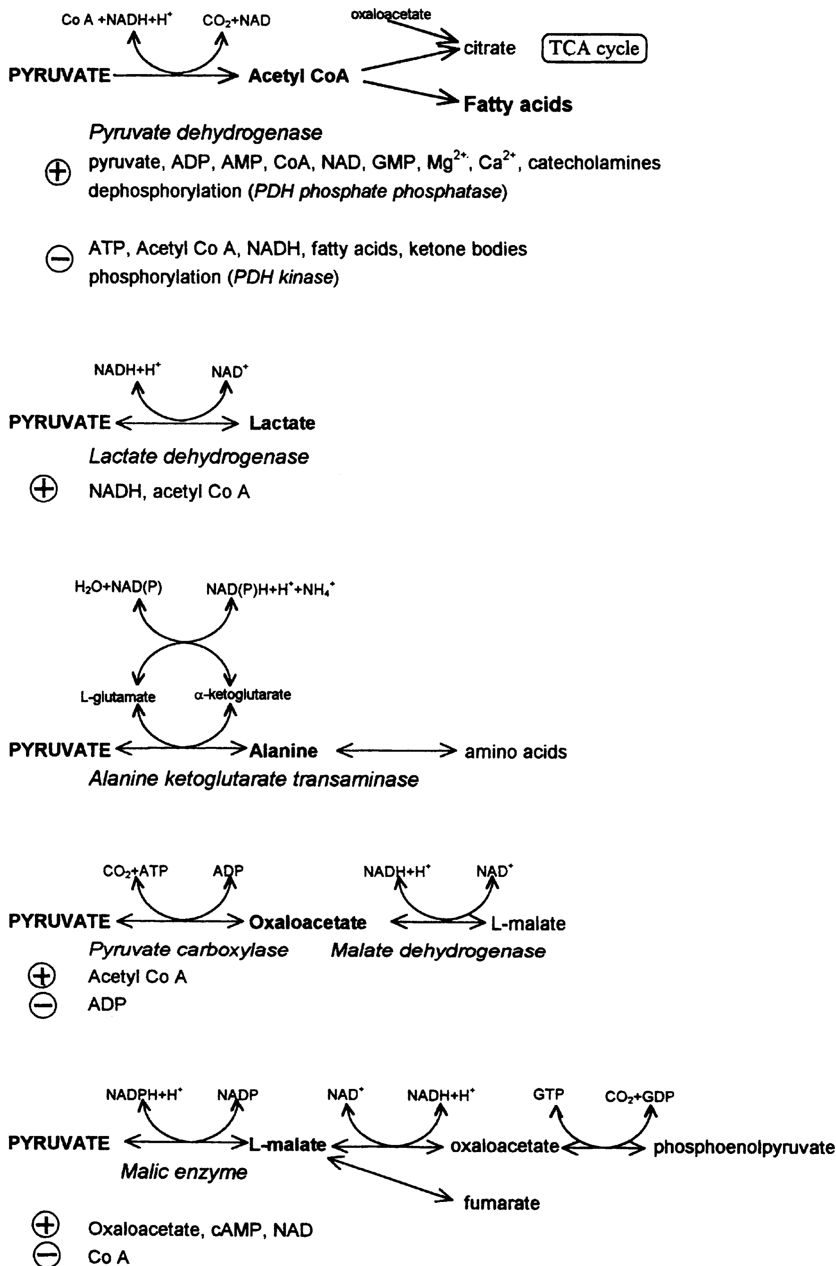

Fig. 4. Fates of pyruvate. Pyruvate is converted to acetyl Co A by pyruvate dehydrogenase (PDH) in the presence of sufficient oxygen, and reduced free fatty acid levels. PDH is a complex of three enzymes which require a number of cofactors. The enzyme complex is tightly regulated by the ratios of NADH/NAD$^+$, acetyl Co A/Co A, and ATP/AMP ratios (end product inhibition), as well as phosphorylation (inactivation – by PDH kinase) and dephosphorylation (activation – by PDH phosphate phosphatase). Under anaerobic conditions, pyruvate is converted to lactate to allow regeneration of NAD$^+$, and continuation of glycolysis. Pyruvate can also be converted to alanine, with the concomitant transamination of glutamate, and the formation of α-ketoglutarate. Pyruvate can also be converted to oxaloacetate by pyruvate carboxylase, and then to malate, or directly to malate by malate dehydrogenase. These are important anaplerotic mechanisms, and are involved in the malate-aspartate shuttle (Fig. 5).

ketoglutarate and oxaloacetate to the TCA cycle. Pyruvate is converted to oxaloacetate in the mitochondria by pyruvate carboxylase (Fig. 4). While activity of this enzyme may be low in heart muscle, some evidence for the activity of this pathway in heart tissue has been found [77].

Other anaplerotic pathways include conversion of phosphoenolpyruvate to oxaloacetate in the cytosol by pyruvate carboxylase, and the contribution of alanine (Fig. 4). Alanine is a major product of glycolysis, and accumulates during ischemia [78]. Alanine can also contribute to amino acid synthesis from glucose. The formation of alanine from pyruvate, with the concomitant transamination of glutamate to α-ketoglutarate, replenishes the latter, in an important anaplerotic mechanism.

Sequential pathways and alternate substrates

Tricarboxylic acid cycle and oxidative phosphorylation
The TCA cycle is the meeting point of substrate metabolism, whereby reducing equivalents for the subsequent generation of ATP by the respiratory chain are produced. Acetyl CoA is the entry point of most substrates into the TCA cycle, while NADH and $FADH_2$ are the end products, which enter oxidative phosphorylation for the formation of high energy phosphates. The TCA cycle is the most important generator of ATP of each substrate, such that after oxidative phosphorylation, an additional 36 ATP can be produced from glucose breakdown (as well as 2 ATP from glycolysis), and from a fatty acid such as palmitate (16 C), 129 ATP can be formed (although the exact number of ATPs produced from this process has been questioned, such that a maximum of 31 ATP from glucose and 104 ATP from palmitate can be produced [56]).

Regulation of NADH/NAD^+, glycolysis and the TCA cycle
Under conditions where acetyl Co A accumulates – when there is insufficient oxygen, or when NADH accumulates – NAD^+ must be regenerated, both to ensure continued glycolysis at the levels of PFK and GAPDH, and maintain turnover of the TCA cycle. The former is cytosolic, the latter mitochondrial.

Lactate dehydrogenase oxidises cytosolic NADH to NAD^+, converting pyruvate to lactate (Fig. 4). As mentioned above, this is a temporary mechanism to allow continued glycolysis.

αGP dehydrogenase also lowers cytosolic NADH levels with the reduction of DHAP to αGP (Fig. 3). However, the importance of this shuttle in the heart has been disputed [79], given the low activity of αGP dehydrogenase in heart muscle [80]. In addition, under normal conditions, the level of αGP is far below the Km for αGP oxidase. In ischemia, however, αGP levels increase significantly [59] and this shuttle mechanism may become important under these conditions [79, 81]. αGP forms the backbone of triacylglycerol synthesis, but the effects of an accumulation of this metabolite in ischemia have not been elucidated.

The malate–aspartate shuttle is perhaps the most important mechanism whereby the cytosolic and mitochondrial levels of NADH are regulated [79] (Fig. 5). There is an overall shift of malate into the mitochondria, and aspartate out of the organelle, although the shuttle is readily reversible. Its direction is determined by the cytosolic to mitochondrial NADH/NAD^+, such that a higher cytosolic ratio drives NADH transport into the mitochondria. This transfer does not utilise energy, but allows production of 3 ATP per NADH by oxidative phosphorylation (although this value is questioned [56]). If oxygen is limiting, NADH accumulates in the mitochondria, and then in the cytosol. This may affect redox-dependent reactions including glycolysis. The malate-aspartate shuttle can be reversed if NADH levels rise in the mitochondria, with the formation of malate. Malate is then transported out of the mitochondria, and converted to pyruvate by NADP-dependent malic enzyme, or to oxaloacetate by MDH (Figs 4 and 5).

Free fatty acids
It has long been known that free fatty acids are the major fuel of the normoxic heart, providing over 60% of the total ATP synthesised. As originally proposed, in terms of the 'glucose-fatty acid cycle' [82], an increased availability of lipid fuels such as in fasting, fat feeding or diabetes leads to decreased carbohydrate utilisation and impairment of insulin action. Possible mechanisms have been extensively documented involving inhibition of carbohydrate metabolism at at least 3 key control sites i.e. glucose entry, hexokinase/PFK and in particular PDH [61, 83, 84].

Although key regulatory sites have not been clearly pinpointed, there is considerable evidence that the interplay between fatty acids and carbohydrates is not entirely one-way i.e. under appropriate conditions, carbohydrate fuels can decrease fatty acid availability [82]. There is now considerable evidence that the carnitine palmitoyltransferase system required to transport activated fatty acids into the mitochondria is a major regulatory site of fuel utilisation in the heart [85–87], with inhibition by malonyl CoA.

Triglycerides are formed from acyl CoA and αGP, and can be synthesised during ischemia (Fig. 3). For each mol produced, 3 mol H^+ are formed, which increases the proton load in ischemia [88]. Endogenous lipids are not normally used as an energy source, given sufficient external substrate, but may be used e.g. in a perfused heart with limited external substrate [18].

Fatty acids have a higher requirement for oxygen than other substrates, but also yield a higher ATP per molecule.

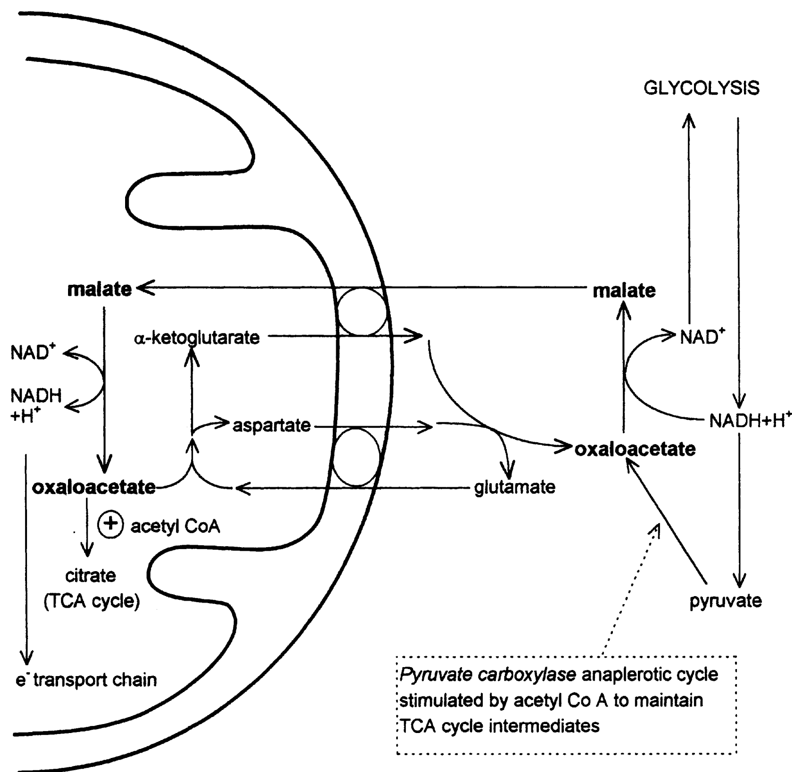

Fig. 5. The malate-aspartate shuttle. Cytosolic accumulation of NADH (e.g. with increased glycolysis) shifts the activity of malate dehydrogenase (MDH) in the direction of malate formation from oxaloacetate. Malate crosses the mitochondrial membrane in exchange for α-ketoglutarate, and is reconverted to oxaloacetate by mitochondrial MDH. NADH is thus regenerated in the mitochondria and can enter the respiratory chain. In addition, oxaloacetate replenishes the TCA cycle, combining with acetyl Co A to form citrate. Oxaloacetate can also combine with glutamate to form α-ketoglutarate and aspartate. α-ketoglutarate drives the entry of malate, as aspartate exits the cell in exchange for glutamate. In the cytosol, the α-ketoglutarate and aspartate recombine to form oxaloacetate and glutamate. There is thus an overall shift of malate into the mitochondria, and aspartate out of the organelle when cytosolic NADH increases. The whole shuttle can reverse, such that malate is transported out of the mitochondria, converted to oxaloacetate, and then to pyruvate. Pyruvate can also contribute to oxaloacetate formation via pyruvate carboxylase, or to malate by malic enzyme. These mechanisms, and the malate-aspartate shuttle, result in the anaplerotic contributions of pyruvate.

Fatty acids are therefore useful when oxygenation is high, but can be 'oxygen-wasting'. In conditions of ischemia, fatty acids and their metabolites exert a toxic effect, as well as consuming available oxygen rapidly. The breakdown products of fatty acids (acyl CoA, acyl carnitine, and lysophosphoglycerides) may be involved in many of the deleterious effects in ischemia, particularly arrhythmogenesis [18]. Fatty acids inhibit glucose oxidation; in turn, glucose provision attenuates many of the deleterious effects associated with fatty acids in ischemia. Thus the relative concentrations of fatty acids and glucose are of major importance in determining the tolerance of a heart to ischemia. However, this topic is largely beyond the scope of this review (see [27, 89–91]).

Myocardial ischemia

Glucose metabolism in ischemia

Hypoxia with maintained coronary flow stimulates glucose utilisation by reversal of the Pasteur effect, with a 20 fold increase in glycolytic flux in dogs *in vivo* [92], and a 3 fold increase in isolated rat hearts perfused with 11 mM glucose [93]. Glycolytic inhibition at the level of PFK-1 by citrate and ATP is removed [94], and glucose uptake is stimulated by increased GLUT 4 translocation [33]. If hypoxia is severe, heart function declines slowly, from insufficient ATP. If glycolysis is inhibited, developed tension dissipates more rapidly and completely [95]. Contracture may develop following ATP depletion [96, 97], an effect

counteracted by glucose provision.

In total ischemia (complete global cessation of flow and absence of oxygen), glycolysis is the sole source of ATP and may be stimulated initially following a reduction in oxygen. While ATP utilisation is reduced by mechanisms including membrane depolarisation and reduction in contractile function, the ATP demands of the myocardium rapidly exceed supply, such that net ATP decreases and Pi increases. Lactate accumulates with reduced washout, which may be deleterious. The metabolic changes occurring with ischemia thus include cessation of aerobic metabolism, onset of anaerobic glycolysis, depletion of creatine phosphate (CP), and accumulation of glycolytic products (lactate, αGP, adenine nucleotide breakdown) [98]. However, after a brief period, the glycolytic flux rate is reduced.

Differing concepts of rate-limiting steps in glycolysis in ischemia

Kübler and Spieckermann [92] found that when pO_2 becomes critical (less than 5 mmHg) in ischemic myocardium in dogs *in vivo*, glycolysis is initially stimulated by reversal of the Pasteur effect, as described above. A build-up of lactate then follows. The subsequent decline in glycolysis was attributed to the limiting effect of increased $[H^+]i$ and reduced ATP for the PFK-1 step, i.e. the conversion of F6P to F1,6-BP. The level of ATP in the tissue at this point (3.5 μmol/g wet wt) was said to be critical, with ischemic injury occurring at lower values.

This concept of glycolytic inhibition was revised by Neely and Rovetto [59, 93, 99, 100] who proposed that glycolytic flux in ischemia was inhibited at GAPDH by an accumulation of end products, specifically lactate, H^+, and NADH. This concept was based on work using an isolated working rat heart. A 60% reduction in coronary flow (from 15 ml/min to 6 ml/min) reduced oxygen consumption and accelerated glucose utilisation by 100%. Using a low flow rate of 0.6 ml/min, glucose utilisation was less than in control conditions (about 50%). After 16 min of low flow ischemia, the hearts were clamped and levels of metabolites assessed. Lactate values were high, suggesting that NADH was increased, because of the equilibrium of the LDH reaction. Increased NADH would, in turn, inhibit GAPDH, which is regulated by the $NADH/NAD^+$ ratio [74]. GAPDH inhibition was shown by an increase in DHAP levels [59] and application of the crossover theorem [101]. To date, it has been widely accepted that glycolytic enzymes are inhibited in ischemia by a build-up of glycolytic metabolites, and glycolytic flux is thus inhibited. However, further analysis of glucose uptake in severe low flow ischemia indicates that this interpretation, is not strictly correct [7, 102]; rather, glycolysis may be limited primarily by substrate supply [103] which in turn is determined by the arterial glucose concentration and the uptake ability of the membrane (i.e. insulin status, GLUT 4 availability etc.). Some modulation by enzyme inhibition is not excluded.

Extraction of glucose (calculated as absolute glucose uptake expressed as a percentage of glucose delivered (= glucose concentration * coronary flow)) from that which is available to the cell increases to about 30% as coronary flow rates fall below about 1 ml/g wet wt/min (in an isolated perfused rat heart) [102], compared to an extraction of about 1% in normal conditions. Absolute uptake does fall significantly in these conditions. *In vivo* experiments show similar findings, with a maintained glucose uptake as flow falls (normal *in vivo* coronary flows 1–2 ml/g wet wt/min; ischemia 0.07–0.15 ml/g wet wt/min) and thus an increased extraction [104]. We did not find evidence of GAP or DHAP accumulation after 15 or 30 min zero flow or low flow (0.2 ml/g wet wt/min) ischemia [72]. These findings indicate firstly, that glycolysis is not necessarily inhibited in ischemia at the level of GAPDH (although there may be some inhibition at PFK-1 as the sugar phosphates, G6P and F6P, accumulate especially with increased cytosolic substrate in the presence of insulin [72, 105] – some inhibition of GAPDH is not excluded, but its role as the rate-limiting step is questioned) but is limited by availability of substrate; secondly, that the ischemic tissue has the capacity to upregulate its ability to take up glucose, either by translocation of glucose transporters to the membrane [34], or by reorientation of the transporters within the sarcolemma [35]; and thirdly, this concept explains observations of 'mismatch' with Positron Emission Tomography (PET), using ^{18}fluorodeoxyglucose i.e. increased glucose uptake in 'hibernating' segments of the heart, which have a moderately impaired coronary flow [106–109]. Support for the concept that ischemia induces upregulation of glucose uptake comes from studies of preconditioning (short periods of ischemia and reperfusion) followed by sustained low flow ischemia, during which glucose uptake is markedly increased [110, 111].

The fate of glucose within the cell may vary with changes in coronary flow, which in turn alters oxygen availability [112]. However, in severe low flow, the availability of oxygen is greatly limited, such that the majority of ATP is derived from glycolysis [7, 11].

Role of glucose utilisation in ischemic myocardium

Specific role of glycolytic ATP

Despite a limitation on the rate of glycolytic flux in ischemia (a reduction in absolute glucose uptake), cell viability may largely be determined by the residual rate of glycolytic flux and the amount of ATP produced [113]. In conditions of metabolic stress – e.g. hypoxia, ischemia – glycolytic ATP appears to have a preferentially effective role as opposed to ATP derived from oxidative phosphorylation [114]. Much

indirect evidence suggests that ATP production within the cell is spatially compartmented [114–116], accounting for differences in the effectiveness of alternate sources of ATP. In ischemia, the processes for transport of ATP within the cell may break down. Thus provision of ATP near to the sites of utilisation becomes a crucial determinant of its effectiveness. A basal level of glycolytic activity is required to prevent irreversible injury [117], with control of cytosolic Ca^{2+} during ischemia [118] and on reperfusion [119], prevention of ischemic contracture [11, 120], inhibition of enzyme release [121], and inhibition of free radical activity [122]. Glycolytic ATP is thought to act in several ways at the membranes: blocking the ATP-dependent K^+ (K_{ATP}) channel [123], maintaining the activity of the Na^+/K^+ ATPase pumps in the sarcolemma [124], sustaining membrane integrity [114, 125], and maintaining Ca^{2+} homeostasis by the SR Ca^{2+} ATPase pumps [116]. These cellular effects modify functional responses to ischemia, with reduced diastolic tension during ischemia [2, 11, 120, 126], and improved functional recovery on reperfusion [2, 127–129].

The beneficial role of glycolytically-derived ATP therefore appears clear-cut. However, during the breakdown of glucose and glycogen, a number of metabolites accumulate which may contribute to some of the deleterious effects of ischemia. The benefit of glycogen availability in particular has been questioned in this regard, especially given the interest generated in the recently described phenomenon of preconditioning. A reduction in glycogen levels is postulated as one of the mechanisms whereby preconditioning may exert its beneficial effects [16].

Thus a crucial balance exists between rates of ATP production and rates of metabolite washout. This balance is determined by the rate of residual coronary flow and the glucose concentration, as well as the level of the endogenous glycolytic substrate, glycogen [2, 7, 11, 72, 127].

Membrane integrity

Glucose utilisation is closely involved in maintaining the integrity of the cell membrane. In isolated rat hearts made hypoxic [121, 130] or perfused with high concentrations of free fatty acids [131], enzyme release, a marker of cell damage, is attenuated by glucose provision. In normoxic isolated rat hearts, iodoacetate (glycolytic inhibitor) increases enzyme release, while cyanide (mitochondrial inhibitor) has no effect [132]. In low flow ischemia, provision of glucose rather than pyruvate, reduces LDH release, despite similar ATP levels [114].

Glucose utilisation may maintain membrane integrity in several ways. Provision of glucose limits phospholipase C degradation of membranes of isolated myocytes [125], and increased phosphorylation with increased ATP availability may also enhance the stability of the phospholipid membrane [132]. Energy is also required for membrane repair. Glucose-derived ATP may maintain the phosphatidic acid cycle, thereby preventing lysophospholipid accumulation which leads to membrane breakdown [133]. The osmotic effects of glucose provision and reduced cell swelling [98], together with reduction of contracture by glycolytic ATP, will limit cell rupture from stretching.

Membrane pump and channel activity

Glycolytic ATP appears to play a major role in ion homeostasis. A large efflux of K^+ occurs in ischemia, which depolarises the membrane and renders the cell inexcitable. This local 'cardioplegic' effect is beneficial in that the ATP demand is reduced. However, the excess extracellular K^+ can precipitate arrhythmias [134, 135]. The ATP-dependent K^+ (K_{ATP}) channel, one of the major channels involved in the efflux of K^+ in ischemia [136, 137], is blocked by ATP. However, the experimental levels to which ATP must fall to allow channel opening in excised patches [136] are well below those in ischemic tissue with a noted K^+ efflux [136]. K^+ efflux through this channel despite relatively small changes in ATP levels can be explained, firstly, by the high density of channels present such that a small change in maximal conductance can result in a large change in action potential shortening and K^+ loss [138]; secondly, by a localised fall in ATP near the membrane allowing for individual channel opening; and thirdly, by a rise in adenosine, lactate, and ADP, which relieve the inhibition by ATP [136, 139, 140]. Glucose-derived ATP, as opposed to that from oxidative phosphorylation, preferentially maintains closure of the K_{ATP} channel, suggesting that this ATP is localised near the membrane [123, 141]. Sarcolemmal-associated glycolytic enzymes may specifically maintain the ratio of ATP/ADP in the vicinity of the K_{ATP} channel [138].

Maintenance of Na^+/K^+ ATPase function may be one of the main mechanisms of protection by glycolytic ATP, as Na^+/K^+ ATPase pump inhibition by ouabain abolishes protection of ischemic hearts by glucose provision [124, 142]. Impaired Na^+/K^+ ATPase function increases osmolarity and cell swelling, as well as precipitating intracellular Ca^{2+} overload from an increased $[Na^+]i$ [142]. Glycolytic enzymes are also functionally coupled to SR Ca^{2+} transport mechanisms [116], as are glycogenolytic enzymes [143]. A preferential role for glycolytic ATP in preserving Ca^{2+} homeostasis by maintained Ca^{2+} re-uptake is thus postulated. In addition, glycolysis is central in maintaining Ca^{2+} homeostasis on reperfusion, possibly by activation of the SR Ca^{2+} ATPase pump [119], the activity of which is affected in stunned hearts [144, 145].

Arrhythmias and free fatty acids

Ventricular arrhythmias generated during ischemia or on reperfusion are complex in origin. Changes in cell membrane integrity, ionic fluxes across the membrane, increases in cytosolic Ca^{2+}, and impairment of conduction, all potentiate

arrhythmogenesis [146]. Glucose provision can combat many of these effects. The vulnerability of the dog heart to arrhythmias is reduced when glucose is administered intravenously [147]. Glucose provision also protects against arrhythmias in a model of regional ischemia in the isolated perfused rat heart [3] while reperfusion arrhythmias are attenuated compared to hearts perfused with acetate or palmitate [114].

Provision of glucose may reduce the arrhythmogenicity of ischemic tissue in several ways, including maintenance of membrane integrity, and pump and channel activity, as discussed above. Glucose provision maintains action potential duration in the isolated perfused rat heart [148], presumably by blocking the K_{ATP} channel, thereby limiting K^+ loss [123]. Glycolytic ATP also reduces cAMP accumulation, inhibits the release of noradrenaline from nerve terminals [149] and lowers LDH release, effects associated with reduced arrhythmias on reperfusion [114]. In addition, maintained glycolysis reduces cytosolic Ca^{2+} accumulation on reperfusion [119], a precipitating factor in reperfusion arrhythmias [150, 151]. Preserved Na^+/K^+ ATPase function [124, 142] would attenuate the increase in $[Na^+]i$, which in turn would reduce $[Ca^{2+}]i$, the major causative agent in arrhythmias [152]. Glucose may also have a free radical scavenging effect [153], important especially on reperfusion in maintaining membrane integrity and reducing arrhythmias [154].

A recent review highlighted the benefit of glucose and insulin in lowering the levels of circulating free fatty acids [155]. Excess free fatty acids are toxic to ischemic hearts, and lead to increased arrhythmias [155], possibly by accumulation of intracellular acylcarnitine and acyl CoA, which may promote intracellular Ca^{2+} overload [155–157]. Lysophospholipids from the breakdown of lipids are arrhythmogenic, with a detergent effect on the membranes [158]. Lipid compounds may also inhibit Ca^{2+} re-uptake mechanisms and activate Ca^{2+} channels [155–157]. Glucose provision counters many of the deleterious effects including arrhythmias associated with free fatty acids in ischemia, by reducing excess circulating free fatty acids, as well as combating the deleterious effects of free fatty acids on Ca^{2+} overload in the cell [155].

Ischemic contracture

Provision of glucose during low flow ischemia reduces ischemic contracture [11]. While there is some controversy over whether or not ischemic contracture is an index of irreversible injury [159, 160], increased contracture is generally considered to reflect increased cell damage [41, 161], and may potentiate injury, further impairing functional recovery.

The ATP level was originally thought to be a major determinant of the time to onset of contracture [161]. In general, a depletion in ATP levels reduces the time to onset; increased ATP availability delays contracture. The onset of contracture has also been linked to cessation of glycolysis, as measured by a levelling-off in the drop in intracellular pH [162]. These studies were performed in hearts exposed to total global ischemia, where the glycolytic flux rate is determined solely by the level of endogenous glycogen. With low flow ischemia (0.5 ml/g wet wt/min), a minimum rate of glycolytic ATP production of at least 2 μmol/min/g wet wt is required to prevent contracture [11]. A graded response to increased glucose concentrations was seen [11]. Therefore the onset of contracture is not associated so much with cessation of glycolysis, as with a fall in glycolytic flux rate below a threshold. Similar or larger rates of ATP production from utilisation of glycogen or non-glycolytic substrates were not effective in delaying contracture [11, 120, 126]. Other mechanisms which increase glycolysis, including glycogen loading [161, 163], inosine (increases pyruvate-to-alanine conversion, and removes glycolytic inhibition by lactate) [164], adenosine [165] and reduced circulating free fatty acids [129] delay the onset of contracture.

The exact mechanism of glycolytic ATP protection against ischemic contracture is thus as yet unclear. Contracture is triggered by the formation of rigor complexes (actin-myosin), which in experimental conditions, bond only at very low ATP concentrations (<100 μM [166]). However, total tissue ATP levels at the onset of contracture are much higher (about 3 μmol/g wet wt). Individual cells can have very low total ATP levels without contracting as long as glycolysis (from glycogen utilisation) is maintained [167]. A drop in ATP to less than 150 μM in isolated myocytes will, however, precipitate contracture [168]. These findings can be resolved by the concept of localised depletion of ATP within the cell (cf. K_{ATP} channel opening), as well as heterogeneous falls in ATP in different myocytes, resulting in dispersed foci of contracture in the muscle [169].

Glycolytic ATP may be essential for direct relaxation of the actin-myosin complexes, reversing the formation of rigor complexes, the so-called 'plasticising' effect [170]. However, some difficulties are associated with this concept, namely that glycolytic i.e. glucose-derived ATP, appears to be functionally associated with the sarcolemma and SR rather than the myofibrils [123]. Only under conditions of high glycolytic flux rates, may sufficient glycolytic ATP diffuse to the myofibrils to increase the rate of relaxation and prevent rigor formation. However, this could theoretically occur only under conditions of relatively unimpaired function i.e. a fairly high residual flow rate, and a high glucose concentration, ensuring a large intracellular ATP production [2, 127]. Glycolytic ATP may therefore be more important in maintaining ion homeostasis (as described above) and attenuating intracellular Ca^{2+} accumulation, rather than ensuring direct relaxation of the cross-bridges by binding to myosin. In addition, glucose utilisation attenuates the

deleterious effects of long-chain acyl carnitine (LCAC) on contracture [171], possibly by counteracting LCAC-induced disruption of Ca^{2+} homeostasis [155, 171]. While Eberli *et al.* [172] propose that Ca^{2+} is not involved in ischemic diastolic dysfunction, in a low flow model with maintained developed pressure, these results do not translate directly to the ischemic heart with severely impaired or no flow, and no developed pressure.

There is also evidence to suggest that pre-ischemic glycogen levels (and thus the rate of glycogenolysis during ischemia) primarily determine the time to onset of contracture (glycogen depletion with acetate [7] or preconditioning [105, 173, 174] hasten contracture; glycogen loading [7] delays contracture), while glucose-derived ATP attenuates overall contracture. The effects of the different glycolytic substrates on contracture may be attributed to compartmentation within the cell.

Mechanical function on reperfusion

Recovery of mechanical function is determined by the degree of ischemic injury, and the conditions on reperfusion [175]. At each point, substrate provision can be altered, affecting eventual recovery. While sufficient levels of ATP are essential for complete restoration of function, there is no direct correlation i e. no 'critical' level of ATP. Increased glucose provision during low flow ischemia increases the functional recovery of hearts [2, 114, 127, 129], mainly by reducing diastolic pressure on reperfusion, a consequence of reduced contracture during ischemia and improved relaxation on reperfusion [170]. Optimal recovery on reperfusion requires the presence of several compounds, one of which should be glucose [8, 176–178]. Glycolysis in early reperfusion appears to be essential in preventing energetic and contractile collapse, and specifically to facilitate Ca^{2+} homeostasis [119, 128]. Excess free radicals, one of the possible causes of stunning and arrhythmias on reperfusion, inhibit glycolysis, and thus further impair Ca^{2+} homeostasis [179]. Inhibition of glycolysis, despite the presence of pyruvate and oxygen, depresses functional recovery severely, with a persistent Ca^{2+} overload [119, 128].

Glycolytic ATP may be required to reduce $[Na^+]i$ by maintaining the activity of the Na^+/K^+ ATPase, thereby resulting in reduced Ca^{2+} overload via the Na^+/Ca^{2+} exchange which predisposes to reperfusion injury [128]. Na^+/K^+ ATPase inhibition during ischemia removes the protective effect of glucose on functional recovery [124, 142]. ATP derived from glycolysis is also functionally associated with the sarcoplasmic reticulum [116], thereby providing a means of restoring Ca^{2+} overload on reperfusion by enhanced SR Ca^{2+} ATPase function.

Palmitate is the preferred substrate on reperfusion, accounting for over 90% of ATP from exogenous substrates. High levels of fatty acids on reperfusion limit glucose utilisation by competitive inhibition [180], primarily by inhibition of glucose oxidation and not of glycolysis [181]. Inhibition of palmitate oxidation with increased glucose oxidation improves functional recovery [177], possibly because stimulation of glucose oxidation speeds the initial rate of return to total oxidative metabolism. A faster repletion of high energy phosphates is then sustained by fatty acid or pyruvate oxidation [182]. Secondly, a reduced intracellular acidosis from improved coupling of glucose oxidation with glycolysis may be beneficial by reducing intracellular Ca^{2+} overload [181, 182]. Increased rates of glycolysis and glucose oxidation with improved coupling of these two pathways, together with reduced fatty acid oxidation, may enhance recovery.

Preconditioning

Glycolytic flux is stimulated in preconditioned hearts subjected to low flow ischemia, and may exert its positive effects by this mechanism [110]. However, additional evidence to support this hypothesis is lacking, given that preconditioning does not seem to be effective when a sustained low residual flow [111, 183] or hypoxia [184] is used as the 'test', despite an increase in glucose uptake. This effect may be attributed either to an excess glycolytic flux in the presence of preconditioning (following increased translocation of GLUT 4 transporters to the membrane, resulting in stimulated uptake at the onset of the 'test' period), with excess metabolite accumulation; or it may be that preconditioning is ineffective when glycolysis is maintained. Preconditioning (with total global ischemia, the usual model investigated) is associated with a reduced tissue glycogen level prior to sustained ischemia. If, however, glycolysis is maintained during low flow ischemia, opening of the K_{ATP} channels [185] and release of adenosine [186], two of the major proposed triggers of preconditioning, may be attenuated. This phenomenon adds to the controversy over the roles of glycogen utilisation and glycolysis in ischemia.

Deleterious effects associated with increased glycolysis

Benefits associated with depleted glycogen levels

Much evidence suggests that ATP derived from glycolysis is beneficial to the ischemic myocardium. However, accumulation of deleterious end products may partly outweigh, or overcome, the benefit. Much controversy arose from a frequently-quoted paper published in 1984 by Neely and Grotyohann [14]. Following glycogen depletion by a brief period of anoxia (10 min) prior to sustained total global ischemia, a greatly improved recovery of function was found on reperfusion. The improved recovery was correlated with

a reduced lactate content at the end of ischemia. It was hypothesised that increased glycolysis is associated with a detrimental effect due to an increase in lactate and other end products. The concept then arose that provision of glucose should be detrimental.

Several other studies have linked beneficial effects to reduced glycogen levels. For example, 2 h perfusion with pyruvate depleted tissue glycogen by 40–50%, and resulted in a significant improvement in functional recovery after 25 min total global ischemia. This result was attributed to reduced $[H^+]i$ accumulation despite lower ATP levels during ischemia [187]. Glycogen-depleted hearts did show a reduced time to onset of contracture although peak contracture was similar to control glucose-perfused hearts. In addition, glycogen reduction by hypoxic perfusion, followed by 25 min total global ischemia, reduced lactate accumulation and improved functional recovery [188].

The possible benefits of glycogen depletion may also apply to preconditioning, as the brief episode(s) of ischemia deplete tissue glycogen prior to the sustained ischemic period. An increased duration of intervening perfusion was correlated with repletion of glycogen stores, but loss of recovery [189]. A more complex study found that recovery of function was improved in preconditioned and pyruvate glycogen-depleted hearts, but only if glucose was not provided to the latter. Glycogen depletion *per se* was not sufficient to explain the beneficial effects of preconditioning, but interventions which limited glycolysis and thus H^+ accumulation were beneficial [190]. We have, however, found no correlation between pre-ischemic glycogen levels (modified by acetate or glucose+insulin perfusion) and protection with preconditioning [105].

Proposed beneficial mechanisms of glycogen depletion

Protons

While intracellular pH always falls in ischemic hearts, the extent of the drop can be modified by changes in the amount of glycogen in the tissue at the onset of ischemia, such that glycogen-depleted hearts show reduced acidosis [191]. Increased $[H^+]i$ increases $[Na^+]i$, and in turn increases $[Ca^{2+}]i$ via the Na^+/H^+ and Na^+/Ca^{2+} exchange mechanisms [152]. Glycogen depletion is associated with reduced $[Na^+]i$ accumulation, linked to reduced H^+ accumulation [192].

Recovery of function is improved, with reduced cytosolic Ca^{2+} overload. However, while some reports have correlated intracellular pH with ischemic injury [193], others have dissociated these effects [191, 194]. The relationship between increased acidosis and increased ischemic injury is not clear.

Lactate

The cellular role of lactate in ischemic damage is unclear. Intracellular lactate accumulation is thought to be deleterious, both directly, and by inhibiting GAPDH following NADH accumulation [14, 73, 74], thereby inhibiting glycolysis. Increased extracellular lactate may be deleterious by inhibiting the $lactate^-/H^+$ co-transporter and increasing H^+ accumulation [195]. Thus a Na^+/H^+ exchange inhibitor on reperfusion reversed the deleterious effects of pre-ischemic lactate perfusion on functional recovery, presumably by reducing final Ca^{2+} overload [195]. However, Cross *et al.* found no change in intracellular pH with increased extracellular lactate [194], contrary to the previous findings [195]. In this model of low flow ischemia (0.5 ml/g wet wt/min) the effects of $lactate/H^+$ transport inhibition may not be as marked because of the maintained washout. The deleterious effects of lactate were attributed to the inhibition of glycolysis by NADH accumulation, which could account for the attenuated fall in pHi. Lactate may also activate K_{ATP} channels [196], thereby shortening the action potential and predisposing to arrhythmias. However, in a myocyte model of anoxia and reoxygenation, Geisbuhler *et al.* [197] reported no deleterious effect of high concentrations of lactate (10 and 50 mM) on anoxic myocytes. Damage was found, however, if pH was lowered. In addition, alterations in pre-ischemic glycogen do not result in a correlation between end-ischemic tissue lactate and functional recovery [198, 199].

Sugar phosphates

Glycolytic inhibition increases diastolic dysfunction in normally perfused hearts [200], attributed to increased sugar phosphates which may impair Ca^{2+} homeostasis. Phosphomonoester resonance, which measures phosphorylated compounds including G6P, F6P, α-GP and AMP, increases during ischemia and has been correlated with reduced recovery [201, 202]. However, glycolytic ATP is linked to Ca^{2+} homeostasis by functional coupling with SR Ca^{2+} ATPase reuptake [116]. A decreased cytosolic Ca^{2+} should thus reduce diastolic impairment. Therefore the relative rates of sugar phosphate accumulation vs. ATP production may be crucial in determining the efficacy of glucose provision. Increased G6P production would also lower ATP levels, as this step (from glucose) consumes an ATP. If glycolysis were inhibited at points just below this reaction, this step would be detrimental as no replacement ATP would be provided [3]. While much of the focus of the deleterious effects of increased glycolysis has been on lactate and protons, more evidence is now available to suggest that sugar phosphate accumulation may be an important factor [105, 190, 200, 201]. We found that with the addition of insulin to hearts perfused with 11 mM glucose, the levels of G6P and F6P rose considerably, and recovery of function was not improved [105, 111]. These factors may account for any detrimental effects of a high glucose concentration, and for some of the controversy surrounding the roles of glucose and glycogen utilisation in ischemia.

Evidence contradicting benefit of glycogen depletion

Taegtmeyer *et al.* [198, 199] have tried to replicate the findings of Neely and Grotyohann [14], with little success. A period of anoxia or substrate-free perfusion prior to 30 min total global ischemia reduced tissue glycogen, but did not improve recovery of function on reperfusion; rather, function was significantly impaired [198]. Interestingly, the anoxic hearts did not show a reduction in tissue lactate despite a 60% decrease in glycogen levels. This lactate could be accounted for by the residual glucose remaining in the tissue following cessation of flow. In substrate-free hearts with no residual glucose, lactate levels decreased substantially. A later publication by the same group [199] found similar results with a shorter period of ischemia (15 min). There was no correlation of lactate build-up with functional recovery, but a depletion of glycogen was detrimental. Postischemic glycogen availability and functional recovery were well correlated. We have also found no reduction in tissue lactate in preconditioned hearts with depleted glycogen, attributed to utilisation of the residual glucose at the onset of ischemia [105].

In addition, several reports show that glycogen loading can benefit hearts exposed to ischemia. Preoperative administration of GIK increased tissue glycogen levels, which were associated with a lower incidence of postoperative hypotension, reduced arrhythmias and fewer complications [203]. Glycogen loading with insulin greatly improves the tolerance of isolated rabbit hearts to ischemia, while glycogen depletion by epinephrine infusion severely impairs ischemic tolerance [204]. Fasting, which increases glycogen content, also increases the resistance of hearts to an ischemic episode [52], with improved recovery of function, reduced membrane damage and reduced loss of adenine nucleotides. Increased glycogen levels following palmitate addition to the perfusate of isolated rabbit hearts significantly improved recovery of function [205] while glycogen loading and lactate perfusion mimicked the protective effects of preconditioning, improving tolerance to ischemia [206].

A recent paper purports to explain the controversy over glycogen utilisation in ischemia suggesting that while glycogen is being utilised, the ATP provided supplies necessary energy [207]. Reperfusion at this relatively early stage results in good recoveries. However, once the glycogen has been used up (in the absence of glucose, in an ischemic model of relatively 'high' coronary flow, 0.5 ml/min/g wet wt), the accumulation of intracellular metabolites outweighs the advantages conveyed by ATP, with impaired functional recovery. A Na^+/H^+ exchange inhibitor on reperfusion after extended ischemia improves functional recovery, suggesting that the $[H^+]i$ was high following glycogen depletion [207]. However, at a lower flow rate, when the rates of ATP demand and metabolite accumulation are very different, the findings might be different.

Glucose vs. glycogen utilisation in ischemia

Major concerns with studies purporting to show deleterious effects of glycolysis are (1) glycogen is confused with glucose as the source of glycolytic ATP and (2) most studies have been done using models of total global ischemia, where glycolytic flux is limited by substrate and may also be inhibited by metabolite build-up. Glucose is therefore not present throughout the ischemic period. If glycogen loading were detrimental, increased glucose utilisation would not necessarily be detrimental; alternatively, if glycogen depletion is beneficial, glucose should not necessarily be removed. In addition, many of the early studies using brief periods of anoxia to deplete glycogen, with subsequent beneficial effects, can now be said to mimic preconditioning [208–211] which may act by different pathways excluding glycolytic involvement. We have attempted to correlate changes induced by preconditioning with changes in glycolysis (both glucose and glycogen) and find no significant relationship [105, 111]. Studies implicating a deleterious effect of glucose must thus be considered carefully in terms of the experimental model, and the source of glycolytic substrate. Thus the residual flow rate and glucose concentration, as well as the functional compartmentation of ATP production within the cell, are major determinants of the response to glycolysis.

Role of residual coronary flow and glucose concentration

The rate of residual coronary flow, together with an optimal glucose concentration, are crucial determinants of the extent of ischemic injury, and the recovery of function [7]. Provision of glucose in rat models of low flow ischemia (0.5 ml/min/g wet wt or 0.06 ml/min/g wet wt) maintains diastolic function, and improves recovery of mechanical function on reperfusion [2, 127]. With a low flow of 0.4–0.5 ml/g wet wt/min, the rate of glycolysis during ischemia is directly related to the degree of ischemic injury i.e. contracture, and functional recovery [129].

At a low residual flow rate (0.2 ml/g wet wt/min), a high glucose concentration (22 mM) with or without insulin may be deleterious [7] with an excess accumulation of end products [7, 105]. A higher coronary flow removes many of the deleterious effects associated with a high glucose concentration [2, 7, 127]. However, in the absence of glucose, even if an alternate substrate such as acetate is present, function is impaired with a higher residual flow [7, 11]. In addition, it must be noted that at no flow rate is the absence of glucose better than the presence of glucose, despite accumulation of glycolytic end products. Thus an optimal glucose concentration (11 mM in the absence of insulin) [7] with the highest possible residual flow rate results in optimal recovery. If insulin is present, a lower concentration of glucose (about 5 mM) is optimal (unpublished data), in accordance with physiological values in blood.

Compartmentation of ATP

Cellular compartmentation

Many cell components are compartmented – e.g. intracellular Ca^{2+} is highly compartmented in the myocyte, distributed among the SR, the Ca^{2+}-binding proteins and the cytosol. The adenine nucleotides including ATP are also strictly compartmented, which becomes especially evident in ischemia [212]. An extensive review also recounted the evidence for the compartmentation of glycolysis, with 'microenvironments' within the cell [213]. A high degree of functional organisation of the glycolytic enzymes, particularly in muscle cells, would allow for efficient 'channelling' of the substrate through its catabolic pathway [65]. Evidence of glycolytic vs. oxidative ATP compartmentation has been found in different cell types e.g. liver [214], vascular smooth muscle [215], and myometrium [216]. Glucose and glycogen metabolism also form two functionally exclusive compartments in the cytoplasm of vascular smooth muscle [217, 218]. This arrangement maximises the efficiency and response time of energy transduction within the smooth muscle cell. In the myocyte, the distances are greater because the cell is much larger, and the demands on energy are also increased with the much greater contractile component of the cell. Thus compartmentation within the myocyte is plausible given the requirement for sustained efficiency of contraction.

Two pools of ATP have been identified in hepatocytes – one cytosolic, and one near the membrane [214], on the basis of ATP utilisation by membrane (Na^+/K^+ ATPase) and cytosolic (ATP-sulfurylase) enzymes. Differences in the mechanisms resulting in ATP depletion indicate that the different elements are not exposed to the same ATP level. Intracellular inhomogeneities of ATP can be attributed to different rates of ATP-producing and ATP-consuming sites, such that ATP produced in large amounts from the mitochondria supply the cytosolic elements. These elements reduce the amount of ATP at the membrane because of rapid utilisation of ATP diffusing from the mitochondria, such that at further distances the ATP is depleted. Thus mitochondrial ATP utilisation by the membrane components appears to be limited by diffusion and steepness of gradients within the cell. This may certainly apply to the large, metabolically active myocyte, although this has not been shown directly.

While compartmentation is difficult to prove (ATP is a ubiquitous compound), this concept explains many observations and has been invoked by several authors to explain observations in the heart [11, 114, 123, 141, 219]. Oxidative phosphorylation and glycogen breakdown (which supplies substrate for the oxidative pathway) may supply ATP largely for contraction, while glycolysis (glucose) may be required for sarcolemmal pump function and membrane integrity [141]. A localisation of energy-producing pathways near energy-consuming processes thereby ensures efficient supply and utilisation of energy. When ATP supply is plentiful, the different systems may overlap. However, if energy supply is limited, the source of energy becomes crucial. In ischemia, the different effects of glycogen-derived, glucose-derived and oxidative ATP on contracture and on functional recovery may be explained by these differences.

Location of enzymes within the cell – basis for compartmentation theory

Oxidative phosphorylation occurs in the mitochondria, which are localised near the myofibrils [220], the primary energy consumers in the myocyte. Inhibition of oxidative phosphorylation has a major depressant effect on tension [95, 123, 221], an effect slowed down by the presence of glucose. Conversely, inhibition of glycolysis with maintained oxidative phosphorylation has a much lesser effect on reduction in tension [123]. These findings and others [10] suggest that ATP from oxidative phosphorylation is used preferentially by the contractile apparatus.

The ATP produced in the mitochondria needs to be transported to the sites of utilisation, a process mediated by the creatine kinase shuttle [222, 223], which maintains a local ratio of ATP/ADP around the myofibrils [224–226], and sustains contraction. In ischemia, CP levels fall and mitochondrial activity is inhibited. ATP becomes trapped in the mitochondria [212], which in turn become major sites of ATP utilisation as the mitochondrial ATP synthase reverses, and becomes an ATPase [227] (this mechanism is not thought to be important in the rat [228]). ATP availability to the myofibrils is thus reduced.

Utilisation of glucose may be localised within the cell. In vascular smooth muscle, an unexpectedly high proportion of glucose is converted to lactate under fully oxygenated conditions. This may be due to a preferential utilisation of glycolytic ATP by the Na^+/K^+ ATPase membrane pump in these cells [115, 215]. Following Na^+/K^+ ATPase inhibition by ouabain, glycolysis (in terms of lactate production) is reduced, with no change in force development. When Na^+/K^+ ATPase activity is stimulated, lactate production is increased [215]. While this mechanism requires a feedback regulation of ATP utilisation on glycolytic flux which is difficult to understand, the evidence for the association of glycolytic enzymes with the membrane is fairly convincing.

The enzymes and substrates of glycolysis were initially thought to be freely dispersed in the aqueous medium of the cytoplasm [213]. However, subsequent studies have found that the glycolytic enzymes are at specific locations within the cell [213]. The glycolytic enzymes, GAPDH and PK, bind to sarcolemma and SR membranes, possibly to charged phospholipids which are more prevalent in the former

membranes and thus show greater binding affinity for the enzymes [229]. Further evidence for physical compartmentation comes from excised inside-out patches of sarcolemmal membrane (with no cytosol present). Addition of substrates for the ATP-producing steps of glycolysis (phophoglycerokinase and pyruvate kinase) block the K_{ATP} channel [123], which can only occur by a localised production of ATP. This finding substantiates the concept that key glycolytic enzymes are located in or near the sarcolemma. In addition, if glycolysis is inhibited in myocytes with maintained oxidative phosphorylation, a large efflux of K^+ is found, with little change in tension [123]. However, if oxidative phosphorylation is inhibited, tension is depressed, but K^+ efflux is much lower. The enzymes of the glycolytic cascade also appear to be associated with the plasma membrane in smooth muscle, allowing isolated vesicles to produce ATP and maintain Ca^{2+} pump function [230], an effect which can be blocked by iodoacetate. Glycolytic enzymes are also functionally coupled to the SR, maintaining Ca^{2+} homeostasis [116], which in turn greatly modifies contraction.

While glycolytic ATP therefore appears to be closely involved with regulating K^+ efflux and Ca^{2+} homeostasis, glycolytic ATP is not necessarily distributed to the contractile apparatus. The mechanisms of ATP transport may be limited in conditions of metabolic impairment such as hypoxia and ischemia. Glycolytic enzymes are not found near myofibrils in any great quantity [213], suggesting that glycolytic ATP is not synthesised in these regions, and thus is of less importance than oxidatively derived ATP for the contractile apparatus.

Glycogen particles are located along the mitochondrial columns between myofibrils, and are also present in the perinuclear sarcoplasm and the SR including the sub-sarcolemmal cisterns [220]. The enzymes for glycogen breakdown (phosphorylases etc.) are also found near the SR in cardiac muscle [143], and form 'metabolons' for the efficient metabolism of glycogen [62].

The substrate supply from glycogen utilisation is coordinated with mitochondrial substrate utilisation whereas glucose utilisation correlates with Na^+/K^+ ATPase activity in smooth muscle cells [215]. Glycogenolysis may be linked to the contractile and cytosolic elements in vascular smooth muscles, rather than membrane functions [230]. This may explain the role of glycogen utilisation as a major determinant of the onset of contracture [7, 105, 161].

Conclusions

The ischemic myocardium can upregulate its ability to extract glucose from that made available to the tissue, which is contrary to previous conceptions that glycolysis is inhibited in ischemia. Thus provision is made to enhance the numerous protective effects of glucose in the ischemic cell. However, excess glycolysis can be detrimental. A balance between substrate supply and ATP production, and metabolite accumulation, must be maintained. In terms of clinical applications, despite the original report showing that administration of GIK had no benefit [12], many subsequent studies argue for a reinvestigation of this therapy [231–234], with a great potential for protection in patients with myocardial infarction.

Glucose seems to exert a beneficial effect mainly at the membranes, regulating intracellular ion homeostasis, while glycogen-derived ATP is present mainly at the myofibrils, and possibly at the SR, thereby attenuating ischemic contracture. The functional distinction between these two glycolytic substrates and the compartmentation within the cell clarifies many controversial issues regarding the benefit or detriment associated with increased glycolysis during ischemia.

References

1. Opie LH: The glucose hypothesis: Relation to acute myocardial ischaemia. J Mol Cell Cardiol 1: 107–114, 1970
2. Apstein CS, Gravino FN, Haudenschild CC: Determinants of a protective effect of glucose and insulin on the ischaemic myocardium. Effects of contractile function, diastolic compliance, metabolism, and ultrastructure during ischaemia and reperfusion. Circ Res 52: 515–526, 1983
3. Bernier M, Hearse DJ: Reperfusion-induced arrhythmias: Mechanisms of protection by glucose and mannitol. Am J Physiol 254: H862–H870, 1988
4. Dalby AJ, Bricknell OL, Opie LH: Effect of glucose-insulin-potassium infusions on epicardial ECG changes and on myocardial metabolic changes after coronary artery ligation in dogs. Cardiovasc Res 15: 588–598, 1981
5. Hess ML, Okabe E, Poland J, Warner M, Stewart JR, Greenfield LJ: Glucose, insulin, potassium protection during the course of hypothermic global ischaemia and reperfusion: A new proposed mechanism by the scavenging of free radicals. J Cardiovasc Pharmacol 5: 35–43, 1983
6. Hoekenga DE, Brainard JR, Hutson JY: Rates of glycolysis and glycogenolysis during ischemia in glucose-insulin-potassium-treated perfused hearts: A C-13, P-32 nuclear magnetic resonance study. Circ Res 62: 1065–1074, 1988
7. King LM, Boucher F, Opie LH: Coronary flow and glucose delivery as determinants of contracture in the ischemic myocardium. J Mol Cell Cardiol 27: 701–720, 1995
8. Mallet RT, Hartman DA, Bünger R: Glucose requirement for postischemic recovery of perfused working heart. Eur J Biochem 188: 481–493, 1990
9. Moreau D, Chardigny JM: The protective role of glucose on ischemic-reperfused hearts: effect of dietary fats. J Mol Cell Cardiol 23: 1165–1176, 1991
10. Opie LH, Bricknell OL: Role of glycolytic flux in effect of glucose in decreasing fatty-acid induced release of lactate dehydrogenase from isolated coronary ligated rat heart. Cardiovasc Res 13: 693–702, 1979
11. Owen P, Dennis S, Opie LH: Glucose flux rate regulates onset of ischemic contracture in globally underperfused rat hearts. Circ Res 66: 344–354, 1990
12. Medical Research Council Working Party on the Treatment of

Myocardial Infarction: Potassium, glucose, and insulin treatment for acute myocardial infarction. Lancet 2: 1355–1360, 1968
13. Hearse DJ, Stewart DA, Braimbridge MV: Myocardial protection during ischemic cardiac arrest. Possible deleterious effects of glucose and mannitol in coronary infusates. J Thorac Cardiovasc Surg 76: 16–23, 1978
14. Neely JR, Grotyohann LW: Role of glycolytic products in damage to ischemic myocardium. Dissociation of adenosine triphosphate levels and recovery of function of reperfused ischemic hearts. Circ Res 55: 816–824, 1984
15. Murry CE, Jennings RB, Reimer KA: Preconditioning with ischaemia: A delay of lethal cell injury in ischaemic myocardium. Circulation 74: 1124–1136, 1986
16. Jennings RB, Murry CE, Reimer KA: Energy metabolism in preconditioned and control myocardium: Effect of total ischemia. J Mol Cell Cardiol 23: 1449–1458, 1991
17. Weiss RG, de Albuquerque C, Chacko VP, Gerstenblith G: Reduced glycogenolysis rather than glycogen depletion contributes to decreased lactate accumulation after preconditioning. Circulation 88: I-379, 1993
18. Opie LH: Substrate and energy metabolism of the heart. In: N Sperelakis (ed). Physiology and Pathophysiology of the Heart. Kluwer Academic Publishers, 1995
19. Alonso MD, Lomako J, Lomako WM, Whelan WJ: A new look at the biogenesis of glycogen. FASEB J 9: 1126–1137, 1995
20. De Barsy T, Hers H-G: Normal metabolism and disorders of carbohydrate metabolism. Bailliere's Clin Endocrin Metab 4: 499–522, 1990
21. Pessin JE, Bell GI: Mammalian facilitative glucose transporter family: Structure and molecular regulation. Annu Rev Physiol 54: 911 –930, 1992
22. James DE: The mammalian facilitative glucose transporter family. News Physiol Sci 10: 67–71, 1995
23. James DE, Strube M, Mueckler M: Molecular cloning and characterisation of an insulin-regulatable glucose transporter. Nature 338: 83–87, 1989
24. Elsas LJ, Longo N: Glucose transporters. Annu Rev Med 43: 377–393, 1992
25. Katz EB, Stenbit AK, Hatton K, DePinho R, Charron MJ: Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. Nature 377: 151–155, 1995
26. Nishimura H, Pallardo FV, Seidner GA, Vannucci S, Simpson IA, Birnbaum MJ: Kinetics of GLUT1 and GLUT4 glucose transporters expressed in *Xenopus* oocytes. J Biol Chem 268: 8514–8520, 1993
27. Taegtmeyer H: Energy metabolism of the heart: From basic concepts to clinical applications. Curr Prob Cardiol 19: 59–113, 1994
28. Myers MG, Sun XJ, White MF: The IRS-1 signalling system. Trends Biochem Sci 19: 289–293, 1994
29. Liu F, Roth RA: Identification of serines-1035/1037 in the kinase domain of the insulin receptor as protein kinase Cα mediated phosphorylation sites. FEBS Lett 352: 389–392, 1994
30. Berridge MJ: Inositol trisphosphate and calcium signalling. Nature 361: 315–325, 1993
31. Kelada ASM, Macauley SL, Proietto J: Cyclic AMP acutely stimulates translocation of the major insulin-regulatable glucose transporter GLUT4. J Biol Chem 267: 7021–7025, 1992
32. Rattigan S, Appleby GJ, Clark MG: Insulin-like action of catecholamines and Ca^{2+} to stimulate glucose transport and GLUT4 translocation in perfused rat heart. Biochem Biophys Acta 1094: 217–223, 1991
33. Wheeler TJ: Translocation of glucose transporters in response to anoxia in heart. J Biol Chem 263: 19447–19454, 1988
34. Sun DQ, Nguyen N, DeGrado TR, Schwaiger M, Brosius FC: Ischemia induces translocation of the insulin-responsive glucose transporter GLUT4 to the plasma membrane of cardiac myocytes. Circulation 89: 793–798, 1994
35. Vannucci SJ, Nishirnura H, Satoh S, Cushman SW, Holman GD, Simpson IA: Cell surface accessibility of GLUT4 glucose transporters in insulin-stimulated rat adipose cells. Modulation by isoprenaline and adenosine. Biochem J 288: 325–330, 1992
36. Law WR, Raymond RM: Adenosine potentiates insulin-stimulated myocardial glucose *in vivo*. Am J Physiol 254: H970–H975, 1988
37. Law WR, McLane MP: Adenosine enhances myocardial glucose uptake only in the presence of insulin. Metab Clin Exper 40: 947–952, 1991
38. Wyatt DA, Edmunds MC, Rubio R, Beme RM, Lasley RD, Mentzer RM: Adenosine stimulates glycolytic flux in isolated perfused rat hearts by A1-adenosine receptors. Am J Physiol 257: H1952–H1957, 1989
39. Angello DA, Beme RM, Coddington NM: Adenosine and insulin mediate glucose uptake in normoxic rat hearts by different mechanisms. Am J Physiol 265: H880–H885, 1993
40. Shanahan MF, Edwards BM: Stimulation of glucose transport in rat cardiac myocytes by guanosine 3′, 5′-monophosphate. Endocrinology 125: 1074–1081, 1989
41. Depré C, Vanoverschelde J-L, Goudemant J-F, Mottet I, Hue L: Protection against ischemic injury by nonvasoactive concentrations of nitric oxide synthase inhibitors in the perfused rabbit heart. Circulation 92: 1911–1918, 1995
42. Morgan HE, Henderson MJ, Regen DM, Park CR: Regulation of glucose uptake in muscle. I. The effects of insulin and anoxia on glucose transport and phosphorylation in the isolated perfused heart of normal rats. J Biol Chem 236: 253–261, 1961
43. Opie LH, Shipp JC, Evans JR, Leboeuf B: Metabolism of glucose-U-^{14}C in perfused rat heart. Am J Physiol 203: 839–843, 1962
44. Hopkins JCA, Radda GK, Clarke K: Glycogen levels modify myocardial glucose uptake and insulin sensitivity. Circulation 92: I-633, 1995
45. Neely JR, Bowman RH, Morgan HE: Effects of ventricular pressure development and palmitate on glucose transport. Am J Physiol 216: 804–811, 1969
46. Goodyear LJ, Hirshman MF, Horton ES: Exercise-induced translocation of skeletal muscle glucose transporters. Am J Physiol 261: E795–E799, 1991
47. Goodwin GW, Arteaga JR, Taegtmeyer H: Glycogen turnover in the isolated working rat heart. J Biol Chem 270: 9234–9240, 1995
48. Henning SL, Wambolt RB, Schonekess BO, Lopaschuk GD, Allard MF: Contribution of glycogen to aerobic myocardial glucose utilization. Circulation 93: 1549–1555, 1996
49. Randle PJ: Regulation of glycolysis and pyruvate oxidation in cardiac muscle. Circ Res 38 (Suppl 1): I8–I15, 1976
50. Lomako J, Lomako WM, Whelan WJ: Proglycogen: a low molecular-weight form of muscle glycogen. FEBS Lett 279: 223–228, 1991
51. Lawrence JC: Signal transduction and protein phosphorylation in the regulation of cellular metabolism by insulin. Annu Rev Physiol 54: 177–193, 1992
52. Schneider CA, Taegtmeyer H: Fasting *in vivo* delays myocardial cell damage after brief periods of ischemia in the isolated working rat heart. Circ Res 68: 1045–1050, 1991
53. McNulty PH, Luba MC: Transient ischemia induces regional myocardial glycogen synthase activation and glycogen synthesis *in vivo*. Am J Physiol 268: H364–H370, 1995
54. Rahimtoola SH: A perspective on three large multicenter randomized clinical trials of coronary bypass surgery for chronic stable angina. Circulation 72 (Suppl V): 123–125, 1985
55. Lamer J: Mechanisms of regulation of glycogen synthesis and degradation. Circ Res 38 (Suppl 1): I2–I7, 1976
56. Brand MD: The stoichiometry of proton pumping and ATP synthesis in mitochondria. Biochemist 16: 20–24, 1994

57. De Jong JW, Cargnoni A, Bradamente S, Curello S, Janssen M, Pasini E, Ceconi C, Bünger R, Ferrari R: Intermittent vs. continuous ischemia decelerates adenylate breakdown and prevents norepinephrine release in reperfused rabbit heart. J Mol Cell Cardiol 27: 659–671, 1995
58. Botker HE, Randsbaek F, Hansen SB, Thomassen A, Nielsen TT: Superiority of acid-extractable glycogen for detection of metabolic changes during myocardial ischaemia. J Mol Cell Cardiol 27: 1325–1332, 1995
59. Rovetto MJ, Lamberton WF, Neely JR: Mechanisms of glycolytic inhibition in ischemic rat hearts. Circ Res 37: 742–751, 1975
60. Neely JR, Whitmer JT, Rovetto MJ: Inhibition of glycolysis in hearts during ischemic perfusion. In: P Harris, RJ Bing, A Fleckenstein (eds). Recent Advances in Studies on Cardiac Structure and Metabolism. University Park Press, Baltimore, 1976
61. Neely JR, Rovetto MJ, Whitmer JT: Rate-limiting steps of carbohydrate and fatty acid metabolism in ischemic hearts. Acta Med Scand 587 (Suppl): 9–15, 1976
62. Srere P: Complexities of metabolic regulation. Trends Biochem Sci 519–520, 1994
63. Kashiwaya Y, Sato K, Tsuchiya N, Thomas S, Fells DA, Veech RL, Passoneau JV: Control of glucose utilization in working perfused rat heart. J Biol Chem 269: 25502–25514, 1994
64. Kübler W, Strasser RH: Signal transduction in myocardial ischaemia. Eur Heart J 15: 437–445, 1994
65. Welch GR, Easterby JS: Metabolic channelling versus free diffusion: Transition-time analysis. TIBS 19: 193–197, 1994
66. Srere PA: Complexes of sequential metabolic enzymes. Annu Rev Biochem 56: 89–124, 1987
67. Manchester J, Kong X, Nerbonne J, Lowry OH, Lawrence JC: Glucose transport and phosphorylation in single cardiac myocytes: Rate-limiting steps in glucose metabolism. Am J Physiol 266: E326–E333, 1994
68. Chappell JD, Robinson BH: Penetration of the mitochondrial membrane by tricarboxylic acid anions. Biochem Soc Symp 27: 123–133, 1968
69. Ui M: A role of phosphofructokinase in pH-dependent regulation of glycolysis. Biochim Biophys Acta 124: 310–322, 1966
70. Lawson JWR, Uyeda K: Effects of insulin and work on fructose 2,6-bisphosphate content and phosphofructokinase activity in perfused rat hearts. J Biol Chem 262: 3165–3173, 1987
71. Hue L, Depré C, Lefebvre V, Rider MH, Veitch K: Regulation of carbohydrate metabolism in muscle and liver. Biochem Soc Trans 23: 311–314, 1995
72. King LM, Opie LH: Substrate availability vs. distributive control of glycolytic flux rate in myocardial ischaemia (abstr). J Moll Cell Cardiol 29: A87, 1997
73. Mochizuki S, Kobayashi K, Neely JR: Effects of L-lactate on glyceraldehyde-3-P dehydrogenase in heart muscle. In: T Kobayashi, Y Ito, G Rona (eds). Recent Advances in Studies on Cardiac Structure and Metabolism. University Park Press, Baltimore, 1978
74. Mochizuki S, Neely JR: Control of glyceraldehyde-3-phosphate dehydrogenase in cardiac muscle. J Mol Cell Cardiol 11: 221–236, 1979
75. Poole RC, Halestrap AP: Transport of lactate and other mono-carboxylates across mammalian plasma membranes. Am J Physiol 264: C761–C782, 1993
76. Gevers W: Generation of protons by metabolic processes in heart cells. J Mol Cell Cardiol 9: 867–874, 1977
77. Peuhkurinen KJ, Hassinen IE: Pyruvate carboxylation as an anaplerotic mechanism in the isolated perfused rat heart. Biochem J 202: 67–76, 1982
78. Peuhkurinen KJ, Takala TES, Nuutinen EM, Hassinen E: Tricarboxylic acid cycle metabolises during ischemia in isolated perfused rat heart. Am J Physiol 24: H281–H288, 1983
79. Safer B, Smith CM, Williamson JR: Control of the transport of reducing equivalents across the mitochondrial membrane in perfused rat heart. J Mol Cell Cardiol 2: 111–124, 1971
80. McGinnis JF, de Vellis J: Glycerol-3 phosphate dehydrogenase isoenzymes in human tissues: Evidence for a heart specific form. J Mol Cell Cardiol 11: 795–802, 1979
81. Isaacs GH, Sacktor B, Murphy TA: The role of α-glycerophosphate cycle in the control of carbohydrate oxidation in heart and in the mechanism of action of thyroid hormone. Biochim Biophys Acta 177: 196–203, 1969
82. Randle PJ, Garland PB, Hales CN, Newsholme EA: The glucose-fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet April 13: 785–789, 1963
83. Shipp JC, Opie LH, Challoner D: Fatty acid and glucose metabolism in the perfused heart. Nature 189: 1018–1019, 1961
84. Randle PJ, Newsholme EA, Garland PB: Regulation of glucose uptake by muscle. Effects of fatty acids, ketone bodies and pyruvate, and of alloxan-diabetes and starvation on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. Biochemistry 93: 652–687, 1964
85. McGarry JD, Mills SE, Long CS, Foster DW: Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. Biochem J 214: 21–28, 1983
86. McMillan JB, Hudson EK, Van Winkle WB: Evidence for malonyl-CoA-sensitive carnitine acyl-CoA transferase activity in sarcoplasmic reticulum of canine heart. J Mol Cell Cardiol 24: 259–268, 1992
87. Bielefeld DR, Vary C, Neely JR: Inhibition of carnitine palmitoyl-CoA transferase activity and fatty acid oxidation by lactate and oxtenicine in cardiac muscle. J Mol Cell Cardiol 17: 619–625, 1985
88. Dennis SC, Gevers W, Opie LH: Protons in ischemia: Where do they come from and where do they go to? J Mol Cell Cardiol 23: 1077–1086, 1991
89. Abdel-Aleem S, Nada MA, Sayed-Ahmed M, Hendrikson SC, St Louis J, Walthall HP, Lowe JE: Regulation of fatty acid oxidation by acetyl-CoA generated from glucose utilisation in isolated myocytes. J Mol Cell Cardiol 28: 825–833, 1996
90. Grynberg A, Demaison L: Fatty acid oxidation in the heart. J Cardiovasc Pharmacol 28 (Suppl 1): S11–S17, 1996
91. Lopaschuk GD, Belke DD, Gamble J, Itoi T, Schönekess BO: Regulation of fatty acid oxidation in the mammalian heart in health and disease. Biochim Biophys Acta 1213: 263–276, 1994
92. Kübler W, Spieckermann PG: Regulation of glycolysis in the ischemic and the anoxic myocardium. J Mol Cell Cardiol 1: 351–377, 1970
93. Rovetto MJ, Whitmer JT, Neely JR: Comparison of the effects of anoxia and whole heart ischemia on carbohydrate utilisation in isolated working rat hearts. Circ Res 32: 699–711, 1973
94. Williamson JR: Glycolytic control mechanisms. II. Kinetics of intermediate changes during the aerobic-anoxic transition in perfused rat heart. J Biol Chem 241: 5026–5036, 1966
95. Allen DG, Orchard CH: Myocardial contractile function during ischemia and hypoxia. Circ Res 60: 153–168, 1987
96. Allen DG, Morris PG, Orchard CH, Pirolo JS: A nuclear magnetic resonance study of metabolism in the ferret heart during hypoxia and inhibition of glycolysis. J Physiol 361: 185–204, 1985
97. Allshire AP, Cobbold PH: Causes and effects of changes in cytosolic free calcium in the hypoxic myocardial cell. In: HM Piper (ed). Pathophysiology of Severe Ischemic Myocardial Injury. Kluwer Academic Publishers, Dordrecht, 1990
98. Jennings RB, Reimer KA: The cell biology of acute myocardial ischaemia. Annu Rev Med 42: 225–246, 1991
99. Neely JR, Rovetto MJ, Whitmer JT, Morgan HE: Effects of ischemia on function and metabolism of the isolated working rat heart. Am J Physiol 225: 651–658, 1973

100. Neely JR, Whitmer JT, Rovetto MJ: Effect of coronary blood flow on glycolytic flux and intracellular pH in isolated rat hearts. Circ Res 37: 733–741, 1975
101. Chance B, Holmes W, Higgins J, Connelly CM: Localization of interaction sites in multicomponent transfer systems: theorems derived from analogues. Nature 182: 1190–1193, 1958
102. King LM, Boucher FR, Opie LH: Glucose uptake in the myocardium with changes in coronary flow and concentration – theoretical basis of positron emission tomography 'mismatch' concept (abstr). Eur Heart J 17 (Suppl): 16, 1996
103. Kohn MC, Garfinkel D: Computer simulation of entry into glycolysis and lactate output in the ischaemic rat heart. J Mol Cell Cardiol 10: 779–796, 1978
104. Stanley WC, Hall JL, Stone CK, Hacker TA: Acute myocardial ischemia causes a transmural gradient in glucose extraction but not glucose uptake. Am J Physiol 262: H91–H96, 1992
105. King LM, Opie LH: Does preconditioning act by glycogen depletion in the isolated rat heart? J Mol Cell Cardiol 28: 2305–2321,1996
106. Tillisch J, Marshall R, Schelbert H, Huang S-C, Phelps M: Reversibility of wall motion abnormalities; preoperative determination using positron tomography, ^{18}fluorodeoxyglucose and $^{13}NH_3$ (abstr). Circulation 68 (Suppl III): III-387, 1983
107. vom Dahl J, Eitzman DT, Al-Aouar ZR, Kanter HL, Hicks RJ, Deeb GM, Kirsh MM, Schwaiger M: Relation of regional function, perfusion, and metabolism in patients with advanced coronary artery disease undergoing surgical revascularization. Circulation 90: 2356–2366, 1994
108. Marshall RC, Tillisch JH, Phelps ME, Huang S-C, Carson R, Henze E, Schelbert HR: Identification and differentiation of resting myocardial ischemia and infarction in man with positron computed tomography, ^{18}F-labelled fluorodeoxyglucose and N-13 ammonia. Circulation 67: 766–778, 1983
109. Gerber BL, Vanoverschelde J-LJ, Bol A, Michel C, Labar D, Wijns W, Melin JA: Myocardial blood flow, glucose uptake, and recruitment of inotropic reserve in chronic left ventricular ischemic dysfunction. Implications for the pathophysiology of chronic myocardial hibernation. Circulation 94: 651–659, 1996
110. Janier MF, Vanoverschelde J-LJ, Bergmann SR: Ischemic preconditioning stimulates anaerobic glycolysis in the isolated rabbit heart. Am J Physiol 267: H1353–H1360, 1994
111. King LM, Opie LH: Low flow ischaemia abolishes the protective effect of preconditioning on stunning (abstr). J Mol Cell Cardiol 27: A264, 1995
112. Bolukoglu H, Goodwin GW, Guthrie PH, Carmical SG, Chen TM, Taegtmeyer H: Metabolic fate of glucose in reversible low-flow ischemia of the isolated working rat heart. Am J Physiol 270: H817–H826, 1996
113. Opie LH: Hypothesis: glycolytic rates control cell viability in ischemia. J Appl Cardiol 3: 407–414, 1988
114. Bricknell OL, Opie LH: Effects of substrates on tissue metabolic changes in the isolated rat heart during underperfusion and on release of lactate dehydrogenase and arrhythmias during reperfusion. Circ Res 43: 102–115, 1978
115. Paul RJ, Bauer M, Pease W: Vascular smooth muscle: Aerobic glycolysis linked to sodium and potassium transport processes. Science 206: 1414–1416, 1979
116. Xu KY, Zweier JL, Becker LC: Functional coupling between glycolysis and sarcoplasmic reticulum Ca^{2+} transport. Circ Res 77: 88–97, 1995
117. Jennings RB, Reimer KA, Steenbergen C, Schaper J: Total ischemia III: Effect of inhibition of anaerobic glycolysis. J Mol Cell Cardiol 21 (Suppl 1): 37–54, 1989
118. Poole-Wilson PA, Harding DP, Buordillon PDV, Tones MA: Calcium out of control. J Mol Cell Cardiol 16: 175–187, 1984
119. Jeremy RW, Koretsune Y, Marban E, Becker LC: Relation between glycolysis and calcium homeostasis in postischemic myocardium. Circ Res 70: 1180–1190, 1992
120. Bricknell OL, Daries PS, Opie LH: A relationship between adenosine triphosphate, glycolysis and ischaemic contracture in the isolated rat heart. J Mol Cell Cardiol 13: 941–945, 1981
121. Hearse DJ, Humphrey SM: Enzyme release during myocardial anoxia: A study of metabolic protection. J Mol Cell Cardiol 7: 463–482, 1975
122. Opie LH: Proposed role of calcium in reperfusion injury. Int J Cardiol 23: 159–164, 1989
123. Weiss JN, Lamp ST: Glycolysis preferentially inhibits ATP-sensitive K^+ channels in isolated guinea pig cardiac myocytes. Science 238: 67–70, 1987
124. Goudemant JF, Brodur G, Mottet I, Demeure R, Melin JA, Vanoverschelde JL: Inhibition of the Na^+-K^+-ATPase abolishes the protection afforded by glycolysis against ischemic injury (abstr). Circulation 92 (Suppl I): I-631, 1995
125. Higgins TJC, Bailey PJ, Allsop D: Interrelationship between cellular metabolic status and susceptibility of heart cells to attack by phospholipase. J Mol Cell Cardiol 14: 645–654, 1982
126. Lipasti JA, Nevalainen TJ, Alanen KA, Tolvanen M: Anaerobic glycolysis and the development of ischaemic contracture in isolated rat heart. Cardiovasc Res 18: 145–148, 1984
127. Eberli FR, Weinberg EO, Grice WN, Horowitz GL, Apstein CS: Protective effect of increased glycolytic substrate against systolic and diastolic dysfunction and increased coronary resistance from prolonged global underperfusion and reperfusion in isolated rabbit hearts perfused with erythrocyte suspensions. Circ Res 68: 466–481, 1991
128. Jeremy RW, Ambrosio G, Pike MM, Jacobus WE, Becker LC: The functional recovery of post-ischaemic myocardium requires glycolysis during early reperfusion. J Mol Cell Cardiol 25: 261–276, 1993
129. Vanoverschelde J-LJ, Janier MF, Bakke JE, Marshall DR, Bergmann SR: Rate of glycolysis during ischemia determines extent of ischemic injury and functional recovery after reperfusion. Am J Physiol 267: H1785–H1794, 1994
130. Hearse DJ, Humphrey SM, Garlick PB: Species variation in myocardial anoxic enzyme release, glucose protection and reoxygenation damage. J Mol Cell Cardiol 8: 329–339, 1976
131. de Leiris J, Opie LH, Lubbe WF: Effects of free fatty acid and enzyme release in experimental glucose on myocardial infarction. Nature 253: 746–747, 1975
132. Higgins TJC, Bailey PJ: The effects of cyanide and iodoacetate intoxication and ischaemia on enzyme release from the perfused rat heart. Biochim Biophys Acta 762: 67–75, 1983
133. Corr PB, Gross RW, Sobel BE: Amphipathic metabolites and membrane dysfunction in ischemic myocardium. Circ Res 55: 135–154, 1984
134. Lubbe WF, Holland RK, Gilchrist AI, Pybus J: Permissive action of potassium on arrhythmogenesis in the rat heart. J Mol Cell Cardiol 18 (Suppl 4): 37–41, 1986
135. Wilde AAM: Role of ATP-sensitive K^+ channel current in ischemic arrhythmias. Cardiovasc Drugs Ther 7: 521–526, 1993
136. Kakei M, Noma A, Shibasaki T: Properties of adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. J Physiol 363: 441–462, 1985
137. Kantor PF, Coetzee WA, Carmeliet EE, Dennis SC, Opie LH: Reduction of ischemic K^+ loss and arrhythmias in rat hearts. Effect of glibenclamide, a sulfonylurea. Circ Res 66: 478–485, 1990
138. Weiss JN, Venkatesh N: Metabolic regulation of cardiac ATP-sensitive K^+ channels. Cardiovasc Drugs Ther 7: 499–505, 1993
139. Han J, So I, Kim E-Y, Earm YE: ATP-sensitive potassium channels

are modulated by intracellular lactate in rabbit ventricular myocytes. Pflugers Archiv 425: 546–548, 1993
140. Opie LH: Modulation of ischemia by the regulation of the ATP-sensitive potassium channel. Cardiovasc Drugs Ther 7: 507–513, 1993
141. Weiss J, Hiltbrand B: Functional compartmentation of glycolytic versus oxidative metabolism in isolated rabbit heart. J Clin Invest 75: 436–447, 1985
142. Cross HR, Radda GK, Clarke K: The role of Na^+/K^+ ATPase activity during low flow ischemia in preventing myocardial injury: A ^{31}P, ^{23}Na and ^{87}RB NMR spectroscopic study. Magn Reson Med 34: 673–685, 1995
143. Entman ML, Kanike K, Goldstein MA, Nelson TE, Bornet EP, Futch TW, Schwartz A: Association of glycogenolysis with cardiac sarcoplasmic reticulum. J Biol Chem 251: 3140–3146, 1976
144. Krause SM, Jacobus WE, Becker LC: Alterations in cardiac sarcoplasmic reticulum calcium transport in the postischemic 'stunned' myocardium. Circ Res 65: 526–530, 1989
145. Limbruno U, Zucchi R, Ronca-Testoni S, Galbani P, Ronca G, Mariani M: Sarcoplasmic reticulum function in 'stunned' myocardium. J Mol Cell Cardiol 21: 1063–1072, 1989
146. Opie LH: Cellular mechanism for ischemic ventricular arrhythmias. Ann Rev Med 41: 231–238, 1990
147. Russell DC, Oliver MF: The effect of intravenous glucose on ventricular vulnerability following acute coronary artery occlusion in the dog. J Mol Cell Cardiol 11: 31–44, 1979
148. Opie LH, Tuschmidt R, Bricknell O, Girardier L: Role of glycolysis in maintenance of the action potential duration and contractile activity in isolated perfused rat heart. J Physiol Paris 76: 821–829, 1980
149. Carlsson L: A crucial role of ongoing anaerobic glycolysis in attenuating acute ischemia induced release of myocardial noradrenaline. J Mol Cell Cardiol 20: 247–253, 1988
150. Tosaki A, Hearse DJ: Protective effect of transient calcium reduction against reperfusion-induced arrhythmias in rat hearts. Am J Physiol 253: H225–H233, 1987
151. Opie LH, Coetzee WA, Dennis SC, Thandroyen FT: A potential role of calcium ions in early ischemic and reperfusion arrhythmias. Ann NY Acad Sci 522: 464–477, 1988
152. Lazdunski M, Frelin C, Vigne P: The sodium/hydrogen exchange system in cardiac cells: its biochemical and pharmacological properties and its role in regulating internal concentrations of sodium and internal pH. J Mol Cell Cardiol 17: 1029–1042, 1985
153. Hess ML, Manson NH: Molecular oxygen: friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. J Mol Cell Cardiol 16: 969–985, 1984
154. Yamada M, Hearse DJ, Curtis MJ: Reperfusion and readmission of oxygen. Pathophysiological relevance of oxygen-derived free radicals to arrhythmogenesis. Circ Res 67: 1211–1224, 1990
155. Oliver MF, Opie LH: Effects of glucose and fatty acids on myocardial ischaemia and arrhythmias. Lancet 343: 155–158, 1994
156. Woodley SL, Ikenouchi H, Barry WH: Lysophosphatidylcholine increases cytosolic calcium in ventricular myocytes by direct action on the sarcolemma. J Mol Cell Cardiol 23: 671–680, 1991
157. Huang JM-C, Xian H, Bacaner M: Long-chain fatty acids activate calcium channels in ventricular myocytes. Proc Natl Acad Sci USA 89: 6452–6456, 1992
158. Corr PB, Gross RW, Sobel BE: Arrhythmogenic amphiphilic lipids and the myocardial cell membrane. J Mol Cell Cardiol 14: 619–626, 1982
159. Vanoverschelde J-LJ, Janier MF, Bergman SR: The relative importance of myocardial energy metabolism compared with ischemic contracture in the determination of ischemic injury in perfused rabbit hearts. Circ Res 74: 817–828, 1994
160. Kolocassides KG, Galiñanes M, Hearse DJ: Preconditioning accelerates contracture and ATP depletion in blood-perfused rat hearts. Am J Physiol 269: H1415–H1420, 1995
161. Hearse DJ, Garlick PB, Humphrey SM: Ischemic contracture of the myocardium: Mechanisms and prevention. Am J Cardiol 39: 986–993, 1977
162. Kingsley PB, Sako KY, Yang MQ, Zimmer SD, Ugurbil K, Foker JE, From AHL: Ischemic contracture begins when anaerobic glycolysis stops: A ^{31}P-NMR study of isolated rat hearts. Am J Physiol 261: H469–H478, 1991
163. Annesley TM, Walker JB: Energy metabolism of skeletal muscle containing cyclocreatine phosphate. Delay in onset of rigor mortis and decreased glycogenolysis in response to ischaemia or epinephrine. J Biol Chem 255: 3924–3930, 1980
164. Lewandowski ED, Johnston DL, Roberts R: Effects of inosine on glycolysis and contracture during myocardial ischemia. Circ Res 69: 578–587, 1991
165. Lasley RD, Mentzer RM: Adenosine increases lactate release and delays onset of contracture during global low flow ischaemia. Cardiovasc Res 27: 96–101, 1993
166. Levy RM, Kushmerick MJ: Induction of contractile activity by rigor complexes in myofibrils. Biochem Biophys Res Commun 76: 512–519, 1977
167. Haworth RA, Nicolaus A, Goknur AB, Berkoff HA: Synchronous depletion of ATP in isolated adult rat heart cells. J Mol Cell Cardiol 20: 837–846, 1988
168. Bowers KC, Allshire AP, Cobbold PH: Bioluminescent measurement in single cardiomyocytes of sudden cytosolic ATP depletion coincident with rigor. J Mol Cell Cardiol 24: 213–218, 1992
169. Harper IS, van der Merwe E, Owen P, Opie LH: Ultrastructural correlates of ischaemic contracture during global subtotal ischaemia in the rat heart. J Exp Path 71: 257–268, 1990
170. Apstein CS: Diastolic dysfunction during ischemia: Role of glycolytic ATP generation. In: W Grossman, BH Lorell (eds). Diastolic Relaxation of the Heart. Kluwer Academic Publishers, Boston, 1994
171. Clarke B, Wyatt KM, May GR, McCormack JG: On the roles of long-chain acyl carnitine accumulation and impaired glucose utilization in ischaemic contracture development and tissue damage in guinea pig heart. J Mol Cell Cardiol 28: 171–181, 1996
172. Eberli FR, Ngoy S, Bernstein EA, Asptein CS: More evidence against myocyte calcium overload as the direct cause of ischemic diastolic dysfunction (abstr). Circulation 86 (Suppl I): I-480, 1992
173. Asimakis GK, Inners-McBride K, Conti VR: Improved postischemic mechanical function after ischemic preconditioning is not due to glycogen depletion (abstr). Circulation 86 (Suppl I): I-31, 1992
174. King LM, Opie LH: Is preconditioning linked to glycogen depletion? (abstr). J Mol Cell Cardiol 27: A149, 1995
175. Opie LH: Reperfusion injury – fad, fashion, or fact? Cardiovasc Drugs Ther 223–224, 1990
176. Sako KY, Kingsley-Hickman PB, From AHL, Ugurbil K, Foker JE: Substrate effects in the post-ischemic myocardium. J Surg Res 44: 430–435, 1988
177. Lopaschuk GD, Spafford MA, Davies NJ, Wall SR: Glucose and palmitate oxidation in isolated working rat hearts reperfused after a period of transient global ischemia. Circ Res 66: 546–553, 1990
178. Hernan de Villalobos D, Taegtmeyer H: Metabolic support for the postischaemic heart. Lancet 345: 1552–1555, 1995
179. Coretti MC, Koretsune Y, Kusuoka H, Chacko VP, Zweier JL, Marban E: Glycolytic inhibition and calcium overload as consequences of exogenously generated free radicals in rabbit hearts. J Clin Invest 88: 1014–1025, 1991
180. Renstrom B, Nells SH, Liedtke AJ: Metabolic oxidation of glucose during early myocardial reperfusion. Circ Res 65: 1094–1101, 1989

181. Lopaschuk GD, Wambolt RB, Barr RL: An imbalance between glycolysis and glucose oxidation is a possible explanation for the detrimental effects of high levels of fatty acids during aerobic reperfusion of ischemic hearts. J Pharmacol Exper Ther 264: 135–144, 1993
182. Merin RG: Myocardial 'stunning' and substrate metabolism. J Card Surg 9 (Suppl 1): 479–481, 1994
183. Cave AC, Silverman AS, Apstein CS: Preconditioning against contractile dysfunction is abolished in the presence of residual flow in the isolated blood perfused rat heart (abstr). J Mol Cell Cardiol 27: A145, 1995
184. Cave AC, Horowitz GL, Apstein CS: Can ischemic preconditioning protect against hypoxia-induced damage? Studies of contractile function in isolated perfused rat hearts. J Mol Cell Cardiol 26: 1471–1486, 1994
185. Speechly-Dick ME, Grover GJ, Yellon DM: Does ischemic preconditioning in the human involve protein kinase C and the ATP-dependent K^+ channel? Studies of contractile function after simulated ischemia in an atrial *in vitro* model. Circ Res 77: 1030–1035, 1995
186. Headrick JP: Ischemic preconditioning: bioenergetic and metabolic changes and the role of endogenous adenosine. J Mol Cell Cardiol 28: 1227–1240, 1996
187. Kupriyanov W, Lakomkin VL, Steinscheider AY, Severina MY, Kapelko VI, Ruuge EK, Saks VA: Relationship between pre-ischaemic ATP and glycogen content and post-ischaemic recovery of rat heart. J Mol Cell Cardiol 20: 1151–1162, 1988
188. Allard MF, Emanuel PG, Russell JA, Bishop SP, Digerness SB, Anderson PG: Preischemic glycogen reduction or glycolytic inhibition improves postischemic recovery of hypertrophied rat hearts. Am J Physiol 267: H66–H74, 1994
189. Wolfe CL, Sievers RE, Vissersen FLJ, Donnelly T: Loss of myocardial protection after preconditioning correlates with the time course of glycogen recovery within the preconditioned segment. Circulation 87: 881–892, 1993
190. Schaefer S, Carr LJ, Prussel E, Ramasamy R: Effects of glycogen depletion on ischemic injury in isolated rat hearts: Insights into preconditioning. Am J Physiol 268: H935–H944, 1995
191. Garlick PB, Radda GK, Seeley PJ: Studies of acidosis in the ischaemic heart by phosphorus nuclear magnetic resonance. Biochem J 184: 547–554, 1979
192. Tani M, Neely JR: Role of intracellular Na^+ in Ca^{2+} overload and depressed recovery of ventricular function of reperfused ischemic rat hearts. Possible involvement of H^+-Na^+ and Na^+-Ca^{2+} exchange. Circ Res 65: 1045–1056, 1989
193. de Albuquerque CP, Gerstenblith G, Weiss RG: Importance of metabolic inhibition and cellular pH in mediating preconditioning contractile and metabolic effects in rat hearts. Circ Res 74: 139–150, 1994
194. Cross HR, Clarke K, Opie LH, Radda GK: Is lactate-induced myocardial ischaemic injury mediated by decreased pH or increased intracellular lactate? J Mol Cell Cardiol 27: 1369–1381, 1995
195. Karmazyn M: Na^+/H^+ exchange inhibitors reverse lactate-induced depression in postischemic ventricular recovery. Cardiovasc Res 108: 50–56, 1993
196. Keung EC, Li Q: Lactate activates ATP-sensitive potassium channels in guinea pig ventricular myocytes. J Clin Invest 88: 1772–1777, 1991
197. Geisbuhler TP, Rovetto MJ: Lactate does not enhance anoxia/reoxygenation damage in adult rat cardiac myocytes. J Mol Cell Cardiol 22: 1325–1335, 1990
198. Lagerstrom CF, Walker WE, Taegtmeyer H: Failure of glycogen depletion to improve left ventricular function of the rabbit heart after hypothermic ischemic arrest. Circ Res 63: 81–86, 1988
199. Goodwin GW, Taegtmeyer H: Metabolic recovery of isolated working rat heart after brief global ischemia. Am J Physiol 267: H462–H470, 1994
200. Kusuoka H, Marban E: Mechanism of the diastolic dysfunction induced by glycolytic inhibition. Does adenosine triphosphate derived from glycolysis play a favoured role in cellular Ca^{2+} homeostasis in ferret myocardium? J Clin Invest 93: 1216–1223, 1994
201. Jeffrey FMH, Storey CJ, Malloy CR: Predicting functional recovery from ischaemia. Basic Res Cardiol 87: 548–558, 1992
202. Schaefer S, Prussel E, Carr LJ: Requirement of glycolytic substrate for metabolic recovery during moderate low flow ischemia. J Mol Cell Cardiol 27: 2167–2176, 1995
203. Oldfield GS, Commerford PJ, Opie LH: Effects of preoperative glucose-insulin-potassium on myocardial glycogen levels and on complications of mitral valve replacement. J Thorac Cardiovasc Surg 91: 874–878, 1986
204. McElroy DD, Walker WE, Taegtmeyer H: Glycogen loading improves left ventricular function of the rabbit heart after hypothermic ischemic arrest. J Appl Cardiol 4: 455–465, 1989
205. Vanoverschelde JL, Janier MF, Bakke JE, Bergmann SR: Increasing myocardial glycogen levels prior to ischemia limits ischemic damage and improves functional recovery following reperfusion (abstr). Circulation 84 (Suppl II): II-216, 1991
206. Doenst T, Guthrie PH, Chemnitius J-M, Zech R, Taegtmeyer H: Fasting, lactate, and insulin improve ischemia tolerance in rat heart: A comparison with ischemic preconditioning. Am J Physiol 270: H1607–H1615, 1996
207. Cross HR, Opie LH, Radda GK, Clarke K: Is a high glycogen content beneficial or detrimental to the ischemic rat heart? A controversy resolved. Circ Res 8: 482–491, 1996
208. Shizukuda Y, Mallet RT, Lee SC, Downey HF: Hypoxic preconditioning of ischaemic canine myocardium. Cardiovasc Res 26: 534–542, 1992
209. Zhai X, Lawson CS, Cave AC, Hearse DJ: Preconditioning and post-ischaemic contractile dysfunction: The role of impaired oxygen delivery vs extracellular metabolise accumulation. J Mol Cell Cardiol 25: 847–857, 1993
210. Lasley RD, Anderson GM, Mentzer RM: Ischaemic and hypoxic preconditioning enhance postischaemic recovery of function in the rat heart. Cardiovasc Res 27: 565–570, 1993
211. Cohen MV, Walsh RS, Goto M, Downey JM: Hypoxia preconditions rabbit myocardium via adenosine and catecholamine release. J Mol Cell Cardiol 27: 1527–1534, 1995
212. Geisbuhler T, Altschuld RA, Trewyn RW, Ansel AZ, Lamka K, Breirley GP: Adenine nucleotide metabolism and compartmentalization in isolated adult rat heart cells. Circ Res 54: 536–546, 1984
213. Ottaway JH, Mowbray J: The role of compartmentation in the control of glycolysis. Curr Topics Cell Regul 107–208, 1977
214. Aw TY, Jones DP: ATP concentration gradients in cytosol of liver cells during hypoxia. Am J Physiol 249: C385–C392, 1985
215. Paul RJ: Functional compartmentalization of oxidative and glycolytic metabolism in vascular smooth muscle. Am J Physiol 244: C399–C409, 1983
216. Rangachari PK, Paton DM, Daniel EE: Aerobic and glycolytic support of sodium pumping and contraction in rat myometrium. Am J Physiol 223: 1009–1015, 1972
217. Lynch RM, Paul RJ: Compartmentation of glycolytic and glycogenolytic metabolism in vascular smooth muscle. Science 222: 1344–1346, 1983
218. Lynch RM, Paul RJ: Compartmentation of carbohydrate metabolism in vascular smooth muscle. Am J Physiol 252: C328–C334, 1987
219. Dennis SC, Hearse DJ, Coltart DJ: Metabolic effects of substrates on the isolated guinea-pig heart in relation to arrhythmias during

reperfusion. Cardiovasc Res 16: 209–219, 1982
220. Navaratnam V: Heart Muscle: Ultrastructural Studies. Cambridge University Press, Cambridge, 1987
221. Doorey AJ, Barry WH: The effects of inhibition of oxidative phosphorylation and glycolysis on contractility and high-energy phosphate content in cultured chick heart cells. Circ Res 53: 192–201, 1983
222. Kammermeier H: Why do cells need phosphocreatine and a phosphocreatine shuttle? J Mol Cell Cardiol 19: 115–118, 1987
223. Meyer RA, Sweeney HL, Kushmerick M: A simple analysis of the 'phosphocreatine shuttle'. Am J Physiol 246: C365–C377, 1984
224. Gudbjarnason S, Mathes P, Ravens KG: Functional compartmentation of ATP and creatine phosphate in heart muscle. J Mol Cell Cardiol 1: 325–330, 1970
225. Korge P, Campbell KB: Local ATP regeneration is important for sarcoplasmic reticulum Ca^{2+} pump function. Am J Physiol 267: C357–C366, 1994
226. Ventura-Clapier R, Veksler V: Myocardial ischemic contracture. Metabolites affect rigor tension development and stiffness. Circ Res 74: 920–929, 1994
227. Rouslin W, Erickson JL, Solaro RJ: Effects of oligomycin and acidosis on rates of ATP depletion in ischemic heart muscle. Am J Physiol 250: H503–H508, 1986
228. Rouslin W, Broge CW, Grupp IL: ATP depletion and mitochondrial function loss during ischemia in slow and fast heart-rate groups. Am J Physiol 259: H1759–H1766, 1990
229. Pierce GN, Philipson KD: Binding of glycolytic enzymes to cardiac sarcolemnal and sarcoplasmic reticular membranes. J Biol Chem 260: 6862–6870, 1985
230. Paul RJ, Hardin CD, Raeymaekers L, Wuytack F, Casteels R: Preferential support of Ca^{2+} uptake in smooth muscle plasma membrane vesicles by an endogenous glycolytic cascade. FASEB J 3: 2298–2301, 1989
231. Opie LH, Bruyneel K, Owen P: Effects of glucose, insulin and potassium infusion on tissue metabolic changes within first hour of myocardial infarction in the baboon. Circulation 52: 49–57, 1975
232. Lazar HL: Enhanced preservation of acutely ischemic myocardium using glucose-insulin-potassium solutions. J Card Surg 9 (Suppl): 474–478, 1994
233. Shangraw RE: Glucose-insulin-potassium regimes during cardiac surgery. Anaesth Anal 77: 864–873, 1993
234. Fath-Ordoubadi P, Kaprelian R, Beatt KJ: Glucose-insulin-potassium therapy for treatment of acute myocardial infarction: is it useful or should it be discarded? (abstr). Europ Heart J 16 (Suppl): 443, 1995
235. Taegtmeyer H: Carbohydrate interconversions and energy production. Circulation 72 (Suppl IV): IV1–IV8, 1985