Cardiac Metabolism

Lead Article

Expert Answers to Three Key Questions

Summaries of Ten Seminal Papers - *P.B. Garlick*　　　99

Bibliography of One Hundred Key Papers　　　111

Metabolic perturbations in ischemic heart disease

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The major metabolic substrates of the normal well-oxygenated myocardium are free fatty acids in the fasted state and glucose in the fed state. In general, the normal myocardium uses whichever fuel is available.

During ischemia, there is a swing toward glucose metabolism and it is proposed that glycolysis provides beneficial glycolytic ATP which has many protective actions, including preservation of sodium pump activity.

Hypothetically, when sodium pump activity stops, cytosolic calcium increases and ischemic contracture, often an irreversible event, occurs. The rise in internal sodium may alter sodium/calcium exchange, thus precipitating contracture.

In the postischemic myocardium, glycolysis is again essential, but there is increasing evidence that citric acid cycle intermediates need to be replenished by anaplerosis.

In diabetic patients, glucose-insulin-potassium (GIK) infusions, followed by subcutaneous insulin, have been shown to reduce mortality over the year following the onset of acute myocardial infarction.

Myocardial ischemia is characterized by impeded blood flow, hence lack of oxygen. The direct consequence of this is a deficit in high-energy phosphate compounds and changes in glycolysis and internal pH. This explains why myocardial ischemia is essentially a metabolic event. We thus need to look at the basic metabolic defects which arise during myocardial ischemia and see how these can be best prevented or aborted.

METABOLISM OF THE NORMAL HUMAN HEART

Metabolic substrates

In man, the major substrates of normal heart metabolism are carbohydrates and lipids. In the fasted state, the blood levels of free fatty acids (FFAs) are high. Rates of uptake of fatty acids are also high during fasting and inhibit the oxidation of glucose by the heart; fatty acids then become the major source of energy. When fatty acids are the predominant fuel, any glucose taken up is increasingly converted to glycogen by the glucose-sparing effect of fatty acid oxidation.

The effect of feeding on the metabolism of the human heart is to shift the metabolism from reliance on fatty acids to reliance on carbohydrates. Experimentally, insulin may be infused while keeping the blood glucose concentration steady, or both glucose and insulin may be infused to provide excess of each. All these data are consonant with the concept that carbohydrates, and particularly glucose, are the major fuels for the heart in the fed state (when both glucose and insulin are available).

During exercise, blood levels of lactate are high and this is taken up by the well-oxygenated myocardium. Triglycerides, not normally an important fuel, can rise

after a high-fat meal of cream and cheese, and can contribute to myocardial oxidative metabolism.

Ketone bodies and amino acids are not normally major components of myocardial oxidative metabolism.

Energy yield

The highest yield of ATP per molecule is from fatty acids such as palmitate. This is because many of the carbon atoms in carbohydrates are partially oxidized due to the presence of the oxygen in the molecule, whereas fatty acid molecules contain little oxygen and, therefore, can yield more ATP for each carbon atom. The disadvantage of fatty acids as a fuel is that for each molecule of ATP produced, they require relatively more oxygen. Experimentally, a heart using fatty acids alone would need about 17% more oxygen than when using glucose alone in order to produce the same amount of ATP.

The molecular explanation for the relatively poor ATP yield of fatty acids per oxygen molecule taken up is that each loop of the fatty acid spiral yields equal amounts of $FADH_2$ and $NADH_2$. $FADH_2$ enters the respiratory chain further along than $NADH_2$, and yields less ATP. In recent years, it has been shown that the energy yield per unit of oxygen uptake (phosphorylation/oxidation or P/O ratio) was lower than previously thought, and therefore that less energy is produced per molecule of substrate. Using glucose as an example, 31 ATP units are produced during full oxidation instead of the previously determined value of 38, and palmitate produces 105 instead of the previously determined 130 ATP units per molecule.

METABOLISM OF THE ISCHEMIC HEART

In myocardial ischemia, myocardial cells are suffering from a lack of oxygen, caused by inadequate coronary blood flow. Ischemia may be temporary and reversible, or permanent and irreversible, leading to myocardial infarction. On the other hand, ischemia may also lead to postischemic stunning, hibernation, and preconditioning. These three entities may be called *"new ischemic syndromes."* Coronary artery disease, generally obstructive in nature, is the usual cause of ischemia. Additional or other causes include: (i) coronary artery spasm or functional "dynamic" obstruction added to organic obstruction; and (ii) increased myocardial oxygen demand resulting from myocardial hypertrophy.
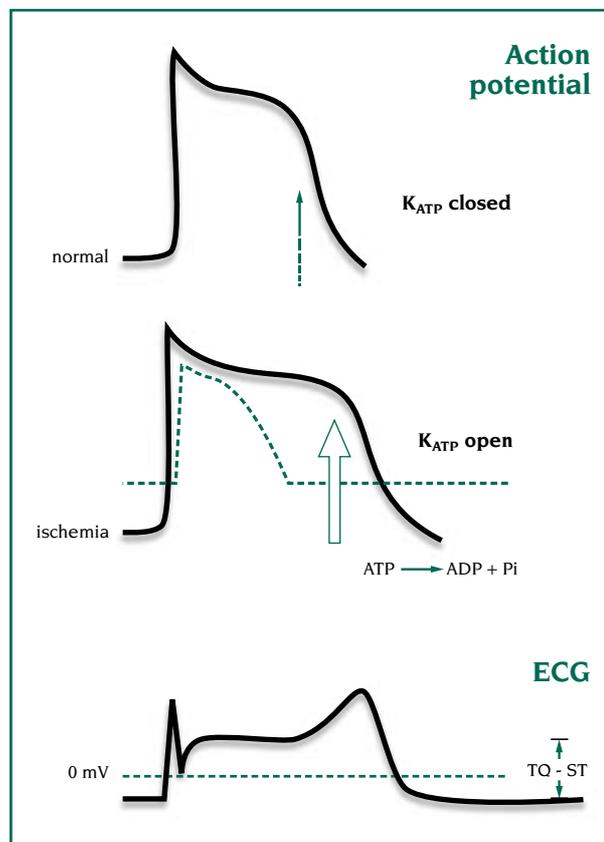
Figure 1. *Changes in the ST segment of the surface electrocardiogram reflect an increase in extracellular potassium in the ischemic zone.*

Mitochondrial O_2 deficit

Central to ischemia is the lack of an adequate oxygen supply to the mitochondria (anaerobiosis), with a consequent fall in the energy available to the cytoplasm. The breakdown of high-energy phosphate compounds accelerates glycolysis and glycogenolysis, so that glycolytic flux is stimulated to a greater extent than its end products, pyruvate and $NADH_2$, can enter the mitochondria for oxidation. The further conversion of pyruvate and $NADH_2$ to lactate explains the production of lactate by the ischemic myocardium. Direct monitoring of enhanced glycolysis in the human ischemic myocardium can be achieved. The increased extraction of labelled glucose ([18F]fluorodeoxyglucose) can be visualized noninvasively by positron emission tomography (PET).

Protons versus ATP

The classic effect of the poor washout of metabolites caused by severe ischemia is the accumulation of protons. The latter are derived not from glycolysis itself, but from the ATP breakdown associated with

anaerobic glycolysis, and also from a variety of metabolic cycles which are proton-producing.[1]
In addition, accumulation of lactate, which is the end product of anaerobic glycolysis, and of CO_2 produced by residual aerobic respiration, may both exert detrimental effects. Hence, the metabolic damage induced by poor washout needs to be balanced against the benefits of increased production of anaerobic ATP.

As coronary flow decreases, there is a bimodal effect on glucose uptake. A modest fall in coronary flow (mild ischemia) increases glucose uptake, and a more major fall (severe ischemia) decreases uptake. Therefore, as the coronary flow rate progressively falls, there will be a critical flow level at which increased glucose uptake switches to decreased uptake. This change is not, however, due to inhibition of accumulated products of glycolysis as previously supposed,[2] but rather to decreased rates of delivery of glucose.[3] This is confirmed by the finding that increased glucose extraction by ischemic tissue reflects reversible ischemia in man, as shown by the use of positron emission tomography with [[18F]fluorodeoxyglucose.

Potassium ions in ischemia

The onset of ischemia is associated with the very rapid loss of potassium ions to the cell exterior, and this loss can be recorded right from onset by a variety of techniques. It is this change, possibly mediated by the ATP-sensitive potassium channel, that is monitored by the electrocardiogram (ECG). Therefore, whenever there are ischemic ST-segment changes on the ECG, whether caused by silent or symptomatic ischemia, there is a potassium loss which reflects inadequate ATP levels or inadequate ATP production. The clinician is, therefore, able to monitor myocardial ischemia accurately *(Figure 1).* For example, in early-phase acute myocardial infarction, an indication for thrombolytic therapy is ST-segment elevation in adjacent precordial leads, reflecting ongoing ischemia (active potassium loss) and potential reversibility. Thus, it is not surprising, given these simple first principles, that a decrease of ST-segment elevation (ST-segment elevation-resolution) is a powerful predictor of the benefit of thrombolysis.[4]

Metabolic aspects of viability of ischemic myocytes

The "Cape Town hypothesis"[5] proposed that an increase in glucose uptake reflects continuing cell viability. In contrast, decreased uptake is associated with loss of viability of the ischemic cells, with damage progressing from reversible to irreversible. On the basis of this hypothesis, cells threatened by ischemia could, according to their
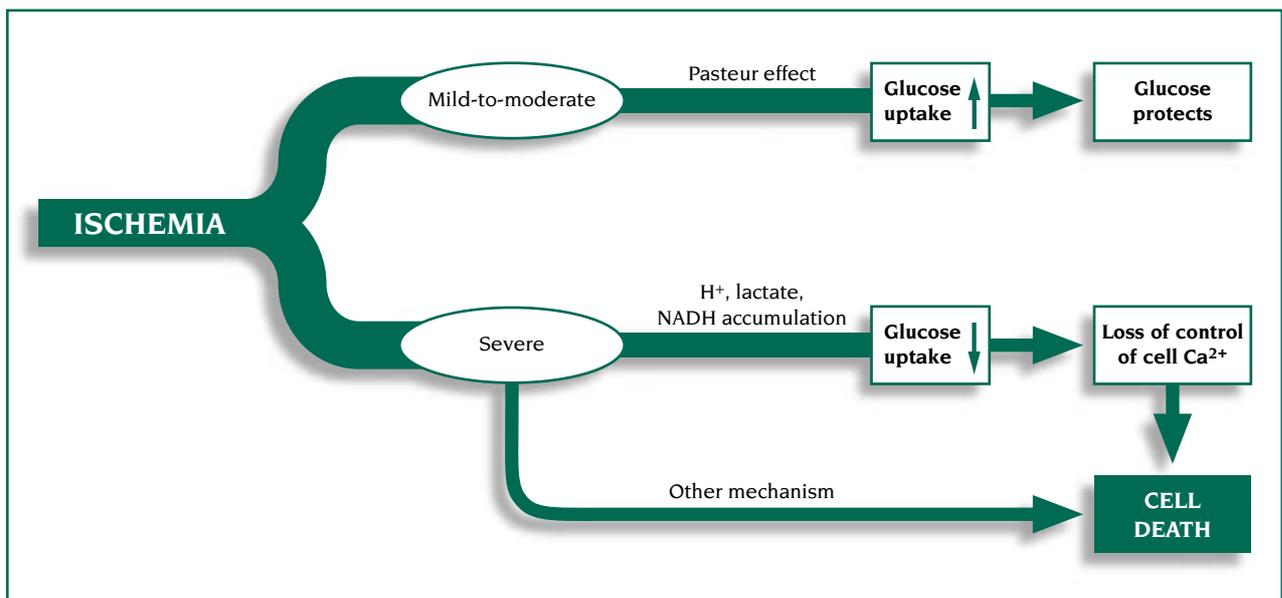
Figure 2. *Hypothesis relating the rate of glycolysis to cell death. During mild-to-moderate ischemia, glucose uptake is increased, providing the benefit of increased glycolytic ATP. During severe ischemia, the rate of glucose delivery becomes limiting.[3] In addition, the accumulation of protons, lactate, and NADH inhibits glycolysis and glucose uptake. Consequently, there is a loss of control of intracellular calcium, resulting in ischemic contracture. Figure © L.H. Opie.*

patterns of glucose uptake, be divided into those with increased values (viable) or decreased values (nonviable). It follows that: (i) any benefits of enhanced glycolysis are likely to be limited to zones of moderate or mild flow restriction where glycolysis is not inhibited *(Figure 2)*; whereas (ii) in zones of severe ischemia, coronary flow would first have to be improved by coronary vasodilation or thrombolysis to achieve the desired effect of increased glycolytic flux.

Contracture as an end point of ischemia

It has been proposed that the rate of production of glycolytic ATP is of paramount importance in the prevention of ischemic contracture.[3,6] Owen et al[7] and Cross et al[6] have shown that the distinguishing feature of hearts in which ischemic contracture did not develop was the production of ATP from glucose at rates of about 2.0 μmol ATP/g/min or higher. When glycolysis ceases or falls below this rate, contracture occurs. Glycolytic ATP is more effective in the prevention of contracture than a similar level of ATP produced by residual oxidative metabolism.

It must, however, be acknowledged that this hypothesis is based on the assumption that it is the rise of cytosolic calcium rather than a fall in ATP that triggers ischemic contracture, an assumption that is still controversial.[8] The confounding effects of accumulation of protons and inorganic phosphate cannot be ignored. For example, within the first minutes after the onset of total global ischemia, cytosolic calcium rises but there is no ischemic contracture, presumably because of the accompanying increase in protons and inorganic phosphate.[9]

Furthermore, accumulation of lipid metabolites in ischemia also determines ischemic contracture.[10] Thus, long-chain acylcarnitine increases ischemic contracture, possibly by an elevation of internal calcium.

Effects of glycolysis

The specific evidence favoring the view that glycolysis lessens ischemic injury is as follows: (i) the rate of glycolysis in the ischemic cell may govern the activity of the sodium pump[11]; furthermore, in isolated myocytes, the increase in rate of glycolysis is more effective than the rate of oxidative phosphorylation in preventing ATP-sensitive K^+ channels from opening; (ii) increasing rates of glycolysis, via raised external glucose concentrations, decrease enzyme release from the ischemic coronary-ligated isolated heart; (iii) glycolysis can help prevent increased resting tension and development of contracture in hypoxic ventricular strips; (iv) enhanced glycolytic flux helps maintain mitochondrial function during ischemia and reperfusion; (v) an increased glycolytic flux achieved by glucose and insulin and proven by enhanced lactate output in the underperfused rabbit heart can prevent ischemic contracture and improve reperfusion function[12]; (vi) glycolytic flux improves

PRINCIPLE	CLASS OF AGENT	SPECIFIC EXAMPLE	CLINICAL STATUS
Ca^{2+} control	Na^+/H^+ inhibitor	Hoe 694	Phase II-III trials, unstable angina
Na^+ pump active	Glycolytic flux	GIK (glucose-insulin-potassium)	Needs megatrial
Cytoprotection	Free radical scavenger	N-acetylcysteine Fructose-1,6-diphosphate Trimetazidine	Safe; may work[24] Safe; may work[30] Multicenter trial[25]
Lipid control	Acyl-CoA transporter	Carnitine, Propionylcarnitine	Post myocardial infarction

Table I. *Some novel approaches to metabolic management of myocardial ischemia.*

the synthesis of membrane phospholipids[13]; and (vii) glucose diminishes contracture induced by long-chain acylcarnitine.[10]

Ischemia and lipid metabolism

In the aerobic myocardium, rates of glycolysis are closely linked with rates of oxidation of fatty acids by the aerobic myocardium. Considering first the aerobic myocardium in situ, the glucose-fatty acid Randle cycle postulates that provision of glucose should decrease delivery of FFAs to the heart because, in vivo, the hyperinsulinemia induced by glucose ingestion inhibits the release of FFAs from adipose tissue. Similar relations appear to exist for glucose and FFA blood levels in patients with acute myocardial infarction. In animals, increased glucose uptake and high circulating levels of insulin help decrease circulating levels of FFAs and their uptake by the myocardium, thus decreasing the extent of necrosis in the ischemic myocardium.

There is substantial evidence that increased rates of delivery of FFAs are potentially harmful to the ischemic myocardium.[14] FFAs have "oxygen-wasting" potential in the aerobic, ischemic, or reperfused myocardium, and provision of glucose rather than FFAs promotes recovery in the postischemic reperfusion period.[15] The latter data may explain why in patients with acute anterior myocardial infarction who are given streptokinase, glucose-insulin-potassium (GIK) improves ventricular function and reduces the segmental wall abnormality.[16] Increased glucose uptake by the myocardium could therefore be achieved by decreasing circulating FFAs, for example, by administration of glucose-insulin, glucose ingestion, carnitine, nicotinic acid, or beta blockade. As the myocardial uptake of FFAs falls, there are substantial beneficial metabolic changes in the epicardial infarct zone and in the peri-infarct zone. Besides decreasing extraction of FFAs, beta blockade may also maintain a more favorable tissue pH in the ischemic zone. The latter effect is probably secondary to the anti-ischemic benefits of reduced heart rate and work induced by beta blockade.

In a variety of circumstances, agents promoting glycolytic flux decrease myocardial ischemic damage, whereas agents promoting lipolysis (fatty acid metabolism) increase damage. Provision of glycolytic flux to a partially ischemic cell decreases the fatty acid-mediated damange and "protects" the sarcolemma from lipid-associated damage.[13]

A different approach is to use agents which are thought to modify the intracellular metabolism of fatty acid intermediates. For example, administration of carnitine may cause ischemic myocardial cells to lose acyl-CoA. The compound propionylcarnitine may have an antianginal effect via enhanced transport of acetyl-CoA to the mitochondria. Oxfenicine stimulates pyruvate dehydrogenase, probably by inhibiting fatty acid oxidation. Conversely, inhibition of long-chain acyl-CoA transport into mitochondria by etomoxir[17] seems to exert a beneficial effect not only by directly inhibiting fatty acid oxidation, but also indirectly by increasing the rate of glycolysis. Similar mechanisms operate in the diabetic heart.[18] The latter findings with etomoxir therefore lend support to the existence of a glucose-fatty acid interaction in the ischemic zone.

METABOLIC MANAGEMENT OF THE ISCHEMIC HEART

In this thrombolytic era, the major aim in early-phase acute myocardial infarction is rapid reperfusion, usually with streptokinase or rtPA, or, in specialized centers, by acute angioplasty. Reperfusion is known to be followed by stunning, a condition in which mechanical function is depressed despite adequate restoration of coronary blood flow.

Reperfusion and promotion of glycolysis

Our data suggest that promotion of glucose flux and glycolysis is an important aim, best achieved by ensuring: (i) a high rate of delivery of glucose and insulin to the ischemic cells; and (ii) removal of end products of glycolysis both by maximizing the coronary flow rate and by metabolic procedures. There is already considerable evidence that increased provision of external glucose can diminish ischemic injury. As shown by King et al,[3] it is the delivery of glucose rather than inhibition of glycolysis that is rate-limiting. Hence the benefits of reperfusion include promotion of glycolysis.

Glucose-insulin-potassium (GIK) therapy in early-phase myocardial infarction

In several trials, the dose of GIK has been shown to be too low and its timing too late. Logically, GIK should be most effective in selected

patients, especially those with impaired glucose tolerance, as shown by a high plasma glucose concentration, exceeding 7.5 mmol/L, on admission. The use of GIK in diabetic patients is important: in diabetics in the acute phase, GIK, followed by subcutaneous insulin for 1 year reduces mortality.[19] GIK is effective in patients with depressed left ventricular function following cardiopulmonary bypass.

Enhancement of glycolysis in myocardial stunning

Stunning has clinical relevance,[20] and, of the many possible experimental approaches to the management of stunning, this author prefers the concept that regulation of cytosolic calcium is paramount. Of specific interest is the view that enhanced glycolysis in the postischemic period promotes mechanical recovery, acting at least in part through better control of the elevated cytosolic calcium levels.[21] These concepts appear to have clinical relevance as shown by the benefits of GIK infusion in patients undergoing thrombolysis with streptokinase.[18]

Replenishment of citric acid cycle intermediates

In addition to enhancing glycolysis, another approach is to replenish the depleted citric acid cycle intermediates by promotion of anaplerosis.[22] Glutamate infusion may serve this purpose. Glutamate enters directly into the citric acid cycle by interacting with alanine and producing 2-oxoglutarate, and it also stimulates insulin secretion.[23]

Free radical inhibition

Although there is extensive experimental evidence for reperfusion injury, and free radicals appear to play a role in this phenomenon, no radical scavenger has yet been able to decrease such injury in patients. However, two findings seem to point in this direction: first, N-acetylcysteine, when given to patients undergoing clinical thrombolysis, diminishes the products of oxidative stress[24]; and second, there is now a major ongoing European trial, the EMIP trial,[25] in patients with acute myocardial infarction and undergoing thrombolysis (EMIP-FR Pilot Study Group, 1993). The agent under investigation is trimetazidine and the hypothesis is that it decreases free radical production on reperfusion. However, because it also decreases ischemic contracture,[26] a successful outcome in the EMIP study would not necessarily prove that free radicals are the cause of reperfusion injury *(see Table I, page 78).*

Inhibition of sodium/calcium exchange

One of the most consistent results, confirmed by many experimental laboratories, is that inhibition of sodium/proton exchange, and indirectly of sodium/calcium exchange, diminishes reperfusion stunning and arrhythmias. Particularly consistent data have been obtained with the specific inhibitor, Hoe 694, which produces beneficial effects, whether given only at the time of reperfusion or before the onset of ischemia.[27,28]

Long-term metabolic support

A closely related concept is that ischemia induces carnitine depletion in the myocardium. Carnitine is a physiological compound that is required for the transport of activated long-chain fatty acids into the mitochondria. In a double-blind trial, carnitine was started within 24 hours of chest pain in selected patients with acute myocardial infarction. This resulted in smaller left ventricular size 12 months later, ie, decreased remodeling.[29]

In order to gain further insight into the relevance of cardiac metabolism to myocardial ischemia, this issue of *Dialogues* addresses three topics: Paolo Camici details the different methods of **measuring cardiac metabolism in man** as well as the main findings in the normal heart and in myocardial ischemia; Carlo Guarnieri answers the question of **how to manipulate cardiac mitochondrial metabolism;** and Gary Lopaschuck discusses the ways of **manipulating fatty acid and carbohydrate metabolism.**

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Cardiac Metabolism

Expert Answers to Three Key Questions

—— ① ——

How should myocardial metabolism be measured in man?

P.G. Camici

—— ② ——

How can cardiac mitochondrial metabolism be manipulated?

C. Guarnieri

—— ③ ——

How can fatty acid and carbohydrate metabolism be manipulated?

G.D. Lopaschuk

How should myocardial metabolism be measured in man?

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Many variables, including food ingestion, circulating hormones, and cardiac workload, affect myocardial metabolism. Important changes in myocardial metabolism are associated with myocardial ischemia. The study of myocardial metabolism by means of different invasive and noninvasive techniques allows a better understanding of both cardiac physiology and pathophysiology in humans.

Any changes in cardiac function are associated with parallel changes in cardiac metabolism. Measuring such changes has important therapeutic implications in myocardial ischemia. This paper reviews the techniques available for measuring cardiac metabolism as well as the main findings in the normal and ischemic heart.

TECHNIQUES FOR STUDYING MYOCARDIAL METABOLISM IN MAN

Invasive procedures

In 1947, Bing[1] started to use coronary sinus catheterization for the study of myocardial metabolism in humans. This procedure implies the combined catheterization of the coronary sinus (CS) and an artery (A) with measurement of substrate concentrations in simultaneously drawn blood samples. From these, the extraction fraction [defined as (A-CS)/A] can be calculated, which reflects the ability of the heart to extract a substrate independently of its arterial level. Conversely, if the required information is the rate of absolute substrate uptake, then an estimate of coronary blood flow (CBF) is necessary, which is generally achieved by the thermodilution technique. This allows the use of the same catheter for blood sampling, CBF measurement, and if needed, for electrical pacing.[2,3] If CBF is known, net myocardial substrate balance can be calculated as: (A-CS) × CBF.[4] If there is simultaneous uptake and release of a substrate, the A-CS difference will be the algebraic sum of the two processes. To obtain absolute rates of substrate uptake or release, the catheter technique must be combined with the use of labelled substrates.[5] Net rates of carbohydrate and lipid oxidation and myocardial energy expenditure can be calculated from classic calorimetric equations if myocardial exchange of oxygen and carbon dioxide is measured.[6]

Positron emission tomography

Positron emission tomography (PET) is a radionuclide imaging technique which enables quantitative assessment of regional myocardial tissue function in vivo.[7] Using the appropriate tracers, labelled with positron emitting isotopes, a variety of functional parameters can be investigated. Because of the availability of positron emitting isotopes of elements which are commonly found in molecules of biological interest (eg, carbon 11, oxygen 15, and nitrogen 13), such compounds may be labelled without alteration of either their chemical structure or biological activity.

PET imaging has been used to probe a variety of cardiac biochemical pathways. Studies have been performed using labelled amino

acids to measure amino acid metabolism and protein turnover rates.[8] However, the majority of PET metabolic studies have focused upon investigation of the pathways involved in energy metabolism and the alterations which occur in disease.

The carbon 11-labelled free fatty acid palmitate ([^{11}C]palmitate) has been proposed as a tracer to probe beta oxidation. The clearance of [^{11}C]palmitate from the myocardium was found to be related to the degree of oxidative metabolism, though absolute quantification of utilization rates was not possible due to the over-complexity of the model required to explain the behavior of [^{11}C]palmitate in tissue. Interpretation of myocardial uptake and clearance of [^{11}C]palmitate is further complicated by the dependence of these two parameters on the prevailing blood flow and dietary state. In ischemic tissue the clearance rates were found to be reduced, suggesting reduced free fatty acid utilization in these regions.[8,9]

Carbon 11-labelled acetate ([^{11}C]acetate) has been advocated as a tracer of tricarboxylic acid cycle activity[10] and has been used as an indirect marker of myocardial oxygen consumption (MVO_2) by PET in both experimental animals[10-13] and humans.[14] A number of studies have shown that the rate constant describing the clearance of [^{11}C]acetate from the myocardium correlates well with catheter measurements of oxygen extraction fraction (OEF) from analysis of arteriovenous differences of blood oxygen content using the Fick principle. Clinical studies using [^{11}C]acetate have demonstrated a decreased clearance rate from infarcted myocardium.[15]

A new method to quantify MVO_2 by inhalation of oxygen 15-labelled molecular oxygen gas ($^{15}O_2$) has

been developed recently.[16] The accuracy of this approach to quantify oxygen extraction fraction (OEF) and MVO_2 has been successfully validated over a wide range of values in experimental animal studies.[17] Studies in six human subjects yielded mean OEF and MVO_2 values of 61±8% and 9.4±1.8 mL/min/100 g, respectively,[16] which are consistent with those values previously reported in man using invasive techniques.

Extensive studies of glucose metabolism have been performed using PET, principally using [^{18}F]-2-fluoro-2-deoxyglucose (FDG). This tracer is transported into the myocyte on the same transsarcolemmal carrier as glucose and is then phosphorylated to FDG-6-phosphate by the enzyme hexokinase. This is essentially a unidirectional reaction, as no glucose-6-phosphatase has yet been identified in cardiac muscle,[18] and results in FDG-6-phosphate accumulation within the myocardium. Thus, although measurement of the myocardial uptake of FDG is proportional to the overall rate of transsarcolemmal transport and hexokinase phosphorylation of circulating glucose by heart muscle, no information about the further intracellular disposal of glucose can be derived from measurements of FDG uptake.

A number of kinetic modelling approaches have been used for the quantification of glucose utilization rates using FDG.[19] The major limitation of these approaches is that quantification of glucose metabolism requires the knowledge of the lumped constant, a factor which relates the kinetic behavior of the FDG to naturally occurring glucose in terms of the relative affinity of each molecule for the transsarcolemmal transporter and for hexokinase. Unfortunately,

the value of the lumped constant in humans under different physiological and pathophysiological conditions is not known, thus making true in vivo quantification of myocardial metabolic rates of glucose very difficult.

METABOLISM IN THE NORMAL HUMAN HEART

Under normal circumstances, any increase in cardiac work is met by a parallel rise in coronary blood flow. If this is the conditio sine qua non for a physiological increase of cardiac performance, other important adjustments in myocardial metabolism accompany and follow any given change in heart function. At rest, in the postabsorptive state, all the major circulating substrates, including free fatty acids (FFAs), lactate, pyruvate, and β-hydroxy-butyrate, are extracted by the heart with the exception of alanine (which is released), glucose and glycerol,[4] for both of which net balances are not different from zero. The respiratory quotient (ie, the ratio between the carbon dioxide released in the coronary veins and the oxygen extracted from the coronary arteries) is approximately 0.7, indicating dominant reliance of heart muscle on lipid oxidation for energy production.[6] Camici et al,[4] showed that if cardiac workload is increased by rapid atrial pacing (heart rate was progressively increased from 76±6 to 159±9 bpm), myocardial oxygen consumption, which was 301±53 µmol/min at baseline, increased to 593±71 µmol/min. During pacing, both myocardial lactate and pyruvate uptake tended to increase before returning to baseline values during recovery. The uptake of β-hydroxybutyrate remained unchanged and alanine continued to be released. Myocardial glucose uptake increased linearly with

pacing to a peak of 11 μmol/min, from which it declined also linearly as a function of recovery time. The rate of change in myocardial glucose uptake followed heart rate closely during pacing, but not during the recovery phase when circulating glucose uptake was apparently in excess of the demand imposed by cardiac workload. In contrast, uptake of circulating FFAs was significant at baseline (10.0±2.5 μmol/min), did not change during pacing, and fell significantly throughout the recovery phase.[4] Under most circumstances, oxidation of lipid fuel gave the major contribution to myocardial energy production. However, during maximal atrial pacing carbohydrate (glucose + lactate + pyruvate + alanine) oxidation rose significantly and contributed more than 62% of the energy produced during this phase. Accordingly, the respiratory quotient increased to almost 0.9. During this phase, carbohydrate oxidation was in excess of their uptake from the circulation, suggesting breakdown of myocardial glycogen stores. This shift can probably be explained by the greater caloric equivalent of oxygen for carbohydrate (5.02 kcal/L) than for lipid (4.66 kcal/L).

Feeding induces a series of metabolic changes in the whole body that have profound effects on myocardial metabolism.[20] Important substrate and hormonal changes are generated after food ingestion. Of these, by far the most important is the increase in the circulating levels of insulin. Concomitant with insulin-induced stimulation of glucose metabolism is a drastic reduction in FFA delivery to tissues due to inhibition of adipose tissue lipolysis by insulin. Therefore, the shift in myocardial substrate utilization which occurs with feeding is the result of the combined actions of insulin at a whole body level.

Recently, new insight into the direct effect of insulin on the human heart has been gained by the study of myocardial metabolism during hyperinsulinemic, euglycemic clamp.[21] In brief, these studies have shown that insulin: (i) specifically enhances myocardial glucose, lactate, and pyruvate uptake; (ii) converts cardiac fuel reliance from fat to carbohydrate (by suppressing lipolysis) with no change in oxygen consumption; and (iii) does not affect cardiac hemodynamics and external work.

EFFECT OF ISCHEMIA ON MYOCARDIAL METABOLISM

Patients with coronary artery disease and stable angina pectoris have a resting myocardial metabolism similar to that in control subjects. All major substrates, including FFAs, glucose, pyruvate, lactate, ketones, and glutamate, are extracted, with the exception of alanine and citrate which are released in small amounts.[22,23] The fraction of energy supplied by lipid oxidation is more than 80%. Significantly, the uptake of carbohydrates exceeds their oxidation.[23] Regional utilization of FFAs and glucose at rest, as assessed with [11C]palmitate and FDG by means of PET, is homogeneous in patients with exercise-induced angina.[24,25]

In these patients, regional myocardial perfusion becomes inadequate during stress.[25] In the regions which demonstrated perfusion defects during exercise, an increased FDG uptake was observed. This is consistent with an increased glycolytic metabolism in the ischemic zone. Furthermore, the augmented glucose uptake in the ischemic territory is sustained well after the reversal of the perfusion defects and it is thought that the glucose is probably being

used to replenish glycogen stores which were depleted during the ischemic episode.[25] During ischemia there will be accumulation of reduced coenzymes. Thus, despite the increase in myocardial glucose utilization, the pyruvate formed through anaerobic glycolysis will not be oxidized, but in the presence of increased amounts of reduced nicotinamide adenine nucleotide (NADH) will be converted to lactate. In addition, a greater amount of alanine will be released through transamination of pyruvate with glutamate serving as the NH_2 donor.[22] In addition, glutamate may be used as an anaerobic fuel through conversion to succinate which is coupled with formation of GTP.[20]

In patients with unstable angina, resting glucose utilization is increased in the absence of symptoms and signs of ischemia.[20] These data suggest the presence of ischemia in these patients which can be alleviated by medical treatment, as evidenced by a decrease in resting myocardial FDG uptake.[26] Of interest was the finding that in patients with unstable angina, increased resting FDG uptake could be observed in myocardial territories subtended by epicardial coronary arteries with noncritical stenoses.

Studies by Marshall et al[27] have indicated that myocardial ischemia and infarction could be distinguished by qualitative PET imaging with FDG and nitrogen 13-labelled ammonia ($^{13}NH_3$), as a flow tracer, acquired after an oral glucose load. Regions which showed a concordant reduction in both $^{13}NH_3$ and FDG uptake ("flow-metabolism match") were considered scarred, whereas regions in which FDG uptake was relatively preserved or increased despite having a $^{13}NH_3$ defect ("flow-metabolism mismatch") were considered ischemic and thus

still viable. Further studies were performed so as to ascertain whether asynergic regions with a "flow-metabolism mismatch" represented reversibly injured myocardium. Preoperative PET scans were performed in 17 patients undergoing coronary artery bypass grafting. Regional wall motion increased after surgery in 35/41 segments displaying "flow-metabolism mismatch" and remained depressed in 24/26 segments demonstrating "flow-metabolism match."[28]

Recently, quantitative PET studies of myocardial blood flow (MBF) with oxygen 15-labelled water ($H_2^{15}O$) and glucose utilization with FDG have been performed to study the pathophysiology of chronic left ventricular (LV) dysfunction in patients with coronary artery disease.[29] Regional MBF (mL/min/g of water-perfusable tissue) and glucose utilization (MRG, μmol/min/g), during hyperinsulinemic euglycemic clamp, were measured in 30 patients before bypass. At baseline, 133 myocardial segments were normal (NOR) and 107 dysfunctional. After revascularization, 59/107 segments improved (IMP) while 48/107 were unchanged (UNC). MBF was 0.92±0.25 in NOR, 0.87±0.31 in IMP (P=NS vs NOR) and 0.82±0.40 in UNC (P<0.05 vs NOR). In 90% of the dysfunctional segments MBF was > 0.42, a cutoff value corresponding to the mean MBF minus 2 SD in NOR. The MRG was 0.71±0.14 in 9 age-matched normal subjects, 0.45±0.19 (P<0.01) in NOR, 0.44±0.14 in IMP (P=NS vs NOR), and 0.34±0.17 in UNC (P<0.01 vs NOR and IMP). The results of this study suggest that resting MBF, measured with $H_2^{15}O$ in chronically dysfunctional segments is not reduced, that the myocardium of these patients is less sensitive to insulin than that

of normal subjects and that dysfunctional segments that improve after revascularization are characterized by higher glucose utilization rates.

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How can cardiac mitochondrial metabolism be manipulated?

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Cardiac mitochondrial metabolism can be manipulated by: (i) addition of metabolites or cofactors; (ii) activation of enzymes or complexes involving generation of reduced equivalents; (iii) control of synthesis of mitochondrial factors or mitochondrial biogenesis. The most effective means of stimulating ATP synthesis when the heart is exposed to high workloads or inotropic agents is through elevation of mitochondrial Ca^{2+} concentrations. Physiologically, this occurs through an increase in electron transport-limiting components or NADH supply via stimulation of Ca^{2+}-dependent mitochondrial dehydrogenases. Drugs such as ubiquinone, diltiazem, and trimetazidine seem to be effective in achieving this goal.

Drugs that reduce oxidative damage and mitochondrial DNA mutations, or genetic manipulations that improve mitochondrial biogenesis, can be considered additional interventions improving cardiac mitochondrial function.

The most remarkable feature that characterizes the cardiac muscle is the stability of the phosphorylation potential in the face of varying workloads, suggesting that several factors are involved in the control of oxidative phosphorylation. It is therefore possible to predict strategies for restoring cardiac mitochondrial function under different physiological or pathological conditions.

CONTROL OF MITOCHONDRIAL ATP SYNTHESIS IN HEART MUSCLE

Under physiological conditions, the heart is fully aerobic and over 90% of its ATP originates from mitochondrial oxidative respiration. The main steps involved in control of cardiac mitochondrial ATP are: (i) delivery of reducing equivalents to the electron transport chain; (ii) transport of electrons along the respiratory chain components associated with proton ejection and O_2 consumption coupled to phosphorylation of ADP to ATP; and (iii) flux of ATP and ADP and other metabolites across the mitochondrial membranes. Transient changes in ATP level are initially buffered by creatine kinase equilibrium; if ATP consumption increases in response to nerve impulses or hormones which enhance the rate and force of contraction of the heart muscle,

increased mitochondrial ATP synthesis is provided by elevating ATP hydrolysis products (ADP, Pi) that are the substrates for the ATP/ADP carrier and oxidative phosphorylation.[1] The first step in the delivery of reducing equivalents into the cytochrome chain is the production of substrates in the extramitochondrial space in an appropriate form for entry into the mitochondria. Any process in the cytosol that could modify the concentrations of these or other transported substrates could alter the delivery of NADH. The major sources of reduced carbons transported into mitochondria are pyruvate and fats. The great variety of results concerning mitochondrial NADH alterations induced in heart muscle by the delivery of different substrates gives no indication as to whether an increase in mitochondrial NADH can stimulate the rate of respiration without an increase in extramitochondrial [ADP] or [Pi].[2] Contrasting results indicate that an increase in the supply of respiratory substrates can stimulate mitochondrial respiration in cardiac muscle by enhancing only the NADH/NAD ratio. In fact, addition of pyruvate, fatty acids, and β-hydroxybutyrate enhances the ATP/ADP ratio without increasing oxygen consumption.[2] Moreover, cardiac mitochondria contain several other dehydrogenases that make the mitochondrial utilization of ketone bodies and amino acid-derived substrates possible in addition to fatty acids and glucose.

Recent NMR studies indicate a nonunique relationship between [ADP] and O_2 consumption, suggesting that other factors are also involved in increasing phosphorylating flux. It has been suggested that elevated [Ca^{2+}] is the signal used to increase phosphorylating flux through the activation of mitochondrial Ca^{2+}-sensitive dehydrogenases.[3]

Within mitochondria, a relatively small increase (in the 0.2-1 mM range) in the matrix calcium content stimulates both respiration and phosphorylation at nonsaturating substrate concentration. This stimulation can be explained by the calcium-dependent activation of three mitochondrial dehydrogenases: α-ketoglutarate dehydrogenase, pyruvate dehydrogenase, and the NAD-linked isocitrate dehydrogenase. Each of these Ca^{2+}-activated dehydrogenases tends to maintain elevated mitochondrial NADH/NAD ratios during periods of elevated tissue workload, allowing a closer balance of energy supply and demand in the cardiac muscle independently of the variation in [ADP] and [Pi].[4] The evaluation of the interrelationship between relative enzyme activity and its contribution to the control of phosphorylating flux has shown that the predominant controlling steps at low concentrations of Ca^{2+} and pyruvate, as well as in state 3 respiration, which is the most physiological condition in the cardiac muscle, appear to be the ATP/ADP carrier, the dehydrogenases, and cytochrome oxidase, while ATP synthase is negligible. In the presence of increased concentrations of mitochondrial Ca^{2+} that activate the dehydro-genases, ATP synthase activity and the ATP/ADP and Pi carriers become the limiting steps controlling oxidative phosphorylation.

Control of heart mitochondrial respiration as a function of oxygen availability has not been clearly defined. In fact, even if most reports suggest that mitochondrial respiratory rate is not affected by O_2 concentrations seen in vivo under normal physiological conditions, increasing O_2 supply results in a small increase in oxygen consumption, but the mechanism of this stimulation is unclear.[2]

Another control mechanism of heart mitochondrial respiration is represented by ATP synthase. The activity of this enzyme is not constant, but varies with ATP demand. In addition to the positive action on ATP synthase by ADP and Pi, there are two regulator proteins[5] that have been isolated from heart mitochondria: IF_1, the inhibitor of F_1 - the extramembrane segment of the mitochondrial ATP synthase - which is reversed by the membrane potential, and another protein, calcium-binding inhibitor (CaBI) with inhibitory properties, which confers Ca^{2+} sensitivity on ATP synthase regulation in the heart, as it is reversed by Ca^{2+}. Recent evidence suggests that control of the ATP/ADP ratio in cardiac muscle is also under the influence of the creatine/phosphocreatine shuttle, involving creatine kinase activity. It has been shown that creatine kinase can specifically induce contact sites between the inner and outer mitochondrial membranes, where there is preferential access for the rapid exchange of generated ATP for the extramitochondrial compartment.[6] This mechanism, which is the same as that of the enzyme hexokinase I, allows the preservation of a high level of the ATP/ADP ratio. Of great interest is the fact that the frequency of contact sites is regulated by a variety of metabolic or hormonal stimuli.[6]

INTERVENTIONS ABLE TO MODIFY MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION IN HEART MUSCLE

On the basis of the aforementioned control mechanisms of oxidative phosphorylation, it is possible to determine potentially effective interventions in order to manipulate mitochondrial metabolism in heart muscle:

1. Addition of metabolites or cofactors;

2. Activation of enzymes or complexes involving generation of reduced equivalents and their utilization;

3. Control of synthesis of mitochondrial factors or genesis of mitochondria.

In heart muscle, relatively little is known about control by respiratory substrate supply, even though it is known that increasing levels of carnitine and CoA and Krebs cycle intermediates exert a significant control at the level of fatty acid consumption and NADH production.[2]
In addition, appropriate high pyruvate or acetate concentrations might enhance the delivery of NADH and activate other parameters of the regulation of oxidative phosphorylation. Moreover, a maximal rate of mitochondrial respiration may be achieved by elevating the concentration of components that limit electron transport, as is the case of coenzyme Q.
Nevertheless, the most effective intervention able to stimulate ATP formation when the cardiac muscle is exposed to high workloads or inotropic agents is the elevation of mitochondrial Ca^{2+} concentrations. Physiologically, this event is produced in response to an increase in cytosolic Ca^{2+} concentration stimulated by exogenous agonists.

Elevated concentrations of mitochondrial Ca^{2+} at micromolar levels stimulate matrix dehydrogenase activity, thereby increasing the NADH supply for oxidative phosphorylation. Agents interfering with the mechanisms involved in mitochondrial Ca^{2+} transport that increase the physiological level of this calcium "activator," represent the most advantageous drugs which are probably capable of sustaining myocardial metabolism when the cardiac muscle is poorly sensitive to inotropic stimuli.

Some drugs, such as diltiazem[7] and trimetazidine,[8] seem to confirm the efficacy of this pharmacological intervention. In fact, diltiazem and other benzodiazepines antagonizing the mitochondrial Na^+-Ca^{2+} exchanger can reduce the mitochondrial Ca^{2+} efflux and therefore elevate the intramitochondrial Ca^{2+} concentration and the rate of oxidative ATP synthesis.[7] Similar effects, but with a different mechanism, are produced by trimetazidine when the drug is added to isolated rat heart mitochondria. In this experimental condition, 100 mM trimetazidine increases the entry of Ca^{2+} through mitochondrial membranes and stimulates ATP formation when a physiological extramitochondrial Ca^{2+} level (100 nM) is employed (unpublished results). On the contrary, when higher cytosolic Ca^{2+} causes a continued electrogenic entry of Ca^{2+} into mitochondria, producing calcium overload and proton motive force ($\Delta\mu_{H+}$) dissipation, ruthenium red, an inhibitor of mitochondrial Ca^{2+} uptake, protects mitochondrial function. Another important process induced by mitochondrial Ca^{2+} overload that could be controlled with pharmacological interventions is the "permeability transition" that opens an inner membrane channel with mitochondrial depolarization coincident with loss of matrix

components such as ADP, Mg^{2+}, and glutathione. Cyclosporin A (0.2 μM), which is a potent inhibitor of pore formation, enhances the ATP/ADP ratio and protects cardiac contractility when injected into isolated ischemic hearts. Even if the precise function of pore opening is not clear at present, the fact that in some conditions it is reversible with recovery of mitochondrial energy-linked functions, seems to suggest a possible physiological role able to reset Ca^{2+}-overloaded mitochondria. The study of compounds acting on the mechanism(s) of pore opening will probably be promising in the field of mitochondrial research.

Protection of mitochondrial function in different pathological cardiac contexts represents an important goal in mitochondrial medicine. Of great interest are the studies investigating the effect of natural or synthetic antioxidants on mitochondrial damage induced by oxidative stress. Favorable results with antioxidants have been reported not only in conditions of mitochondrial dysfunction induced acutely by reactive oxygen (eg, ischemia-reperfusion, drugs), but also in subjects with age-related disorders, or in patients with classic mitochondrial diseases due to deficiencies in mitochondrial factors. Alpha-tocopherol and coenzyme Q occupy a prominent place in these attempts. Moreover, it should be underlined that protection with antioxidants represents a long-term investment able to preserve the cardiac mitochondria against chemical oxidative modifications in mitochondrial DNA (mit DNA). In fact, this damage appears to reduce the efficiency of cardiac oxidative phosphorylation, for example, in ageing and atherosclerosis. Recent findings

suggest that the cumulative increase in oxygen free radical damage in mit DNA closely correlates with a large number of mit DNA mutations and deletions. Accumulation of hydroxyl radical adducts in mit DNA, such as 8-hydroxydeoxyguanosine (8-OH-dG), would play a role in yielding double-strand separation and deletion. Especially in postmitotic cells, such as cardiac cells, the mitochondrial genome mutations accumulate, escaping the excision repair and cellular selection by mitosis. These events, that are present in normal ageing and can be exacerbated in patients with coronary occlusion and primary cardiomyopathy, are associated with a progressive decline in bioenergetic activities and programmed cell death (apoptosis).[9] The possibility of controlling oxygen free radical damage in the cardiac cell, particularly at mit DNA level, with antioxidants or compounds able to protect mit DNA, or genetic treatments that enhance endogenous mitochondrial antioxidant capacities, will represent reliable modalities of protection of the cardiac muscle, at levels other than that of its bioenergetic behavior. In this regard, the fact that some cytokines can specifically induce the synthesis of mitochondrial manganese-dependent superoxide dismutase has considerable implications.

Recent advances in the mechanisms leading to cell apoptosis have revealed the presence, in the mitochondrial membranes, of the proto-oncogene Bcl-2, which blocks programmed cell death induced in mitochondria by peroxide formation and lipid membrane peroxidation, and a homologue protein (Bax), which accelerates cell death.

Therefore, the Bcl-2/Bax ratio predetermines a cell's life or death in response to an apoptotic stimulus such as an oxidative stress. In cells overexpressing the oncogene Bcl-2, much more TNF is required to induce apoptosis, and mitochondria are protected against TNF-induced damage. Such information could be useful to devise future genetic therapy for ageing and degenerative diseases associated with mit DNA mutations in cardiac cells.

In the past few years, exciting studies have clarified several mechanisms responsible for the expression of the mitochondrial genome and its control by the nuclear genome, and the molecular machinery of mitochondrial protein import and mitochondrial biogenesis.[10] The results have opened the way for new experiments of genetic manipulation of mammalian mitochondria, such as the repopulation of human mit DNA-less cells with exogenous mitochondria by functional complementation. Transfection of cardiac cells with factors able to promote the transcription of mitochondrial genome and/or the expression of nuclear factors regulating individual mitochondrial genes will probably be the most important gene therapy approach for restoring the number and activity of mitochondria in the cardiac muscle, particularly in pathological conditions.

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How can fatty acid and carbohydrate metabolism be manipulated?

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*Despite important advances over the last two decades, heart disease remains a major cause of morbidity and mortality in Western society.
As a result, in addition to optimizing existing approaches, new approaches are needed to treat heart disease.
Agents which alter energy metabolism in the heart offer an exciting new approach to treating ischemic heart disease and other cardiovascular complications.
A preponderance of experimental and clinical data now support the concept that shifting energy substrate preference away from fatty acid metabolism and towards glucose metabolism is a novel and effective approach to treating cardiovascular disease.*

In many forms of cardiovascular disease (both ischemic and nonischemic) increasing myocardial glucose metabolism can benefit heart function and/or lessen tissue injury. Unfortunately, fatty acids are very effective competitors of glucose as a source of energy and, under a variety of pathological conditions, high levels of circulating fatty acids markedly decrease glucose use by the heart.[1-3] Experimental and clinical studies have now convincingly demonstrated that shifting the source of energy production away from fatty acid metabolism and towards glucose metabolism can benefit the heart. For instance, lowering circulating fatty acid levels during and following ischemia is an indirect approach to lessening the dependence of the heart on fatty acids. Pharmacologically, a number of promising agents have also been introduced that act by directly stimulating cardiac glucose metabolism or by indirectly stimulating glucose metabolism secondary to inhibition of cardiac fatty acid metabolism.

These agents include dichloroacetate, ranolazine, trimetazidine, etomoxir, oxfenicine, methylpalmoxirate, L-carnitine, and propionyl L-carnitine. A number of these agents have been shown in widespread clinical trials to be effective in treating ischemic heart disease (ie, trimetazidine, ranolazine, L-carnitine, and propionyl L-carnitine). As a result, optimizing energy metabolism has considerable promise as a novel approach to treating cardiovascular disease.

INTERACTION BETWEEN CARBOHYDRATE AND FATTY ACID METABOLISM IN THE HEART

The chemical energy that is required to maintain contractile function in the heart is derived from ATP, which in the heart is produced primarily from the metabolism of both carbohydrates and fatty acids *(see article by L.H. Opie).*

Glucose metabolism can be divided into two main components, glycolysis and glucose oxidation. Glycolysis, which is the first part of the glucose metabolic pathway, has the advantage of producing ATP without the requirement of oxygen. While glycolysis usually contributes only 5% to 10% of the overall ATP supply in the normal aerobic heart, glycolytic ATP production appears to have a special role in maintaining ion homeostasis within the myocyte *(see article by L.H. Opie).*

The other main component of glucose metabolism is glucose oxidation, in which pyruvate derived from glycolysis is taken up by the mitochondria and fed into an enzyme complex called the pyruvate dehydrogenase complex.

The product of this enzyme complex is acetyl-CoA, which is further metabolized by the mitochondria and results in ATP production.

Oxidation of fatty acids is the other major source of mitochondrial acetyl-CoA production. While the metabolism of fatty acids is a major and important source of ATP production in the heart, fatty acids require more oxygen than glucose to produce an equivalent amount of ATP. As a result, with regard to oxygen consumption, fatty acids are not as efficient as glucose as a source of energy. Furthermore, as the contribution of fatty acid oxidation as a source of acetyl-CoA production increases, the contribution of glucose oxidation as a source of acetyl-CoA decreases. This is not desirable (especially during and following an episode of myocardial ischemia) since products of glycolysis can accumulate (ie, lactate and protons). This can become a serious problem since clearance of these glycolytic by-products requires a greater consumption of ATP for noncontractile purposes, which further decreases cardiac efficiency.

Circulating fatty acid levels increase following an acute myocardial infarction or during cardiac surgery, so that during and following ischemia the heart muscle can be exposed to very high concentrations of fatty acids.[4] The detrimental effects of high plasma fatty acid levels on mechanical and electro-physiological characteristics of the heart following ischemia-reperfusion have been recognized for over 20 years.[4] High plasma fatty acid concentrations have also been shown to increase the severity of ischemic damage in a number of different experimental animal models of cardiac ischemia, and have also been linked to a depression of mechanical function during aerobic reperfusion of

previously ischemic hearts *(see 3 for review).* During reperfusion of the heart following ischemia fatty acid oxidation can quickly recover and dominate as a source of ATP production. These high rates of fatty acid oxidation contribute to a marked decrease in cardiac efficiency during reperfusion. However, if glucose oxidation is stimulated during reperfusion, a significant increase in cardiac efficiency results, with a parallel improvement in cardiac function.[3,5-9]

A number of different approaches can be used to manipulate energy metabolism in the heart. This involves both indirect measures, as well as a class of agents which directly act on the heart to shift energy substrate preference away from fatty acid metabolism and towards glucose metabolism. A list of pharmacological and nonpharmacological approaches to shifting energy substrate preference in the heart is shown in *Table I.*

Table I. SUMMARY OF PHARMACOLOGICAL AND NONPHARMACOLOGICAL APPROACHES TO SHIFTING ENERGY SUBSTRATE PREFERENCE IN THE HEART

	EFFECT ON ENERGY METABOLISM	EFFECT ON ISCHEMIC INJURY
DIRECT APPROACH		
Dichloroacetate	▲ glucose oxidation ▼ fatty acid oxidation	▼ ▼
Ranolazine	▲ glucose oxidation ▼ fatty acid oxidation	▼ ▼
Trimetazidine	▲ mitochondrial oxidative metabolism	▼
L-carnitine	▲ glucose oxidation ▼ fatty acid oxidation	▼ ▼
Propionyl L-carnitine	▲ glucose oxidation ▼ fatty acid oxidation	▼ ▼
CPT 1 inhibition (etomoxir, oxfenicine, methylpalmoxirate)	▲ glucose oxidation ▼ fatty acid oxidation	▼ ▼
INDIRECT APPROACH		
Glucose/insulin	▲ glucose use due to decreased circulating fatty acids	▼
Nicotinic acid	▲ glucose use due to decreased circulating fatty acids	▼
β-Blockers	▲ glucose use due to decreased circulating fatty acids	▼

INDIRECT APPROACHES TO DECREASING FATTY ACID METABOLISM AND INCREASING GLUCOSE METABOLISM IN THE HEART

One way to increase glucose metabolism and decrease fatty acid metabolism in the heart is to decrease circulating fatty acid levels. This can be achieved by administration of glucose-insulin solutions. While early clinical studies using this approach met with limited success *(see 4 for review),* carefully controlled adequate trials to determine the benefit of increasing blood glucose or decreasing blood fatty acid levels have not yet been carried out. Another indirect approach to lowering fatty acid levels is with the use of nicotinic acid, although this approach is associated with a number of significant side effects.

Catecholamine release of fatty acids from adipocytes is a major source of the elevated levels of fatty acids that occur during ischemia. β-Blockers can blunt this response and decrease circulating fatty acid levels. Whether a lowering of fatty acids contributes to the demonstrated benefits of β-blockade during and following myocardial ischemia has yet to be established.

A large body of experimental and clinical evidence has shown that high levels of circulating fatty acids are detrimental to the ischemic heart. This has led a number of investigators to propose the reevaluation of approaches to lowering circulating fatty acid levels during the prehospital phase of acute myocardial infarction, as well as during standard thrombolytic regimens.[4,10] It is hoped that further clinical investigation along this line will demonstrate the benefits of this approach in the early phases

of developing infarction, as well as in the postinfarction period or the treatment of patients with unstable angina.[4] Another approach to decreasing the detrimental effects of fatty acids is to directly modify energy preference in the heart. Pharmacological agents that are effective in doing this are discussed in the following section.

DIRECT APPROACHES TO DECREASING FATTY ACID OXIDATION AND INCREASING GLUCOSE METABOLISM IN THE HEART

Dichloroacetate

One strategy to stimulate myocardial glucose metabolism is to directly stimulate the rate-limiting enzyme for glucose oxidation, the pyruvate dehydrogenase complex. An agent that effectively does this is dichloroacetate, which by increasing the amount of pyruvate dehydrogenase in the active form, will markedly increase glucose oxidation. Dichloroacetate will also bring about a concomitant decrease in cardiac fatty acid oxidation. This is thought to occur firstly by increased glucose oxidation effectively competing with fatty acid oxidation as a source of mitochondrial acetyl-CoA (called the glucose-fatty acid cycle or Randle cycle), and secondly by an additional mechanism which involves a shuttling of acetyl-CoA groups out of the mitochondria, which eventually can lead to the inhibition of mitochondrial fatty acid uptake.[3]

In a number of experimental studies, dichloroacetate has been shown to dramatically improve recovery of mechanical function following ischemia.[3,5,11] The clinical efficacy of dichloroacetate as an anti-ischemic agent has also been shown. In patients with

coronary artery disease dichloroacetate has been shown to increase left ventricular stroke volume *(see 11 for review).* Unfortunately, while dichloroacetate is very efficacious as a stimulator of glucose oxidation, it is not a particularly potent drug, and blood levels of dichloroacetate need to approach millimolar levels in order to increase myocardial glucose oxidation. Another limitation of this agent is its short half-life following either oral or IV administration.[11] Both these problems and the fact that dichloroacetate is not under patent protection suggest that this agent is unlikely to find widespread clinical use. However, it remains a very effective research tool for delineating the mechanisms as to why stimulation of glucose metabolism can benefit the ischemic and reperfused ischemic myocardium.

Ranolazine

Ranolazine is a novel anti-ischemic agent which has demonstrated beneficial effects on the ischemic heart without altering hemodynamics or baseline contractile parameters. It is a piperazine-based molecule that does not act as a β-blocker, a calcium channel blocker, or a vasodilator. It has, however, been found in both in vivo and in vitro experimental studies to have cardioprotective actions in the ischemic and reperfused ischemic heart *(see 9 for example).* Clinical trials have confirmed the experimental studies and have shown that this compound is a well-tolerated molecule that has significant anti-ischemic properties. In patients with chronic stable angina, ranolazine decreases the frequency of anginal attacks and lowers nitroglycerin consumption. Ranolazine also significantly increases treadmill exercise times to angina and to 1-mm ST-segment

depression. These improvements in treadmill exercise parameters are both comparable in magnitude, and additive to, those of β-blockers and calcium channel antagonists, and occur in the absence of any change in heart rate or decrease in blood pressure. During exercise testing of angina patients ranolazine is also able to prolong exercise duration to a significantly greater extent than atenolol.

Recent evidence has shown that ranolazine acts by optimizing energy metabolism. Experimental studies have shown that under a number of different perfusion conditions ranolazine significantly decreases fatty acid oxidation in the heart and increases glucose oxidation.[9] Furthermore, ranolazine effectively stimulates glucose oxidation under both normoxic and ischemic conditions. During low-flow ischemia the anti-ischemic effects of ranolazine are associated with increases in the amount of the pyruvate dehydrogenase complex in its active form. The mechanism whereby ranolazine promotes glucose oxidation is as yet unknown, but may occur due to a direct inhibition of fatty acid β-oxidation.[12] The resultant lowering of acetyl-CoA production from fatty acid β-oxidation[12] would then relieve the inhibition of pyruvate dehydrogenase, resulting in an increase in glucose oxidation.

Trimetazidine

Trimetazidine is a novel agent that has anti-ischemic properties which are independent of hemodynamic changes. Like ranolazine, trimetazidine also belongs to the piperazine group of compounds, and is structurally similar to ranolazine. Trimetazidine has now been approved in a large number of countries as a cellular anti-ischemic agent for the treatment of angina. Experimentally, trimetazidine has been shown to decrease ST-segment elevation during coronary artery occlusion of rabbit hearts, and will decrease infarct size following variable periods of coronary artery occlusion in dogs. Clinically, double-blind crossover trials have shown that trimetazidine is as effective as nifedipine in the treatment of stable angina. In one particular trial,[13] trimetazidine was also found to have a lower incidence of side effects compared to nifedipine. In addition to showing antianginal efficacy in a number of other trials, trimetazidine has also been shown to have cardioprotective effects during coronary artery bypass graft surgery[14] and during percutaneous transluminal coronary angioplasty.[15] A comparison of trimetazidine and propranolol in patients with stable angina has also been made in a double-blind Trimetazidine European Multicenter Study (TEMS).[16] This trial, which involved 19 centers in 10 European countries, determined that trimetazidine had similar efficacy as propranolol in treating stable angina pectoris, but that the beneficial effects of trimetazidine occurred without hemodynamic changes.

Despite its cardioprotective properties, the actual mechanism(s) by which trimetazidine acts have not been completely delineated. However, experimental studies suggest that trimetazidine acts by changing myocardial energy substrate preference.[17] Trimetazidine reduces the extent of acidosis during ischemia and also preserves mitochondrial function. Improvement of cardiac energetics with trimetazidine has been demonstrated, with an increase in mitochondrial oxidative metabolism and a decrease in acidosis.

Studies in isolated mitochondria have shown that trimetazidine inhibits oxidative phosphorylation of fatty acid substrates,[17] suggesting that, like ranolazine, this agent may act to inhibit fatty acid oxidation. Trimetazidine pretreatment of myocytes also increased the resistance of the cells to hypoxic stress.[17] Since we recently demonstrated that ranolazine, which is structurally similar to trimetazidine, is effective in shifting energy substrate preference from fatty acid oxidation to glucose oxidation,[9] it is possible that trimetazidine may also exert its beneficial effects by improving glucose oxidation in the heart. This possibility remains to be determined.

Carnitine palmitoyltransferase 1 (CPT 1) inhibitors

An alternative approach to achieving a switch in energy substrate preference away from fatty acid metabolism and towards glucose metabolism is to inhibit fatty acid uptake by the mitochondria. The key enzyme involved in this process is carnitine palmitoyl transferase 1 (CPT 1). Inhibition of CPT 1 leads to a reduction in fatty acid oxidation and an increase in glucose oxidation.[7] A number of CPT 1 inhibitors (etomoxir, oxfenicine, and methylpalmoxirate) have also been shown to have anti-ischemic efficacy as a result. However, long-term administration of such agents has been found to be associated with toxicity problems, as well as, in particular, with development of cardiac hypertrophy.

L-carnitine and propionyl L-carnitine

An important step in the oxidation of fatty acids is the translocation of

fatty acids into the inner mitochondrial space by a L-carnitine-mediated transport. In addition to this critical metabolic role, L-carnitine is also important in regulating glucose oxidation in the heart.[6] L-carnitine increases glucose oxidation secondary to an increase in pyruvate dehydrogenase complex activity, which results from an L-carnitine-mediated lowering of the intramitochondrial acetyl-CoA /CoA ratio. Propionyl L-carnitine is an L-carnitine analog that has similar effects on myocardial glucose oxidation.[8] This naturally occurring compound may also have beneficial effects on replenishing intramitochondrial Krebs cycle intermediates.

Anti-ischemic effects of L-carnitine and propionyl L-carnitine (PLC) have been shown in both experimental and clinical studies, and beneficial effects of these compounds have been seen on functional and hemodynamic parameters of failing hearts. Animal studies with L-carnitine and propionyl L-carnitine have shown these agents to be effective cardioprotective agents in a number of different models of experimental ischemia (in vitro and in vivo). These compounds can also directly affect muscle mechanics, improving global cardiac dynamics in failing hearts. The main effect of propionyl L-carnitine on cardiac mechanics is the ability to correct alterations in contractility (relaxation) indexes after pressure or volume overload.
In the pressure-overloaded conscious animal the correlation between the magnitude of cardiac hypertrophy and PLC efficacy strongly suggests that PLC restores depressed cardiac function to normal. The supplementation of the myocardium with carnitine or propionyl L-carnitine results in an increased tissue carnitine content,

which lessens the severity of ischemic injury and improves the recovery of heart function during reperfusion.

Clinically, both L-carnitine and propionyl L-carnitine have been shown to have anti-ischemic properties. Both compounds are effective antianginal agents that can reduce ST-segment depression and left ventricular end-diastolic pressure during stress testing in patients with coronary artery disease. In addition, cardioprotective effects of these compounds have been observed following aortocoronary bypass grafting and following acute myocardial infarction. In a recent multicenter trial, L-carnitine treatment initiated at an early stage after acute myocardial infarction and continued for 12 months was found to attenuate left ventricular dilation and result in smaller left ventricular volumes.[18] L-carnitine and propionyl L-carnitine have also been shown to benefit cardiac mechanics in clinical studies. For instance, in NYHA class II heart failure patients, propionyl L-carnitine improved exercise capacity by 1 month after starting treatment. In these patients L-carnitine also increased shortening fraction and ejection fraction.

In addition to direct cardiac effects, L-carnitine and propionyl L-carnitine also have the potential to alter skeletal muscle function. A recent multicenter trial in patients with intermittent claudication showed that propionyl L-carnitine significantly improved maximal walking distance on treadmill performance tests.[19]

Whether L-carnitine and propionyl L-carnitine increase glucose oxidation in skeletal muscle in a manner similar to that seen in the heart remains to be determined.

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Cardiac Metabolism

Summaries of Ten Seminal Papers

1

Energy metabolism of the heart: from basic
concepts to clinical applications

H. Taegtmeyer. *Curr Probl Cardiol.* 1994

2

Role of glycolytic products in damage
to ischemic myocardium.
Dissociation of adenosine triphosphate levels
and recovery of function of reperfused
ischemic hearts

J.R. Neely and L.W. Grotyohann. *Circ Res.* 1984

3

The glucose-fatty acid cycle.
Its role in insulin sensitivity and the metabolic
disturbances of diabetes mellitus

P.J. Randle and others. *Lancet.* 1963

4

Ultrastructural damage associated
with reoxygenation of the anoxic myocardium

D.J. Hearse and others. *J Mol Cell Cardiol.* 1975

5

Myocardial metabolism in ischemic heart disease:
basic principles and applications to imaging by
positron emission tomography

P. Camici and others. *Prog Cardiovasc Dis.* 1989

6

Effects of glucose and fatty acids on myocardial
ischaemia and arrhythmias

M.F. Oliver and L.H. Opie. *Lancet.* 1994

7

Determinants of a protective effect of glucose
and insulin on the ischemic myocardium.
Effects on contractile function,
diastolic compliance, metabolism,
and ultrastructure during ischemia and reperfusion

C.S. Apstein and others. *Circ Res.* 1983

8

Myocardial ischemia - observations,
definitions and speculations

R.B. Jennings
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reperfusion

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10

Glucose and palmitate oxidation in isolated
working rat hearts reperfused after a period
of global ischemia

G.D. Lopaschuk and others. *Circ Res.* 1990

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Energy metabolism of the heart: from basic concepts to clinical applications

H. Taegtmeyer

For all those interested in the heart, whether from a clinical or an experimental viewpoint, this review should be compulsory reading. Taegtmeyer's approach to metabolism is innovative and thought-provoking and the clarity of his language is strong evidence that not all scientists and clinicians (Taegtmeyer himself being both) are linguistically unaware! Conscious of the numerous reviews of cardiac energy metabolism in the literature, the author restricts himself to aspects of clinical relevance and underlines the importance of the energy-generating reactions by calculating that, over 24 hours, the human heart produces and uses 35 kg of ATP, more than 10 000 times the amount of its own ATP stores! He stresses that it is the turnover of ATP, rather than the ATP content, that is of paramount importance in metabolic control.

Taking a totally novel approach, Taegtmeyer discusses the myriad of metabolic pathways in the myocardium in the context of energy transfer in biological systems in general. He invokes three simplifying principles: the first two laws of thermodynamics, the concept of metabolite recycling, and the fact that the heart synthesizes very few compounds "for export." He then elaborates upon each of these principles. Taegtmeyer devotes the rest of the review to the clinical relevance of myocardial metabolism. He begins by acknowledging that detailed information about metabolic pathways is not an essential prerequisite for the cardiologist, but continues by saying that "metabolism comes under scrutiny when coronary arteries are no longer obstructed and the heart fails to contract." He also points out that, with the advent of new, nondestructive imaging techniques, such as nuclear magnetic resonance (NMR) spectroscopy and positron emission tomography (PET), it is now possible to trace metabolic pathways in vivo in a way that has never before been possible. He gives a very brief outline of NMR and PET methodology and a useful table comparing the two techniques in terms of spatial and temporal resolution, specificity, radiation exposure, and cost. The final part of the review is divided into four main sections, dealing with carbohydrates, fatty acids, ketone bodies, and amino acids, each of which is subdivided into smaller sections that deal first with the biochemical facts (or experimental evidence) and, subsequently with the clinical relevance.

In the carbohydrate section, he begins by paying (a well-deserved) tribute to the rat by saying that "the isolated, perfused rat heart has contributed more than any other model in cardiovascular research to the current understanding of glucose transport and phosphorylation, control of glycolysis, and glucose oxidation."

He illustrates this in terms of glucose transport into the cell with a discussion of the recent data on the recruitability of the GLUT 4 transporter and he suggests that, once glucose is inside the cell and phosphorylated, to glucose-6-phosphate (G6P), it is sent on a "detour" to glycogen, before entering glycolysis or the pentose phosphate pathway. He points out that when [18]FDG is used as a glucose analogue in PET studies, it can only trace the uptake and phosphorylation steps because the product, [18]FDG-6-phosphate, cannot be metabolized further. In this regard, he warns that one should be careful not to equate enhanced FDG uptake with enhanced glycolytic flux.

Taegtmeyer finishes his scholarly work with 224 references and a dedication to the past and present members of his laboratory, whom he terms his "motley crew of enthusiasts"; one suspects that each member of this motley crew is very grateful for their association with such an original and eloquent cardiologist.

1994

Yasser Arafat and Menachem Begin shake hands, Conchita Martinez wins the Ladies' Singles at Wimbledon, and Donald Duck celebrates his 60th birthday (June 9)

Role of glycolytic products in damage to ischemic myocardium. Dissociation of adenosine triphosphate levels and recovery of function of reperfused ischemic hearts

J.R. Neely, L.W. Grotyohann

In this paper, the authors describe experiments which they carried out in painstaking detail (using approximately 400 rats!), to elucidate the importance of glycolysis in ischemia. In constrast to Apstein et al *(see summary on page 106)*, Neely and Grotyohann manipulated levels of the intracellular substrate, glycogen, rather than the extracellular substrate, glucose.

The isolated, perfused rat heart was used, instrumented with a left ventricular catheter, and the percentage recovery of the rate-pressure product was used as the index of postischemic contractile function. Different degrees of ischemia were induced and the tissue levels of ATP, ADP, AMP, phosphocreatine (PCr), glycogen, and lactate were measured; this was achieved by analysis of perchloric acid extracts of hearts that had been freeze-clamped in liquid nitrogen at different times throughout the protocols.

Results from the first group of experiments, using control hearts, control buffer Ca^{2+} levels (2.5 mM), and zero-flow ischemia, agreed with much previously reported data, and showed that contractile function and tissue levels of ATP and PCr all decreased progressively with increasing periods of ischemia (20-45 minutes). They also showed that ATP, PCr, and contractile function all increase during reperfusion, albeit never reaching preischemic values. A graph of postischemic developed pressure against ATP content showed an almost linear relationship below 10 μmol•g dry weight^{-1} (control content is 24 μmol•g dry weight^{-1}). Recovery is proportional to ATP content.

The novel aspects of the experiments reported are concerned with the deleterious effects of calcium and lactate. In a model of low-flow anoxia (8% of control flow rate), it was found that decreasing the buffer calcium concentration resulted in a greater recovery of contractile function upon reperfusion. A buffer calcium of 1.25 mM (physiological) resulted in 88% recovery after 75 minutes of ischemia, compared with only 7% recovery in 2.5 mM Ca^{2+}. In this low-flow model, there was no relationship between ATP content and postischemic recovery; a large range of recoveries was observed (7% to 54%) that

corresponded to a narrow range of ATP contents (4-6 μmol•g dry weight^{-1}). A comparison of the recoveries seen in the low-flow experiments with those in the zero-flow groups suggested to the authors that a harmful metabolic product was building up under the latter conditions. Since the obvious candidate was lactate, which, during ischemia, results from glycogenolysis, they investigated the effects of changing the preischemic glycogen level and of directly adding lactate to the perfusion buffer. The data from these experiments indicate that a decreased level of preischemic glycogen resulted in a much improved postischemic recovery; decreasing the glycogen from 120 to 20 μmol glucose equivalents•g dry weight^{-1} increased recovery from 28% to 68%. Recovery during reperfusion showed no correlation with ATP content during this time but did show a negative correlation with lactate levels occurring during ischemia. The addition of different amounts of lactate to the perfusion buffer before ischemia confirmed this correlation; changing lactate levels during reperfusion was ineffective.

Thus, the authors conclude that, during reperfusion, "the ability of the heart to recover ventricular function was largely independent of ATP levels" and that their studies suggest "a major role of anaerobic glycolytic products (lactate, hydrogen ions, or NADH) in ischemic damage to the heart."

1984

Indira Gandhi is assassinated,
Carl Lewis wins four gold medals at the
Los Angeles Olympics, and the first Virgin Atlantic
flight goes to New York (£99, single)

The glucose-fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus

P.J. Randle, P.B. Garland, C.N. Hales, E.A. Newsholme

Lancet. 1963;13:785-789

This is one of the key papers from the "good old days of biochemistry" when regulatory mechanisms were proposed on the basis of a small amount of data and a large amount of thought. The authors propose that a glucose-fatty acid cycle exists in muscle and adipose tissue and that this cycle essentially controls the relative concentrations of these two major substrates in the blood. This cycle, they suggest, would account for the switching of the myocardium from predominantly carbohydrate metabolism in the fed state to predominantly fat metabolism in the fasted state and vice versa. There are two tenets central to the operation of the cycle. The first is that a high uptake of glucose into adipose tissue will restrict the net breakdown of triglycerides to fatty acids, and thus also restrict the fatty acid concentration in the blood, and the second is that a high concentration of fatty acids in the blood will increase the uptake of fatty acids into muscle tissue but restrict the uptake (and oxidation) of glucose. Randle and colleagues provide both clinical and experimental evidence to support these proposals.

Evidence from the literature supports the concept that fatty acid release from adipose tissue varies with blood glucose. Triglyceride breakdown was measured, either in man or in experimental animals, by the determination of the plasma levels of what the authors call nonesterified fatty acids (NEFAs). Today, these are known simply as free fatty acids (FFAs). Diabetes, starvation, and catecholamines caused an increase in fatty acid release, and glucose, insulin, and panhypopituitarism caused a decrease. The authors themselves determined the amount of triglyceride breakdown in muscle or adipose tissue by measuring the release of glycerol. (This approach had been validated by Vaughan two years previously for tissues that could not metabolize glycerol, due, as we now know, to the lack of glycerokinase.) Again, starvation and alloxan diabetes caused an increase in glyceride breakdown, while tissues from hypophysectomized animals showed a decrease. Evidence that fatty acids restrict the uptake and oxidation of glucose is then provided. The clinical evidence cited involves the effects of conditions, such as diabetes or starvation, in which the blood fatty acid concentration is known to be increased (see above). Thus, diabetes and starvation both impair the effect of insulin on glucose uptake by muscle and also cause an increase in myocardial glycogen stores. (Evidence for the latter came from a paper published in 1913!) Results from the authors' own experiments on glucose uptake into rat heart and diaphragm are, essentially, in agreement with these clinical observations: they show that, in the presence of insulin, fatty acids decrease glucose uptake and cause an increase in the intracellular concentrations of glucose, glucose-6-phosphate, and glycogen.

A final section on the possible mechanisms involved in the inhibition of glycolysis by fatty acids is very interesting. In 1963, it was already known that the three "checkpoints" in glycolysis were membrane transport of glucose, phosphorylation of glucose (by hexokinase), and phosphorylation of fructose-6-phosphate (by phosphofructokinase, PFK). It was also known that some of these reactions were controlled by the energy status of the cell, namely, the level of adenosine triphosphate, ATP, or its breakdown products, adenosine monophosphate, AMP, and inorganic phosphate, P_i. Although the authors correctly surmised that neither starvation nor diabetes caused alterations in the levels of ATP or AMP, the correct answer, namely, that citrate caused allosteric inhibition of PFK, eluded them.

1963

J.F. Kennedy is assassinated,
the "Profumo affair" scandalizes Britain,
and Alfred Hitchcock's "The Birds" is screened

Ultrastructural damage associated with reoxygenation of the anoxic myocardium

D.J. Hearse, S.M. Humphrey, W.G. Nayler, A. Slade, D. Border

J Mol Cell Cardiol. 1975;7:315-324

Hearse and colleagues, in this key paper, present clear, electron microscopic evidence for the damage that occurs to the subcellular structures of the myocardium in anoxia, and more importantly, upon readmission of oxygen. It was already known that cytosolic enzymes were released from the heart under these conditions; the experiments reported in this paper, however, extended this work to investigate myocardial ultrastructure during anoxic substrate-free perfusion, during anoxic perfusion in the presence of glucose, and during reoxygenation.

A brief diversion inside a simplified myocardial cell may help the reader understand the significance of the ultrastructural changes observed by the authors. Central to the cell, both geographically and functionally, are the mitochondria and the myofibrils; the former provide the energy for contraction and the latter are the contractile machinery. Marking the boundary of each cell and controlling the entry and exit of assorted ions and molecules is the sarcolemma (or plasma membrane), composed of phospholipids and proteins; beyond that is the basement membrane composed mainly of collagen and assorted glycoproteins. At various points along its circumference, the sarcolemma becomes involuted and forms the T-tubules, thus bringing the extracellular space into close juxtaposition with the center of the cell.

Isolated rat hearts were perfused with anoxic, substrate-free buffer at 37°C. In one group of hearts, perfusion was continued for 7 hours and the release of the enzyme creatine phosphokinase, CPK (now known as creatine kinase, CK) was determined in effluent samples every 15 minutes. The profile was approximately linear, with effluent enzyme activities reaching 150 mIU•mL^{-1} coronary flow after 6 hours. The inclusion of glucose (11 mM) in the buffer decreased this enzyme release by 75%. Reoxygenation at 150 minutes caused an explosive enzyme release (a 100-fold increase in 2 minutes) that subsequently subsided to normal levels within about 20 minutes.

The electron micrographs show the ultrastructural changes beautifully. After 150 minutes of anoxia, slight damage to the cells has already occurred: the T-tubules are dilated, and the sarcolemma, although still intact, is distorted; all other structures are normal. At this time, dark, electron-dense granules are visible in the mitochondria and the cytosol (calcium phosphate deposits, the authors suggest). After a further 30 minutes of anoxia, there is, in addition, some edema and some distortion of the myofibrils although the mitochondrial membrane and the sarcolemma remain intact. The presence of glucose during anoxia prevented the occurrence of these changes. The effects of reoxygenation are dramatic: the sarcolemma becomes fragmented, the basement membrane is lost, the electron-dense granules disappear, and many of the mitochondria become markedly swollen. The mitochondrial membranes themselves, however, remain intact, even after 30 minutes.

These studies show that oxygen-related damage to the myocardium starts at the periphery of the cell and gradually "moves inside." The observation that the mitochondrial membranes always remain intact correlates with the absence of any mitochondrial enzymes in the effluent, while the massive release of cytosolic enzymes (eg, CK) and the loss of the electron-dense granules on reoxygenation correlate with the damage observed to the sarcolemma. The authors speculate that the reoxygenation damage may be caused by large, rapid shifts of ions, such as calcium, across the plasma membrane.

1975

Charlie Chaplin is knighted by Queen Elizabeth II, Steven Spielberg's "Jaws" is screened, and the cost of a first class stamp in the USA increases from 10 to 13 cents

Myocardial metabolism in ischemic heart disease: basic principles and applications to imaging by positron emission tomography

P. Camici, E. Ferrannini, L.H. Opie

This review is useful for people who have insufficient time (or motivation) to wade through a hefty tome on cardiac metabolism in health and disease; if readers become inspired to learn more about the subject, they can always refer to the 77 references! The section on metabolic imaging by positron emission tomography (PET) is short, but interesting.

The authors start with an acknowledgement of Richard Bing and his pioneering work on coronary sinus catheterization in 1947. In their discussion of the method he used to determine the substrate utilization of the human myocardium, the authors highlight an important problem which also applies to the "new," noninvasive techniques used in metabolic biochemistry today (eg, nuclear magnetic resonance (NMR) spectroscopy, PET). It is the fact that it is extremely straightforward to acquire a relative measurement of a metabolite concentration but that it is often quite difficult (and very time-consuming) to acquire an absolute measurement. In terms of the arteriovenous (AV) difference method, if one wants to obtain a qualitative view of which substrates are used, there are no problems. However, as the authors point out, for absolute determinations, one also requires an accurate measure of blood flow and of the release rates for the substrate.

The authors review a large amount of clinical data obtained by themselves and others, from AV difference measurements; these indicate, not surprisingly, that the fasting human heart has a net uptake of (in decreasing order) free fatty acids (FFA), glucose, lactate, ketone bodies, amino acids, and pyruvate while the fed heart has a net uptake of glucose, lactate, and pyruvate. The shift from mainly FFA metabolism in the fasted state to mainly glucose metabolism in the fed state is effected by the glucose-FFA acid cycle of Randle et al *(see summary on page 102).*

In the last section of the review, a very brief summary of the principles of PET is given, followed by some examples of clinical PET images using ^{82}Rb, a flow marker, and [^{18}F]2-fluorodeoxyglucose (FDG),

a glucose analogue. The authors highlight the fact that the increase in the glycolytic rate observed under conditions of decreased flow forms the basis for the visualization of ischemia by the PET technique. At rest, patients with stable angina had PET scans that were identical to those of control subjects, but upon exercise, the patients had nonuniform uptake of the tracers and showed a greater increase in ^{18}FDG uptake in the ischemic region than in the nonischemic region. During the postexercise recovery period, an increase in ^{18}FDG uptake was still observed in the previously ischemic region, even though flow had returned to normal; the authors suggest this may be due to increased glycogen synthesis. The "mismatch" between ^{18}FDG uptake and that of a flow marker can be used to predict the recovery of regional myocardial function in patients prior to coronary artery bypass surgery; the reason for this is that the glycolytic rate will be increased in moderately ischemic (salvageable) tissue but not in totally ischemic (infarcted) tissue. (Although PET is a very exciting technique for visualizing cardiac ischemia, one needs to be aware that the ability of ^{18}FDG to follow glucose uptake has only been fully validated in the brain, a "fussy eater," and not in the omnivorous heart where the varying use of competing substrates may complicate the analysis.)

1989

The "Velvet Revolution" marks the end of Communist party rule in Czechoslovakia, the Berlin Wall is demolished, and Emperor Hirohito of Japan dies

Effects of glucose and fatty acids on myocardial ischaemia and arrhythmias

M.F. Oliver, L.H.Opie

Oliver and Opie, in this short review, essentially give us an update on the facts about the beneficial effects of glucose and the detrimental effects of free fatty acids (FFAs) as fuel sources for the ischemic myocardium. It revisits old territory in terms of the facts but provides up-to-date evidence for mechanisms.

The authors remind their readers that the rise in plasma free fatty acids during and immediately after infarction (caused by the increase in circulating catecholamines) was first observed over 35 years ago. They also point out that it is when the FFA concentration exceeds the FFA-binding capacity of plasma albumin that an increase in the generation of arrhythmias occurs. These early findings were summarized as indicating that "provision of glucose is 'good' and that of a raised circulating FFA concentration is 'bad' for the ischemic myocardium." The authors feel that, despite this knowledge, the clinical management of acute ischemia is rarely attempted from a metabolic viewpoint. They cite the lack of nontoxic lipolytic agents, the nonreproducibility of the early glucose-insulin-potassium (GIK) trials, and the complex relationship between fatty acids and arrhythmogenesis as reasons for this.

Oliver and Opie suggest that many of the early studies on GIK, in which no beneficial effects were observed, were flawed in that, firstly, the patients often arrived at the hospital after the acute ischemic phase had already occurred (when metabolic damage was no longer reversible) and, secondly, the concentrations of the glucose, insulin, and potassium that were infused were often inadequately controlled. Although experimental studies (on isolated hearts) have also produced conflicting evidence about the benefits of increased glycolysis, the authors point out that the more clinically relevant models usually showed beneficial effects.

The mechanisms by which fatty acids generate arrhythmias are discussed in the light of fairly recent data about the actions of fatty acid derivatives on various cation movements in the heart. It has been known for many years, and reviewed many times, that ischemia inhibits the oxidation of fatty acids (a mitochondrial event), thus causing an increased concentration of acylcarnitine in the cytosol. Recently, this molecule has been shown to inhibit two ion transport mechanisms in the sarcolemma, the Na^+-K^+ pump and the Na^+-Ca^{2+} exchanger, and one in the sarcoplasmic reticulum, the Ca^{2+} pump. Thus, fatty acids, via acylcarnitine and inhibition of ion transport, could increase cytosolic calcium and thus promote arrhythmogenesis.

The review continues with a discussion of the four key metabolic interventions that could be beneficial after an acute ischemic episode. These are: a decrease in noradrenaline release; a decrease in FFA availability; the prevention of calcium overload and the provision of membrane-related ATP. Preliminary evidence, both clinical and experimental, is also mentioned for the relevance of the type of fat in the diet, namely that an increase in the ratio of polyunsaturated-to-saturated fats can be of benefit in terms of a decreased incidence of arrhythmias during ischemia.

The authors conclude this review by saying that "adequate clinical trials of GIK or antilipolytic treatment have not been done. These agents should be evaluated during the prehospital phase of acute myocardial infarction, and added to standard thrombolytic regimens."

1994

The first democratic elections are held in South Africa, Brazil wins the World Cup, and the 50th anniversary of the D-Day Landings is celebrated

Determinants of a protective effect of glucose and insulin on the ischemic myocardium. Effects on contractile function, diastolic compliance, metabolism, and ultrastructure during ischemia and reperfusion

C.S. Apstein, F.N. Gravino, C.C. Haudenschild

Apstein and colleagues address the question of whether "glucose plus insulin" therapy can protect the ischemic heart against damage. The authors are acutely aware that the literature on this topic is extensive but confusing and they highlight two factors that they consider important: the first is the degree of ischemia to which the heart is subjected, which obviously affects the washout of potentially damaging metabolic products, and the second is the endogenous glycogen content which could potentially affect the importance of the exogenously supplied glucose.

The isolated, perfused rabbit heart was used, with a left ventricular balloon to make the preparation isovolumic. The buffer always contained lactate (1 mM) and, in addition, either normal glucose levels (5 mM) or high levels of both glucose and insulin (25 mM glucose plus 100 U•L^{-1} insulin) in what is termed the G+I group. Two degrees of ischemia were defined: either "moderate" ischemia (3% of control flow rate) or "severe" ischemia (0.3% of control flow rate). Hearts were reperfused at the end of ischemia and the effects on contractile function, ultrastructure, and metabolism were determined. Lactate was determined in the effluent (as a measure of the glycolytic rate) throughout the protocol and ATP and PCr were quantified at the end of ischemia.

Both moderate and severe ischemia caused an increase in the rate of glycolysis in all hearts for the first 30 minutes of flow reduction. Subsequently, there was a steady decrease in glycolytic activity in control hearts and the development of ischemic contracture mirrored this decrease. By the end of ischemia, the ATP and PCr contents of all hearts were, not surprisingly, decreased, although the G+I treatment had limited the ATP decrease in the moderately ischemic group. The G+I treatment also improved postischemic contractile function (67% vs 45%) in the moderately ischemic hearts, but had no effect in the severely ischemic group. Ultrastructural damage, which was prevented by G+I treatment only in the moderately ischemic group, consisted of disruption to the myofibrils, formation of contraction bands, and mitochondrial swelling.

The data show that, under conditions of reduced flow, there is a marked initial increase in the glycolytic rate, due, presumably, to the hypoxia induced in the cells. The authors propose that only during moderate ischemia, when flow is sufficient to wash out the lactate[1] produced, can this high glycolytic rate be sustained, although the data on this point are slightly confusing. The authors point out that it is only after about 30-40 minutes of ischemia that different rates of glycolysis are seen in the G+I groups, compared to controls. They attribute this to the preferential use of the endogenous glycogen stores in the initial period, stores that only become depleted, according to the literature, after approximately 30 minutes. The authors conclude that G+I treatment is protective against moderate ischemia, but ineffective against severe ischemia.

The authors are acutely aware that there are innumerable differences between their experimental system of isolated, buffer-perfused rabbit hearts exposed to global, low-flow ischemia and the hospital patient with an acute coronary occlusion, but feel that, when emergency reperfusion is anticipated, the use of G+I would not be inappropriate.

[1] *It is now known to be the protons, produced from ATP hydrolysis, rather than the lactate per se, that inhibit glycolysis during ischemia.*

1983

Lech Walesa wins the Nobel Peace Prize,
Australia wins the America's Cup,
and the final episode of "M.A.S.H."
is broadcast in the USA

Myocardial ischemia - observations, definitions and speculations

R.B. Jennings

J Mol Cell Cardiol. 1970;1:345-349

In this short editorial, Jennings reviews the essential features of myocardial ischemia, and what is known about its metabolic and functional consequences, from both clinical observations and experimental data. The author begins with a literal definition of the word "ischemia" as meaning "to hold back blood" (from the Greek), and says that obstructions can occur either by the formation of a thrombus or by the narrowing of coronary vessels. He first defines ischemia as developing "whenever the flow of arterial blood through the diseased vessels is reduced to a volume below that required by the myocardium for adequate function." However, since cardiac function, and thus coronary flow, vary, both between individuals and in a single individual throughout the day, such a definition, he points out, is of little predictive value. The author, therefore, refines his original definition by saying that "ischemia occurs whenever the arterial blood flow is insufficient to provide enough oxygen to prevent intracellular respiration from shifting from the aerobic to the anaerobic form." Although the author was probably unaware of it at the time, this is a very concise expression of the theory underlying myocardial viability studies using positron emission tomography (PET).

After these initial, vital definitions, the author goes on to give a qualitative description of the biochemical events that follow the onset of myocardial ischemia. The cells become cyanotic and the oxygen tension decreases; glycolysis becomes the main pathway for the generation of ATP, with endogenous glycogen as the initial substrate and lactate as the end product. The poor rate of ATP generation from this process, however, causes a decrease in cellular ATP and creatine phosphate,[1] a concomitant rise in ADP, AMP, P_i, and creatine, and ultimately, contractile failure. Electrocardiographic changes appear simultaneously, due to metabolically induced changes in the potential across the cell membrane. If coronary flow is not restored to the tissue, the cells will eventually die, and it is only at this point, following cell death, that the affected tissue should be termed "infarcted." Dead tissue should not be referred to as ischemic tissue,

since, as Jennings stresses, the word ischemia "carries a connotation of continued, albeit diminished, function, as well as potential viability."

The author then proposes what has subsequently become known as the "border zone" hypothesis of infarction, namely, that there is a continuum of death after an occlusive event, such that the central area is probably dead, and thus infarcted, tissue, while the outermost zones are probably intermittently ischemic for hours or even days before either dying or being salvaged. At this point, Jennings reminds his readers that ischemia is not the sine qua non of cell death and that nonischemic cells can die too. Since the two modes of death have different underlying causes and different metabolic results, he stresses that the two should be carefully distinguished from one another. He points out that the nonischemic version should be termed "necrosis" and that the ischemic version should be termed "infarction." Unfortunately, not all authors follow this useful advice.

Jennings concludes his editorial by stating that "knowledge of the general characteristics of metabolism in ischemic cells as well as knowledge of the factors which alter the metabolism of these cells is important relative to developing therapeutic measures designed to prevent or reduce cell death in patients with acute myocardial ischemic injury."

[1] *An incorrect name, in fact, since it is not a phosphate grouping - it should be, and now usually is, called phosphocreatine, PCr.*

1970

Alexander Solzhenitsyn wins the Nobel prize for literature, Concorde makes its maiden flight, and the Baltimore Orioles defeat the Cincinnati Reds (4-1) to win the World Series

Metabolic changes during post-ischaemic reperfusion

R. Ferrari, S. Curello, A. Cargnoni, E. Condorelli, S. Belloli, A. Albertini, O. Visioli

Ferrari and colleagues investigate, in a whirlwind fashion, some of the metabolic events that occur during ischemia and lead to irreversible damage. They begin in the clinic, with a group of patients undergoing intracoronary thrombolysis, and then move to the laboratory, where firstly, they study groups of isolated, perfused hearts and, secondly, groups of isolated mitochondria!

Clinically, nine patients were selected. These patients were diagnosed as having transmural acute myocardial infarction, and angiography revealed occlusion of the left anterior descending (LAD) or circumflex coronary arteries. Thrombolysis (with urokinase) was initiated within 5 hours of the onset of symptoms and electrocardiographic, anatomical, metabolic, and functional parameters were followed for the next 50-70 minutes. Arterial and coronary sinus concentrations of glucose, lactate, free fatty acids, oxygen, and creatine kinase were measured in all patients. Sadly for the reader, the authors present the metabolic data for only two of the patients, chosen on the basis of similar coronary anatomy but differing durations of ischemia. In this extremely small sample, it can be seen that, before thrombolysis, both patients have a complete occlusion of the LAD, elevation of their ST-segments, small arteriovenous (AV) differences for glucose and fatty acids, and a higher concentration of lactate in the coronary sinus than in arterial blood, indicating a large amount of anaerobic metabolism. In the patient receiving thrombolytic therapy 160 minutes after the onset of symptoms, there was an almost immediate rise in the venous concentrations of lactate and creatine kinase; these subsequently decreased until, 50 minutes after thrombolysis, the heart was restored to aerobic metabolism, ie, it showed a positive AV difference for lactate. The left ventricular ejection fraction increased from 35% to 55% (1 month after the event). In contrast to this successful treatment, when thrombolysis was carried out 335 minutes after the onset of symptoms (in the other patient), the heart was still anaerobic 70 minutes later and there was no improvement in the ejection fraction (it changed from 30% to 26%, 1 month later).

In the experiments on isolated rabbit hearts, low-flow ischemia (4% of control flow rate) was induced for 120 minutes and reperfusion was initiated after either 30 minutes or 90 minutes (to simulate the two clinical cases). When reperfusion occurred after 30 minutes, there was a total recovery of contractile activity, a complete return to aerobic metabolism (as indicated by lactate measurements), and a small, but transient, release of creatine kinase; thus, the ischemic changes that had occurred were fully reversible. In contrast, reperfusion after 90 minutes led to exacerbation of the deleterious changes that had already occurred during ischemia. Two of these changes were a decrease in total tissue magnesium and an increase in mitochondrial calcium.

Results from the experiments on isolated mitochondria indicated that magnesium inhibits calcium uptake. The authors, therefore, propose that the calcium influx and the magnesium efflux, which occur in the myocardial cells upon reperfusion, cause the mitochondria to take up massive amounts of calcium (an ATP-requiring process). A final group of experiments, in which ischemic hearts were initially reperfused with a "high magnesium-low calcium" buffer, showed improvements in all the parameters measured.

The authors conclude that "the outcome of ischemia is not determined only by events occurring during the ischemic period. Conditions of reperfusion influence the capacity of recovery and a reduction of mitochondrial calcium accumulation may be beneficial even after prolonged ischemia."

1988

Australia celebrates its bicentennial, Stefan Edberg wins the Men's Singles at Wimbledon, and Benazir Bhutto is elected Prime Minister of Pakistan

Glucose and palmitate oxidation in isolated working rat hearts reperfused after a period of global ischemia

G.D. Lopaschuk, M.A. Spafford, N.J. Davies, S.R. Wall

Circ Res. 1990;66:546-553

Addressing the issue of which substrate is used preferentially by the myocardium during postischemic reperfusion, the authors of this paper rightly acknowledge that quite a number of studies have already been carried out in an attempt to resolve this issue (in dogs, pigs, and humans). However, they point out that the lack of agreement of the results is probably due to a lack of control over such factors as hormonal status, myocardial workload, concentration of fatty acids in the blood, and the variation in the endogenous pools of glycogen and triglycerides. The authors have chosen to use the isolated, working rat heart in order to have some control over these variables.

In their studies, Lopaschuk and colleagues investigated groups of hearts perfused with glucose alone, or with glucose and 1.2 mM palmitate (the blood level that is reached clinically immediately post infarction). By labelling either the glucose or the palmitate with ^{14}C and following the production of $^{14}CO_2$, they were able to calculate the oxidation rates of either of the substrates. Control perfusion periods of 60 minutes were compared with 60 minutes of reperfusion following 25 minutes of ischemia.

It was found that the rate of glucose oxidation during reperfusion was the same as that found under control conditions and that the presence of palmitate decreased both these glucose oxidation rates by 90%. Although palmitate oxidation rates during reperfusion were not different from control rates, the incorporation of palmitate into triglycerides doubled during reperfusion. The authors point out that this increased incorporation of fatty acids into triglycerides may account for some of the positron emission tomography (PET) data obtained from reperfused myocardium, in which the blood clearance of [^{11}C]palmitate was found to decrease in an unexplained manner. It also underlines the importance of conducting detailed, in vitro metabolic studies in order to understand clinical PET data.

In terms of contractile recovery, when glucose was the sole substrate provided, the hearts recovered 100% after a 25-minute period of ischemia. In the presence of glucose and 1.2 mM palmitate, this recovery was decreased by 33%; addition of etomoxir (a blocker of fatty acid utilization) under these conditions increased glucose oxidation and restored postischemic recovery to 100%.

The authors demonstrate that, in the isolated rat heart, fatty acids are the preferred substrates of the heart during postischemic reperfusion, just as they are under normal, aerobic conditions. This finding is in agreement with many other groups. In contrast to the previous reports in the literature, however, which showed either increased or decreased rates of fatty acid oxidation postischemically (compared to control rates), Lopaschuk et al find no change in fatty acid utilization. They point out that this discrepancy is probably a result of differences in experimental conditions. However, if fatty acid utilization is blocked post-ischemically, with a metabolic inhibitor, the resultant increase in glucose oxidation leads to an improvement in contractile function. They propose that the restricted use of glucose under normal, postischemic conditions may be one of the underlying causes of myocardial "stunning." They also suggest that "the control of markedly elevated plasma fatty acid levels or stimulation of glucose oxidation in the reperfused ischemic myocardium may be of clinical benefit."

1990

Nelson Mandela is freed,
John Major becomes Prime Minister
of Great Britain, and Saddam Hussein
invades Kuwait

Cardiac Metabolism

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