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This study was designed to determine the effects of elevated fatty acid and lowered glucose concentrations on myocardial contractile function and substrate selection during hypoperfusion. Coronary perfusion pressure (CPP) was lowered in the left anterior descending coronary artery of open-chest anesthetized dogs. Glucose uptake, fatty acid uptake, and percent segment shortening (%SS) were determined with normal arterial FFA concentrations (Group 1) or with elevated concentrations (Groups 2 and 3). When glucose was removed by dialysis in Group 3, FFA uptake increased and glucose uptake decreased relative to Group 1 at 40 mmHg CPP ($p<0.05$). Oxygen consumption significantly increased ($p<0.05$); however, %SS was unchanged. Thus, although the myocardium switches from fatty acid to glucose metabolism to increase oxygen utilization efficiency during hypoperfusion, blocking this switch does not contribute to a further decrease in myocardial contractile function.

THE EFFECTS OF HYPERLIPIDEMIA AND HYPOGLYCEMIA ON MYOCARDIAL CONTRACTILE FUNCTION AND OXYGEN UTILIZATION DURING CORONARY HYPOPERFUSION

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CHAPTER I

INTRODUCTION

Myocardial metabolic substrate selection has been investigated extensively for years. The two major substrates used for energy by the heart have been shown to be carbohydrates (glucose, lactate) and fatty acids. Although ketone bodies and amino acids can also be utilized, neither is a major substrate under normal circumstances (26). Since Randle *et al.* proposed the glucose/fatty acid cycle in 1963 (38), much time and effort has been devoted to the elucidation of the control mechanism(s) involved. This cycle suggests that glucose and fatty acid metabolism are strongly coupled (38). This interaction is discussed in more detail below. At increased blood glucose levels, as after a meal rich in carbohydrates, myocardial glucose uptake will increase, while fatty acid uptake will fall. Conversely, during fasting, fatty acid uptake is increased, and glucose utilization is minimal (11). This cycle also suggests the myocyte has the potential to preferentially utilize glucose as the major oxidative substrate in the presence of limited oxygen supply (15). This differs from normoxic conditions, where fatty acid β-oxidation accounts for 60–100% of myocardial oxygen usage (see figure 1) (43).

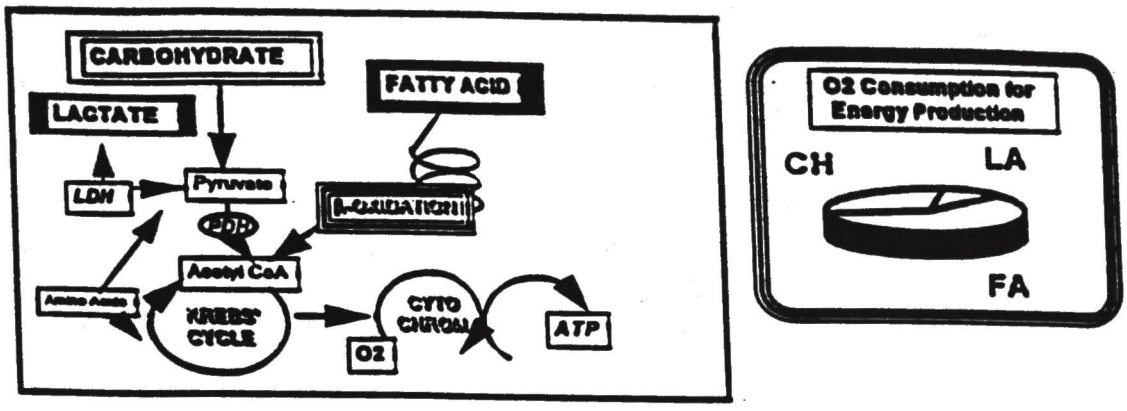

Figure 1. Diagram of the major metabolic pathways involved in ATP formation by the myocardium. Inset demonstrates the relative percentage of total oxygen consumption accounted for by oxidation of the major substrates of the heart. Adapted from Grynberg *et al.*, J. Cardiovas. Pharm. 28:S11-S17, 1996. CH = Carbohydrate, FA = Fatty Acid, LA = Lactate.

The heart cell seems to be able to select the substrate that is most appropriate for a given metabolic condition, and has the potential to adjust its substrate selection quickly. Many complex interactions between the glucose and fatty acid oxidative metabolic pathways exist, and to this date the exact importance of selective metabolism has not been determined. It has been well accepted, but the actual significance of this "Randle cycle" is not clear.

With recent advances in research, substrate selection can be more closely analyzed. Radioactive labeling provides the ability to determine the origin of carbon atoms within the acetyl-CoA or Krebs cycle intermediates, allowing determination of the relative amount of each substrate used by the myocardium (8, 34). Positron Emission Tomography (PET) has also emerged as a

noninvasive technique to access fatty acid metabolism (3). Further improvements in techniques and measurements will provide even more insight into myocardial metabolism.

Fatty Acid Uptake and Utilization

Fatty acids are the preferential substrate for the heart under normal conditions. The energy yield per substrate molecule is far higher than other substrates. Complete oxidation of one molecule of palmitate (C-16), for example, will yield 130 ATP molecules, whereas a molecule of glucose yields only 38 (26). Fatty acids are highly utilized in the heart cells to meet the high energy demands, especially after a high fat intake (see Figure 2).

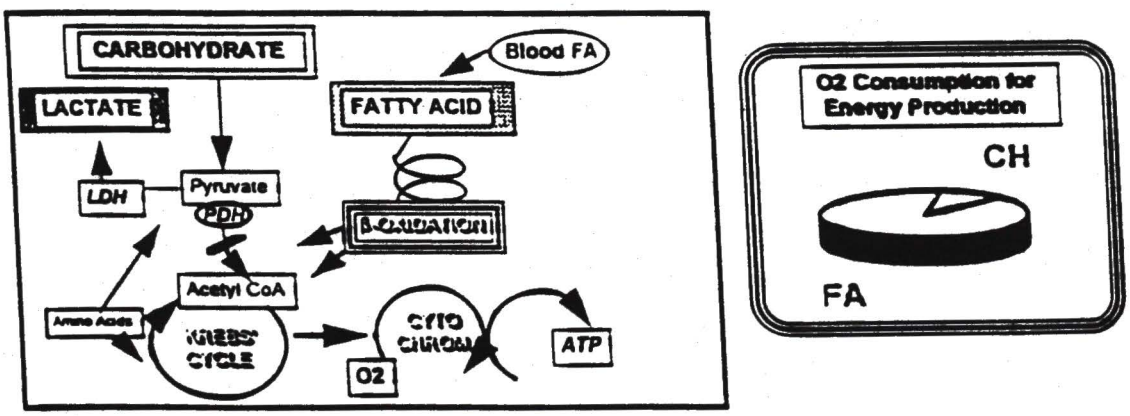

Figure 2. Diagram of the major metabolic pathways involved in ATP formation by the myocardium after a large intake of fat. Inset demonstrates the relative percentage of total oxygen consumption accounted for by oxidation of the major substrates of the heart. Adapted from Grynberg *et al.*, J. Cardiovas. Pharm. 28:S11-S17, 1996. CH = Carbohydrate, FA = Fatty Acid.

Upon digestion, fatty acids are transported to the heart as free fatty acid bound to the protein albumin or as triglycerides within lipoproteins and chylomicrons. Lipoprotein lipase, released by myocytes and transported by unknown mechanisms to the lumenal endothelial membranes of the heart vessels, causes release and hydrolysis of the triglycerides (48). Fatty acids cross the lumenal surface membrane and travel through the interstitium bound to the protein albumin. They then enter the myocyte by an undetermined mechanism (2, 15). Since albumin cannot traverse the sarcolemma, there is evidence for an active process for release of free fatty from the albumin complex, followed by an active or passive fatty acid uptake by the myocyte (44).

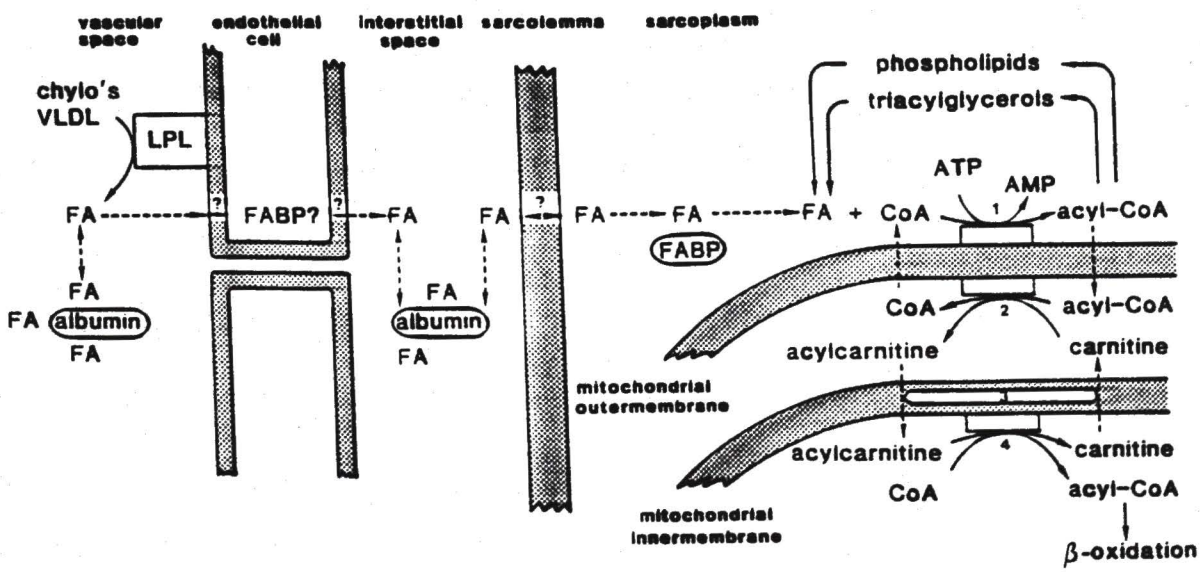

Figure 3. Schematic representation of the uptake, transport, and activation of fatty acids (FA) in the myocardium. Numbers refer to enzymes: 1) acyl-CoA synthetase, 2) carnitine acyltransferase I, 3) carnitine acylcarnitine translocase, 4) carnitine acyltransferase II. Adapted from Van der Vusse, *et al.*, Physiol. Rev. 72 (4): 881-940, 1992.

Once inside the cytosol, the now free fatty acids bind to a fatty acid binding protein (FABP) and are either activated into fatty acyl-CoA, grouped as triglycerides, or converted into phospholipids for membrane incorporation (48). The fatty acyl-CoA is translocated by carnitine palmitoyl-transferase I (CPT I) located in the outer mitochondrial membrane into an association with carnitine within the mitochondrial matrix (see Figure 3). The fatty acid, through the action of carnitine palmitoyl-transferase II (CPT II), is then translocated into the mitochondrial matrix for β-oxidation (26). The regulation of this fatty acid uptake and utilization is far from certain, and much debate exists over the exact regulatory mechanisms.

The rate of fatty acid uptake and oxidation by the heart muscle is largely dependent upon three main factors: free fatty acid plasma concentration and availability, rate of uptake across the outer mitochondrial membrane into the matrix, and the actual rate of acetyl-CoA flux through the citric acid cycle (7). Fatty acid utilization depends largely upon plasma fatty acid concentrations and the plasma fatty acid: albumin ratio (7, 30). With an increase in plasma concentrations and this ratio, fatty acids are more loosely bound to albumin, and the rate of uptake by the cells is increased (30).

Mitochondrial uptake of fatty acids is largely dependent upon the activity of CPT I, which has been shown to be the rate-limiting step for fatty acyl-CoA import into the mitochondrial matrix (26, 48). Thus, CPT-I has been implicated as one of the major sites of fatty acid metabolic control. Malonyl-CoA is a strong inhibitor of this enzyme, and may be one of the more important factors limiting fatty acid uptake and oxidation by the myocyte (1, 26, 39). L-carnitine inhibition of CPT-I and malonyl-CoA production by acetyl-CoA carboxylase are other sources of regulation of fatty acid utilization at the site of fatty acid uptake into the mitochondria. In addition, the overall rate of citric acid cycle flux, subject to control by several mechanisms, plays a role in the rate of β-oxidation (7). Pyruvate dehydrogenase (PDH) also contributes to this regulation and its activation inhibits fatty acid utilization, although the exact mechanism is unknown (26).

High rates of fatty acid metabolism have been demonstrated to inhibit glucose uptake and oxidation (43). Utilizing the above mechanisms, and others that may be unknown, the myocyte regulates the amount of energy derived from β-oxidation or from glycolysis. During periods of low blood flow, hypoxia, or ischemia, these mechanisms have been shown to decrease fatty acid utilization in greyhound dogs (36). The beneficial effects of decreased fatty acid utilization remain to be determined.

Glucose Uptake and Utilization

Glucose utilization as a metabolic substrate is also highly regulated. Glucose uptake is known to be dependent on the transmembrane gradient, which is affected by glucose concentration, and on the concentration and activity of glucose transporters associated with the sarcolemmal membrane (43).

During post-prandial conditions, the heart still utilizes fatty acids; however, with plasma glucose levels elevated, plasma glucose metabolism is enhanced relative to fasting levels (see Figure 4). When glucose was elevated to 150 mg/100ml, similar to levels found after a carbohydrate meal, myocardial glucose uptake increased from 14.3 μmol/min to 29.2 μmol/min (51). This demonstrates

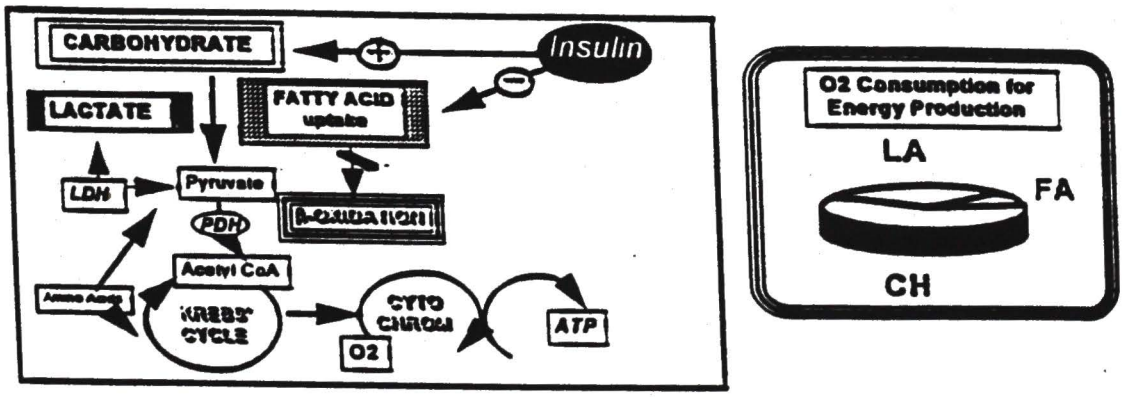

Figure 4. Diagram of the major metabolic pathways involved in ATP formation by the myocardium after a carbohydrate meal. Inset demonstrates the relative percentage of total oxygen consumption accounted for by oxidation of the major substrates of the heart. Adapted from Grynberg *et al.*, J. Cardiovas. Pharm. 28:S11-S17, 1996. CH = Carbohydrate, FA = Fatty Acid, LA = Lactate.

the concentration-related response in uptake seen also with fatty acids. Increased insulin levels after a meal also contribute to the increase in glucose and decrease in fatty acid utilization. PDH becomes active, β-oxidation is reduced, and glucose can account for 60 – 100% of the oxygen consumption (11). The action of insulin is to increase GLUT-4 and GLUT-1 glucose transporters into the sarcolemmal membrane, making more available for glucose retrieval (4). Glucose transporter concentration may be the most important regulator of glucose utilization, as indicated by the insulin-mediated increase in glucose uptake values (31, 46, 47).

The intricate regulations of the Randle cycle once again play an important role with glucose metabolism. High fatty acid concentrations have been shown to inhibit glucose uptake and oxidation in humans, while lowering these levels have increased glucose uptake (43). The preferential utilization of fatty acids in these conditions involves inhibition of carbohydrate utilization by decreased glucose transport, as well as increased glycogen synthesis (7). Fatty acids may also restrict glucose oxidation directly by inhibiting several enzymatic steps in the glycolytic pathway (23, 27, 30). End products associated with fatty acid oxidative metabolism, such as NADH and acetyl-CoA, are known inhibitors of glucose utilization. These end products inhibit PDH and therefore inhibit glycolysis. Phosphofructokinase (PFK) is another key glycolytic enzyme that is

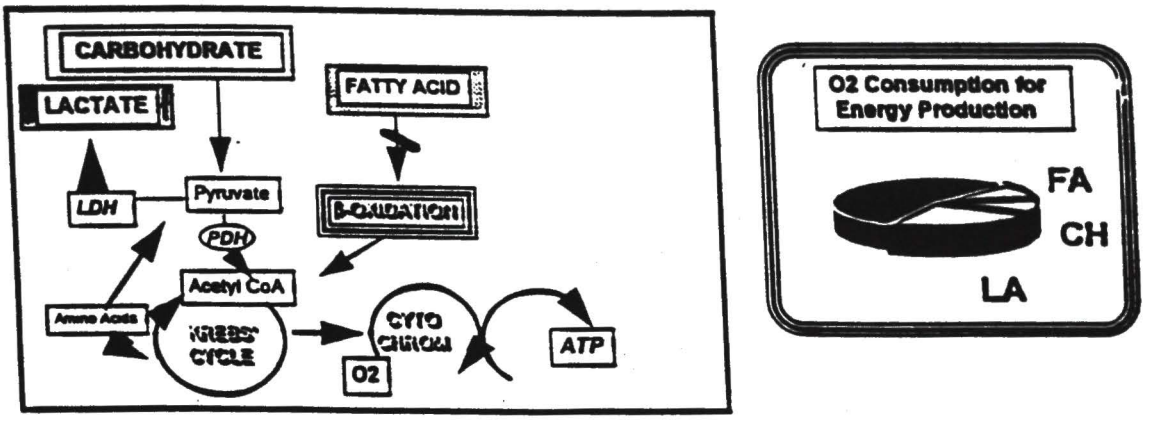

Figure 5. Diagram of the major metabolic pathways involved in ATP formation by the myocardium during mild ischemia (~60 % reduction in flow). Inset demonstrates the relative percentage of total oxygen consumption accounted for by oxidation of the major substrates of the heart. Adapted from Grynberg *et al.*, J. Cardiovas. Pharm. 28:S11-S17, 1996. CH = Carbohydrate, FA = Fatty Acid, LA = Lactate.

subject to inhibition by citrate during increased fatty acid metabolism (15). Glucose oxidative metabolism can potentially be beneficial in conditions of oxygen deprivation. Glucose is usually available, and the myocyte may prefer glucose as a substrate. In hypoxic or ischemic conditions, however, the heart may lose its intrinsic ability to select the substrate that may be more oxygen efficient (30, 43, 48). Under these circumstances, lactate and glucose account for a large portion of the oxygen consumption, and fatty acid β-oxidation is greatly reduced by mechanisms previously discussed (see Figure 5). This may result in an increased efficiency of oxygen utilization by the heart as described below. To date, however, the relative importance of substrate selection has yet to be confirmed.

Oxygen Considerations

The pathways of oxidation for glucose and fatty acids differ in terms of oxygen requirements. ATP production from glucose is less oxygen-consuming than from fatty acids. Oxidation of palmitic acid yields 129 moles of ATP per mole of substrate, whereas glucose yields 38. However, palmitate oxidation uses 46 atoms of oxygen per mole of substrate, and glucose uses only 12 (11). These data lead to the calculation of an ATP/O (P/O) ratio of 2.83 for fatty acids and 3.17 for glucose (30). Therefore, the ratio would increase about 12% if substrate oxidation shifts from 100% fatty acid to 100% glucose utilization (33).

The increased oxygen demand associated with fatty acid β-oxidation is easily met under normal metabolic conditions, but may be detrimental during low-flow or ischemic conditions. This present study will closely analyze these conditions and the effects observed by directly influencing substrate availability.

Detrimental Effects of Fatty Acid Oxidative Metabolism

Fatty acid utilization has been shown in numerous studies to increase myocardial oxygen consumption (MVO_2) (12, 20, 40, 49). This is not surprising knowing the oxygen requirements for fatty acid oxidation. In light of this, we can

expect several detrimental effects of fatty acid metabolism, especially during hindered oxygen delivery. The proposed mechanisms of these harmful effects include: (1) accumulation of toxic intermediates of fatty acid metabolism, (2) inhibition of glucose utilization during ischemia and/or reperfusion, and (3) uncoupling of oxidative metabolism from electron transfer (15).

The accumulation of toxic intermediates such as NADH and acetyl-CoA causes profound problems for the oxygen-deprived myocardium. This build-up of intermediates is easily observed during hypoxic or ischemic conditions, resulting in inhibition of β-oxidation (15). The arrhythmogenic, cardiac depressant, and oxygen wasting effects of elevated fatty acid levels are probably due to this build-up of intermediates (7). Accumulation of the long-chain acyl-CoA and acylcarnitine intermediates tends to alter the calcium homeostasis within the cell, resulting in diminished contractile properties of the myocyte sarcomeres (15). Elevated concentrations of these intermediates have been shown to correlate with the amount of damaged mitochondria (9).

Many studies have documented the inhibition of glucose metabolism by fatty acid utilization, primarily due to an inhibition of PDH. In accordance with the Randle cycle, increased plasma fatty acid concentrations have been found to decrease glucose uptake in human hearts by as much as 30% (27, 38).

Decreased glucose oxidation enhances the fatty acid flux through β-oxidation, again leading to accumulation of toxic intermediates and depletion of the energy stores within the heart cells. Anaerobic glycolysis produces a limited supply of energy, and under hypoxic conditions, this energy is hypothesized to be used to maintain the functional integrity of the cell. This energy is thought to be used to maintain the sarcolemmal membrane, enzymes, and various ion pumps, a process termed "functional compartmentalization" (15). Enhanced fatty acid and decreased glucose oxidative metabolism may cause energy wasting (described below), and this energy used for maintaining cellular integrity may be diminished (15).

High levels of fatty acids during hypoperfusion have been shown to cause energy wasting, mainly due to the increased triglyceride pool turnover rate (33, 48). Fatty acids repeatedly enter triglyceride synthesis and lipolysis in a futile cycle that uses 7 ATP molecules for each triglyceride molecule (15, 48). The exact mechanisms of control for this cycle are unknown, but it is thought that increased fatty acid oxidation intermediates and increased glycerol-3-phosphate levels due to glycogen/glucose degradation may be responsible (48).

During moderate coronary hypoperfusion, the myocardium demonstrates protective mechanisms to maintain cell viability. Although the oxygen demands

by the myocyte during hypoperfusion are lessened by the diminished contractile function called "hibernation", the energy stores in the myocardium are initially reduced (15, 43). Under these circumstances, myocardial contractile work is reduced to balance the oxygen supply, and leads to a partial recovery of the energy stores. Flow restoration will bring about a return to normal contractile function (43). During this hypoperfusion, it would follow that fatty acid metabolism may be detrimental as the myocardium seeks to conserve oxygen. However, even during hypoperfusion with a 60% reduction in coronary blood flow in swine hearts, it has been shown that most of the myocardial energy needs is supplied by exogenous fatty acid metabolism (21, 22, 43).

Reperfusion following more severe hypoperfusion or ischemia can also lead to impairment of heart function. The post-ischemic myocyte is slow to recover mechanical function due to a variety of reasons, and it is characterized by increased oxygen consumption for the amount of work produced (43). Mitochondrial damage that was sustained during the ischemic period is slow to be repaired (27). Intracellular pH contributes heavily to the decrease in function during reperfusion (43). Elevated $[H^+]$ is not able to immediately return to normal levels. $[H^+]$ that accumulates during ischemic acidosis can exchange with Na^+ in the Na^+/H^+-transporter, and Na^+ will then exchange with Ca^{2+} through the Na^+/Ca^{2+}- exchanger. This can lead to an overload of Ca^{2+} within the

myocardium during reperfusion (26). This Ca^{2+} overload has been proposed to be a major cause of cellular damage, although a direct mechanism has not been established (45). Many other factors contribute to the impaired function during reperfusion, but the important consideration is that metabolic stability is difficult to obtain after a period when enzymes, intermediates, and ionic concentrations have become significantly altered. Arrhythmias are often observed during this critical period, as toxic metabolic intermediates have accumulated and ionic balances have been compromised. However, fatty acids have been shown to be necessary for cellular metabolism during this period, as some studies found impaired contractile function while inhibiting β-oxidation during reperfusion (15). In addition, reperfused rat hearts have been shown to preferentially consume fatty acids after 25-60 minutes of ischemia (10, 24).

The effects of plasma fatty acid elevation are not always definite. In addition to the above cited results, high fatty acid levels and elevated fatty acid: albumin ratios have been found to be responsible for the precipitation of cardiac arrhythmias (18, 19, 42, 50). Opposite results have been obtained, however, in other studies that failed to show a significant effect of elevated plasma fatty acids on cardiac electrical behavior (6, 17, 28, 29, 32). Therefore, it is necessary to further explore this area.

Regional and Global Myocardial Function

Many experiments have been devoted to examining the substrate utilization by the heart. Studies have concentrated on substrate uptake, substrate selection, oxygen extraction, and oxygen efficiency changes that may occur during periods during limited oxygen supply. Detailed oxidative analyses have revealed the increased oxygen expenditures required by fatty acid β-oxidation versus glycolysis. It is known that fatty acid metabolism increases oxygen consumption and decreases the efficiency of oxygen utilization, but less has been determined when attempting to correlate these results with the declining regional contractile and mechanical function of the working myocardium during hypoperfusion and ischemia.

Animal studies have shown impaired ventricular function during ischemia in the presence of excess fatty acids (15). Another study documented a decrease in myocardial function and an increase in oxygen consumption during reperfusion following ischemia in swine hearts with increased plasma fatty acid levels (20). Henderson *et al.* described depressed contractility and increased resting force in rat papillary muscles with excess fatty acid plasma concentrations during hypoxia and anoxia (13). Henderson *et al.* also demonstrated depressed contractility and increased diastolic pressure in rat

hearts perfused with excess fatty acids, but later cited the possibility that the hearts received lower coronary blood flow with increased oxygen demands (14).

However, other studies have not observed detrimental mechanical and contractile function due to fatty acid utilization during hypoperfusion. Ichihara and Neely failed to find any significant differences in the decreases observed in mechanical function of ischemic rat hearts in the presence of excess palmitate vs. normal concentrations (16). Pacold et al. demonstrated that Intralipid and heparin infusions actually caused an increase in left ventricular ejection fraction in patients with coronary artery disease (37). Burkhoff *et al.* reported that fatty acids increased MVO_2 compared to glucose, but the increase in oxygen demand was not work-related (5). Therefore, regional and global myocardial function parameters may or may not be significantly diminished with elevated fatty acid concentrations and increased fatty acid metabolism. Certainly, it has yet to be confirmed if the observed decrease in myocardial contractile properties during hypoperfusion or ischemia can be further reduced by a forced increase in fatty acid metabolism.

Clinical Applications

Within the clinical setting, many beneficial effects of substrate selection have yet to be confirmed. Substantial modifications in fatty acid and glucose metabolism have been found in patients following myocardial infarction, such as increased circulating fatty acid levels or glucose intolerance. Animal studies have indicated that these changes play a role in determining infarct size and frequency of arrhythmias. However, it has been shown that during myocardial infarction and subsequent recovery, plasma fatty acid levels tend to be higher, which exacerbates the detrimental effects caused by impaired oxygen delivery (33). Elevated fatty acid levels were found in rabbit hearts subjected to ischemia (25) and in rat hearts during myocardial stunning (8). Both studies showed impairment of recovery. Fatty acid metabolic inhibitors have even delayed the onset of angina in patients with coronary artery disease (15).

Glucose oxidative metabolism may prove beneficial in the clinical setting. Glucose-Insulin-Potassium (GIK) therapy introduced by Sodi-Pallares *et al.* has drawn clinical interest for over 30 years, possibly by increasing glucose utilization by the heart (41, 46). GIK infusions in patients following coronary bypass operations decreased the required inotropic balloon pump support needed post-operatively (15). Although these beneficial effects may be inconclusive, studies

do seem to implicate a possible use for substrate selection therapy in the diseased heart.

Recent reviews cite that increases in fatty acid usage during ischemia and reperfusion leads to a worsening of the outcome, suggesting a therapeutic application for substrate selection during myocardial infarction or coronary artery stenosis (43). It would be to our advantage to thoroughly explore the effects of changes in myocardial substrate utilization for possible clinical applications that may limit the detrimental effects of myocardial hypoxic and ischemic damage.

Conclusion / Rationale

With the wealth of information regarding glucose/fatty acid metabolism at our disposal, the Randle cycle remains surprisingly elusive. The relative importance of myocardial substrate selection and changes that occur during limited oxygen delivery are still controversial (15). The metabolic switch, which has been documented for years, remains unclear. The mechanisms responsible are undetermined. Cellular processes may be responsible for directly influencing the metabolic switch, or feedback mechanisms that exist among the oxidative pathways involved may be the controlling factor. The major focus of attention is the oxygen requirements by the different metabolic substrates available.

However, the ~12% increase in the P\O ratio may not prove to be a major benefit of metabolic selection or in maintaining contractile function even in the hypoperfused myocardium.

The working myocardium must always maintain a strict balance between oxygen supply and demand for proper function. To keep this balance during hypoxic conditions, the heart must either decrease myocardial power or increase oxygen utilization efficiency. Oxygen utilization efficiency has been defined as the ratio of a power index (heart rate x left ventricular pressure x % segment shortening) of the myocardium to its oxygen consumption. Recently, our laboratory has studied mechanical function in the hypoperfused dog model. Tune found that insulin treatment maintained contractile function in hypoperfused dog hearts with decreased O_2 delivery. Thus, under these conditions, he demonstrated increased oxygen utilization efficiency. Tune suggested that the more oxygen efficient P/O ratio resulted from a decrease in fatty acid oxidation and an increase in glucose uptake and utilization, but he was unable to rule out other possible positive inotropic effects of insulin (46). The results inspired my studies to closely examine the possibility of a metabolic switch from fatty acid to glucose metabolism during coronary hypoperfusion.

Specific Aims

The first specific aim of my investigation was to determine the effects of substrate selection on oxygen consumption. We hypothesized that after we remove glucose from the coronary perfusate blood, we would prevent the switch from fatty acid to glucose metabolism during hypoperfusion. This would increase oxygen consumption and decrease oxygen utilization efficiency. Moderate regional coronary hypoperfusion was produced by decreasing coronary perfusion pressure (CPP) from 100 to 60, 50, and 40 mmHg in the left anterior descending coronary artery of open chest, anesthetized dogs. After analyzing the results, we found an increased oxygen consumption during coronary hypoperfusion when glucose was not available, relative to control groups with normal blood levels of glucose. Therefore, we concluded that forced fatty acid β-oxidation during moderate ischemia causes an increase in oxygen usage by the myocardium. We also concluded that with normal arterial glucose concentrations, glucose utilization might be enhanced during hypoperfusion. This may act to lower the oxygen consumption during hypoperfusion.

The second aim for my experimental procedures was to attempt to quantify the metabolic switch that occurs during coronary hypoperfusion by directly measuring the myocardial uptake of fatty acids, glucose, and lactate. My hypothesis suggested that we would see a reduction in fatty acid uptake during

hypoperfusion and a concomitant increase in glucose uptake. This could not occur when glucose was removed by dialysis. Thus, we would prevent the expected metabolic switch. A model for hypoperfusion was generated as above, lowering CPP from 100 to 60, 50 and 40 mmHg. We found an increase in fatty acid uptake during hypoperfusion when glucose was removed from the arterial blood. We also succeeded in reducing glucose uptake to essentially zero by dialysis. We have thus concluded that with normal blood glucose levels, the heart will utilize glucose as a metabolic substrate during hypoperfusion perhaps preferentially to fatty acids, and removing arterial glucose supplies can prevent this switch.

A third specific aim was to analyze the effects of glucose removal from the coronary blood supply on regional contractile function, force generation, and global cardiac mechanical function. We hypothesized that glucose removal would prevent the switch to glucose metabolism, and that fatty acid utilization would be less oxygen efficient leading to decreased contractile function, less force generation, and a global reduction in general cardiac function. We generated a hypoperfused model as described above. Our results showed no significant difference between groups in any of the myocardial function or force measurements that we analyzed. We concluded that preventing the use of glucose during coronary hypoperfusion produces no significant decline in

function, and that the metabolic switch observed during moderate ischemia does not necessarily improve the contractile status of the oxygen-deprived myocardium.

Significance

The research community has devoted much time, money, and energy to study the metabolic substrate selection by the myocardium during times of impaired oxygen delivery. Our results have shown, by forcing fatty acid metabolism, that this substrate selection does not play a significant role in improving contractile function during hypoperfusion. Even with an increase in oxygen utilization efficiency provided by glucose metabolism, the ~12% increase in ATP produced per O atom molecule does not make an impact on regional contractility, at least not during the initial phase of coronary hypoperfusion. In addition, it should be noted that this ~12% increase would only be attained if the heart switched from *100%* fatty acid metabolism to *100%* glucose metabolism, and these conditions are never observed.

Our results and conclusions demonstrate that substrate selection is not an area that shows promise towards therapeutic applications. Although the detrimental effects of elevated plasma fatty acids cannot be denied, myocardial contractile function is not depressed by forced fatty acid metabolism during

hypoperfusion. Thus, enhanced glucose utilization can not improve myocardial dysfunction, and does not have potential for clinical application during hypoxia or coronary artery stenosis.

CHAPTER II

THE EFFECTS OF HYPERLIPIDEMIA AND HYPOGLYCEMIA ON MYOCARDIAL CONTRACTILE FUNCTION AND OXYGEN UTILIZATION DURING CORONARY HYPOPERFUSION

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ABSTRACT

This study was designed to determine changes in myocardial contractile function and metabolic substrate selection during moderate coronary hypoperfusion in the presence of elevated plasma fatty acid (FFA) concentrations and reduced glucose concentrations. Coronary perfusion pressure (CPP) was sequentially lowered from 100 to 60, 50, and 40 mmHg in the left anterior descending coronary artery of sodium pentobarbital-anesthetized, open-chest dogs. Regional glucose uptake (GU), fatty acid uptake (FAU), percent segment shortening (%SS), oxygen consumption (MVO_2), and oxygen utilization efficiency (O_2UE = [%SS * peak left ventricular pressure * heart rate] / MVO_2) were determined with normal arterial plasma FFA concentrations of 300 ± 31 μMol (Group 1, n = 16) or with elevated FFA concentrations of 1210 ± 32 μMol (Group 2, n =10, and Group 3, n = 10). FFA were elevated by infusing 10% Intralipid® at 0.04 ml/min/kg. In Group 3, glucose in the coronary perfusate blood was reduced from 3.49 ± 0.17 to 0.26 ± 0.10 mM by dialysis. At 40 mmHg CPP, myocardial function and metabolic variables were similar in groups 1 and 2. In group 3, FA uptake increased ($p<0.05$), glucose uptake decreased ($p<0.05$), MVO_2 increased ($p<0.05$), and O_2UE decreased ($p>0.05$), but %SS was unchanged relative to group 1. Thus, glucose utilization provides an increase in the efficiency of oxygen usage and plays a role in the reduction of oxygen consumption during hypoperfusion. Blocking this increase in

oxygen utilization efficiency by preventing glucose uptake and oxidation does not, however, contribute to a reduction in regional myocardial contractile function.

Index terms: fatty acid metabolism, glucose, dialysis, myocardial oxygen consumption, ischemia

INTRODUCTION

The working myocardium can utilize glucose, fatty acids, amino acids, pyruvate, and ketone bodies for energy. Since Randle proposed the glucose/fatty acid cycle in 1963 (25), much effort has been expended to elucidate the underlying mechanisms and possible benefits of substrate selection by the heart. Myocardial substrate utilization is known to change with substrate availability (20, 27). After a meal high in carbohydrates, glucose is the primary myocardial energy source. Fatty acids are oxidized during fasting or after a fatty meal. Lactate is used to a greater extent when its blood concentration increases, such as during exercise (4). Although the heart metabolizes fatty acids preferentially under many conditions, it will switch to carbohydrates, a more oxygen efficient substrate, during hypoperfusion (4, 23, 30).

The heart can change its choice of metabolic substrate quickly during limited oxygen supply, as the cellular ATP reserve can maintain contraction for only several beats. Most oxygen consumed by the ischemic myocardium is used for carbohydrate metabolism, and fatty acid uptake and oxidation are limited (4). The mechanism of this switch from fatty acid to glucose oxidation remains undetermined, but may be

attributed to several factors. The switch may result from inhibition of β-oxidation by accumulation of intermediate metabolites, or the myocyte may have some other inherent capability to conserve oxygen by using a more oxygen-efficient substrate (9). In any case, it is generally considered to be beneficial for ischemic myocardium to preferentially utilize glucose as a source of energy (30).

When the heart is exposed to higher levels of circulating fatty acids, fatty acid uptake and utilization is enhanced (4). In the normally perfused heart, hyperlipidemia causes arrhythmias, depressed contractility and decreased aortic pressure (5, 15). During reperfusion following ischemia, elevated fatty acid levels cause increased myocardial oxygen consumption (26, 32). Hyperlipidemia during reperfusion also depresses cardiac function, and causes oxygen wasting, arrhythmias and a lower fibrillation threshold (2, 11, 13, 22). These findings have led to the conclusion that enhanced fatty acid oxidation and the resulting decrease in the efficiency of oxygen utilization have negative effects during normal conditions and during reperfusion following ischemia (30). To date, however, no studies have examined the effects of elevated fatty acid concentrations on myocardial contractile function of the intact, beating, hypoperfused heart.

For this study, we sought to determine if hyperlipidemia would affect the switch to glucose as an energy source during myocardial hypoperfusion. Furthermore, we sought to determine if preventing this metabolic switch to glucose and forcing the heart to use fatty acids during hypoperfusion would further compromise myocardial contractile shortening. Glucose metabolism was prevented by removing glucose from the coronary arterial blood with a dialysis system. This provided a novel model of isolated coronary hypoglycemia in which to examine regional contractile function during hypoperfusion. To date, no studies have used selective coronary hypoglycemia to investigate the metabolic and contractile function changes caused by enhanced lipid metabolism during moderate myocardial ischemia.

The ATP/O ratio for glucose oxidation is higher than that of fatty acid β-oxidation, indicating more oxygen-efficient ATP production (1, 4). However, using glucose as a substrate may not have a major beneficial impact since the increase in ATP produced per O atom consumed is modest, only about 12%. This investigation examined the impact of altering myocardial substrate selection on regional contractile shortening. We examined functional data during hypoperfusion in the presence of elevated plasma fatty acid levels. We further examined the effects of

hypoglycemia on myocardial contractile function, in a model where fatty acids were elevated and glucose was not available for oxidation during hypoperfusion.

We have found that as perfusion pressure falls from normal (100 mmHg), oxygen consumption is reduced. Glucose uptake is unchanged and fatty acid uptake is reduced. Equivalent results were observed during hyperlipidemia. Therefore, the myocardium will devote its limited oxygen supply during hypoperfusion to glucose metabolism, even with the potential for increased fatty acid uptake associated with higher fatty acid concentrations. As we forced the heart to use fatty acids as an energy source during hypoperfusion by removing glucose as an option, we observed an increase in MVO_2. We failed however, to observe any further decrease in regional contractile shortening. Thus, we have shown that, although resulting in a lower ATP/O ratio and higher oxygen consumption during hypoperfusion, coronary hypoglycemia in the presence of elevated plasma fatty acids will not have a significant detrimental impact on myocardial contractile function.

METHODS

Surgical Preparation

Experiments were performed on mongrel dogs of either sex. The dogs were anesthetized with pentobarbital sodium (30 mg/kg body weight) and supplemental anesthetic was administered to maintain stable anesthesia. A tracheotomy was performed to ventilate the dog with room air and supplemental oxygen. Arterial blood samples were frequently analyzed for PO_2, PCO_2, and pH, and ventilation was adjusted to maintain these variables within the normal limits of 80 - 140 mmHg, 35 - 45 mmHg, and 7.35 - 7.45, respectively. Sodium bicarbonate was given intravenously to maintain normal pH when PCO_2 was within normal limits. Rectal temperature was measured, and a water-circulating heating pad maintained normal body temperature.

Vinyl catheters were used to cannulate the femoral vessels. The right femoral venous catheter was used for supplemental infusions (additional anesthetic, bicarbonate, etc.). The right femoral artery catheter, attached to a transducer, was advanced to the descending aorta and used to measure aortic pressure. Blood for pressure-controlled LAD perfusion was withdrawn from the left femoral artery and fatty acids were infused in a catheter in the left femoral vein of groups 2, 3, and 4.

A left thoracotomy was performed to expose the heart and, after opening the pericardium, a Millar catheter-tip pressure transducer was inserted through the left atrial appendage and advanced across the mitral valve for measuring left ventricular pressure. The rate of left ventricular pressure development (dP/dt) was obtained by electronic differentiation of the left ventricular pressure signal. The LAD was then carefully isolated, and, after heparinization (500 U/kg), cannulated with a stainless steel cannula for controlled perfusion. The anterior ventricular vein was cannulated for collection of venous blood from the LAD perfused region.

To obtain regional myocardial function measurements, piezoelectric crystals were implanted within the mid-myocardial layer. They were positioned perpendicular to the main axis of the heart, about 1 cm apart. Segment lengths at the beginning of the positive deflection of the dP/dt record were considered end diastolic, and those measured 20 ms before the peak negative deflection were considered end systolic.

Blood Sample Collection

Arterial and venous blood samples were collected to examine arterio-venous differences. Arterial samples were collected from the coronary perfusion line, and venous samples were collected from the cannulation of the anterior

ventricular vein. This technique has been shown to sample venous blood originating from the perfused area without significant contamination from other blood supply (31). All samples were collected anaerobically and kept in an ice bath until analysis.

Blood Glucose

To maintain normal levels of glucose in the blood in groups 1 and 2, we infused a 30% D-glucose solution into the right femoral venous catheter. With frequent sampling and glucose determinations, we were able to maintain blood glucose levels within the normal physiological limits (70 - 100 mg/dl) by adjusting the glucose infusion rate. Glucose was measured using a Yellow Springs Instrument 2300 STAT L-Lactate analyzer. Sub-normal blood glucose concentrations (group 3 animals) were accomplished by passing the blood through a dialyzing system in series with the LAD perfusion system.

Dialysis and Glucose Removal

The dialysis system consisted of an ultrafiltration tube (Spectrum laboratories hollow fiber tangential flow MiniKros® ultrafiltration module, 10 kD rating, 8000 cm^2 surface area) in series with the extracorporeal perfusion system. The hollow fibers of the tube were bathed in a continuous flow of a dialysate solution, allowing passive diffusion between the blood and dialysate of any

substance 10 kD or smaller. The dialysate solution contained ionic concentrations equivalent to normal plasma ionic concentrations, and was made just prior to the start of the experimental protocol. One liter of the dialysate solution contained 120 mEq NaCl, 1.2 mEq $MgSO_4$, 2.5 mEq CaCl, 4 mEq KH_2PO_4, and 20 mEq $NaHCO_3$. It was then adjusted to a normal blood pH value of 7.4 by appropriate addition of $NaHCO_3$ or HCl, if necessary. The hydrostatic pressure of the solution was adjusted to prevent absorption or filtration across the membrane.

Fatty Acid Elevation

Normal fatty acid concentrations in our subjects after an overnight fast ranged from 0.400 to 0.700 mmol/l. To increase these levels, we infused Pharmacia & Upjohn Co. Intralipid® 10% at 0.04 ml/kg/min into the left femoral venous catheter after obtaining baseline samples. This infusion of Intralipid® raised arterial plasma fatty acid concentrations to three times normal, i.e. to about 1.20 mmol/l after about one hour of infusion. Intralipid® 10% is a 10% intravenous fat emulsion used clinically as a source for calories and essential fatty acids. It contains linoleic (44-62%), oleic (19-30%), palmitic (7-14%), linolenic (4-11%), and stearic (1.4-5.5%) acids. It also contains phospholipids, glycerin, and water, and is adjusted to a pH of 8.0.

Non-esterified (free) fatty acids were measured in each of the blood samples collected during the experimental protocol. After collection, the samples were placed in a plastic microcentrifuge tube and centrifuged for five minutes to isolate the plasma. The plasma was withdrawn and placed into another vial, which was immediately immersed in liquid nitrogen. The samples were then stored at -80° C until analysis. Fatty acid analyses were performed with a Wako NEFA C kit for the determination of non-esterified fatty acids in plasma.

LAD-perfused Area

Before termination of the experiment, Evan's Blue dye (approximately 1.5 ml of 2.5% concentration) was infused into the LAD in order to delineate the perfused territory. This territory was then carefully excised and weighed in order to normalize the coronary flow per gram of tissue mass.

Experimental Protocols

Group 1: Control, Normal Glucose, Normal Fatty Acid (n=16). The canine subject was prepared as indicated above. Coronary perfusion pressure was maintained at 100 mmHg for twenty minutes to allow stabilization and recovery from surgical procedures. Arterial and venous samples were collected during the last five minutes for the baseline values. D-glucose (30%) infusion was then initiated and adjusted to maintain plasma glucose within the normal limits of 70 -

100 mg/dl. Arterial blood samples were taken intermittently and analyzed for glucose, so that the infusion rate could be adjusted as necessary to stabilize plasma glucose concentrations. Arterial and venous samples were collected at fifteen and thirty minutes of this period. Coronary perfusion pressure was then lowered from 100 to 60, 50, and 40 mmHg and maintained for thirty minutes at each pressure. Arterial and venous samples were taken during the last five minutes of each period.

Group 2: Normal Glucose, Hyperlipidemia (n=10). Group 2 protocol was similar to group 1 protocol, but with elevated fatty acid concentrations. We began infusion of Intralipid® 10% as described above (0.04 ml/kg/min). During the process of fatty acid elevation (about 30 minutes), arterial and venous samples were collected at fifteen and thirty minutes for determination of control fatty acid uptake values. With glucose and fatty acid concentrations at desired levels, infusions of glucose and Intralipid® were maintained throughout the remainder of the experiment.

Group 3: Dialysis, Hypoglycemia, Hyperlipidemia (n=10). Group 3 protocol was similar to group 2 protocol, with the exception that coronary perfusate blood was passed through the dialyzing system (described above) prior to controlled perfusion pressure into the LAD. This dialysis was initiated at

the same time in the protocol as the glucose infusion was started in group 1 and 2 protocols, and it was continued throughout the experiment. Glucose was not supplemented. As a result, the LAD blood perfusate had elevated fatty acid concentrations and lowered glucose levels relative to that of group 1 dogs.

Group 4: Dialysis Control, Normal glucose, Hyperlipidemia (n=5). Group 4 protocol was identical to that of group 3 protocol, but the dialysate solution contained normal plasma glucose levels. Thus, glucose was not removed from the coronary blood perfusate, although the dialysis could have potentially removed or altered other substance concentrations. In this protocol, therefore, coronary perfusate blood had normal glucose and elevated fatty acids.

Statistical Analyses

All values are expressed as means ± SEM. One-way analysis of variances (ANOVA) were utilized to examine differences between all groups and between experimental conditions within groups. A Student-Newman-Keuls multiple comparison tests was performed if significance was obtained. Statistical significance was assumed at $p<0.05$.

RESULTS

Table 1 presents hemodynamic data of the four groups. Post-treatment values for aortic pressure (AoP), heart rate (HR), LAD coronary blood flow (CBF), and systolic left ventricular pressure (LVP_{max}) are listed at LAD coronary perfusion pressures of 100 (baseline), 60, 50, and 40 mmHg. Treatment caused no significant difference between any of the variables at CPP 100. Reduction of regional coronary perfusion pressure caused no significant changes in aortic pressure, heart rate, or systolic left ventricular pressure in any group. Coronary blood flow, normalized per gram tissue mass, declined significantly at CPP of 60, 50, and 40 in all groups when compared to baseline flow values ($p<0.05$), but there was no statistical difference among treatment groups. At CPP 40, LAD flow varied from 35% (group 3) to 27% (group 2) of the respective flows observed at CPP 100. This demonstrated a reduction in perfusion pressure below the autoregulatory range of the left coronary circulation.

Fatty acid uptake values are reported in Figure 1. Uptake values in the control group declined significantly upon reduction of CPP to 60 ($p<0.05$), and remained lower at CPP 50 and 40 mmHg. At CPP 100, FA uptake tended to be higher than pre-treatment in groups 2, 3, and 4, probably due to the elevated fatty acid concentrations in these groups. At CPP 60, FA uptake in these groups

was not significantly reduced from CPP 100, and were significantly higher than in the control group at the same CPP ($p<0.05$). At CPP 50 in these same groups, elevated fatty acid concentrations maintained FA uptake values not significantly lower than treated CPP 100. At CPP 40 mmHg, FA uptake of groups 1, 2, and 4 fell to near 10% of the respective rates at 100 mmHg CPP ($p<0.05$). However, hypoglycemia resulted in FA uptake that did not significantly fall as perfusion pressure was reduced. These uptake values of group 3 were significantly higher than those of all other groups at CPP 40 ($p<0.05$). Thus, hyperlipidemia increases the fatty acid uptake at lower perfusion pressures compared to the control group (CPP 60), and tends to increase FA uptake at CPP 50, but did not affect fatty acid uptake at CPP 40. Hypoglycemia caused significant increases in fatty acid uptake at all reduced perfusion pressures when compared to the control group.

Glucose uptake data are shown in Figure 2. Glucose uptake, although tending to be lower as pressure was reduced, was not significantly different from CPP 100 values in groups 1, 2, and 4. In addition, there was no significant difference in glucose uptake between these groups at any CPP. Since FA uptake fell in these groups, glucose became the preferred substrate during hypoperfusion. Group 3 animals, with highly reduced glucose concentrations in the coronary perfusate, had significantly lower glucose uptake at all CPP post-

treatment ($p<0.05$). At CPP of 40 mmHg, this group demonstrated essentially zero glucose uptake, which was significantly lower than that of all other groups ($p<0.05$). In this group FA uptake was well maintained at all CPP (Figure 1). Hyperlipidemia did not affect glucose uptake at any CPP. Hypoglycemia, as expected, significantly reduced glucose uptake at all perfusion pressures.

Oxygen consumption by the myocardium is shown in Figure 3. This oxygen usage tended to decrease in all groups as CPP was reduced, and significant reductions were noted in groups 1, 2, and 4 at 40 mmHg ($p<0.05$). In group 3 hearts, oxygen consumption values were not significantly reduced at CPP 40, and were significantly higher than that of the other three groups ($p<0.05$). Thus, hyperlipidemia caused no significant change of oxygen consumption at any CPP; however, hypoglycemia had a tendency to increase oxygen consumption at all perfusion pressures, and significantly increased these values at CPP 40.

Lactate uptake data are shown in Figure 4. Lactate uptake was significantly reduced as CPP was lowered from 100 to 60 mmHg in all groups. Lactate uptake remained significantly lower than the respective CPP 100 values at CPP 50 and 40 in all groups. Lactate uptake values, however, were not significantly different between the four groups at CPP 40. Hyperlipidemia and

hypoglycemia had no significant effect on lactate uptake at either 100 or 40 mmHg CPP.

Myocardial % segmental shortening is shown in Figure 5. This index of contractile function was not significantly reduced in any group as CPP was lowered to 60 and 50 mmHg. At CPP 40 mmHg, contractile function was reduced significantly from baseline in all groups ($p<0.05$). Among the four groups, however, there were no significant differences in segment shortening at any CPP. Neither hyperlipidemia nor hypoglycemia produced significant effects on contractile function at any CPP.

DISCUSSION

This study was designed to test the effects of hyperlipidemia on myocardial contractile function during hypoperfusion and to examine the effects of isolated coronary hypoglycemia on contractile function under these conditions. Using dialysis in series with an extracorporeal perfusion system, we were able to remove glucose from the coronary perfusate blood and prevent glucose uptake by the working myocardium. Energy for contraction and force development of the dialysis group was thus derived from sources other than intracoronary glucose.

By measuring substrate uptake and oxygen consumption, we have demonstrated the ability of the heart to switch metabolic substrates during moderate ischemia. Glucose uptake did not decline significantly as CPP was reduced to 40 mmHg, as might be expected due to the significant reduction in flow. In contrast, fatty acid uptake declined to a greater extent than did blood flow. Therefore, we have found an increased glucose extraction ratio and a decreased fatty acid extraction ratio during coronary hypoperfusion as expected from the literature. Thus, the heart seems to prefer carbohydrate metabolism during periods of limited oxygen delivery. Interestingly, this glucose preference during hypoperfusion was unaltered by hyperlipidemia.

Our experimental protocols produced data that allow us to arrive at important conclusions: 1) elevated plasma fatty acid concentrations do not affect the substrate selection of the working myocardium during hypoperfusion; 2) regional coronary hypoglycemia in the presence of elevated fatty acid concentrations prevents glucose uptake and forces fatty acid utilization by the myocardium; and 3) this forced fatty acid metabolism during hypoperfusion causes no detrimental effects on contractile function by the myocardium. Thus, even though glucose is a more oxygen-efficient substrate, the small increase in the P/O ratio resulting from preferential use of glucose during moderate ischemia does not contribute to improved myocardial function relative to a condition where fatty acids are used. Many studies have focused on the determination of the optimal substrate available for use by the heart for ATP generation, especially for possible clinical application during coronary stenosis or other partial reduction of the coronary blood supply. Our findings show that hypoglycemic perfusion does not impair regional myocardial contractile function relative to normoglycemic perfusion.

We elevated plasma free fatty acid concentrations, and the measured basal fatty acid concentration values obtained were similar to those reported previously (11, 12, 21, 33). The elevated fatty acid levels of groups 2, 3, and 4 tended to increase fatty acid uptake by the myocardium at the control CPP of

100 mmHg. FA uptake has been previously described to be a function of plasma concentration (30). With these elevated levels and the diminished glucose supply in group 3, we were able to force the myocytes to utilize fatty acids.

The protocols followed in the current study have allowed the isolation of substrate selection as the dependent variable affecting heart function. We were unable to find a decrease in performance by forcing an increased fatty acid oxidative rate during hypoperfusion. Our hypoperfused model generated a mild ischemia at a perfusion pressure of 40 mmHg, while allowing a "washout" of metabolic waste products. Our model examined conditions similar to that of coronary artery stenosis or hypoxic conditions. In other studies, however, elevated fatty acid concentrations have been shown detrimental (2, 9).

Oxygen consumption has been shown to increase in the presence of fatty acids (5, 15, 26, 32), and elevated fatty acids contribute to arrhythmias, depression of cardiac function, oxygen wasting, and a lower fibrillation threshold (2, 11, 13, 22). Feuvray et al. found that these high levels of free fatty acids in the plasma have also increased mitochondrial damage following ischemia (3). Liedtke et al. demonstrated that reduced fatty acid metabolism during ischemia lessened the decline in mechanical function (16). Fatty acids inhibit glucose metabolism, and prevent recovery of mechanical function during reperfusion

following ischemia, although the mechanisms are unclear (17). These studies, however, focused on the effects of fatty acids during periods of ischemia and reperfusion, and are not undisputed. Most et al. have shown that increased fatty acid levels do not affect myocardial injury obtained after acute coronary artery occlusion in pigs (18). A further study demonstrated that elevated fatty acid concentrations did not provoke arrhythmias after occlusion using the same model (19).

We have devised a dialysis method that removes glucose efficiently from the blood, and we have used this method to explore the impact that the myocardial energy supply may have on heart function. Glucose utilization has been shown to increase during periods of limited oxygen supply, and by removing this option, we have isolated the effects that result from diminished glucose metabolism. To date, no studies have attempted to remove glucose as an available substrate to the working myocardium. Using the dialysis system, we maintained normal serum ionic concentrations and prevented glucose uptake by the myocardium.

The dialysis method provided an excellent model, as coronary perfusate blood was identical to normal systemic blood for almost all variables measured. Thus, glucose levels were diminished without altering concentrations of most

other substances. The only exception was lactate, which was removed less efficiently than glucose. Arterial lactate values were reduced to about half of systemic values. Lactate uptake, however, remained unchanged. It must be noted that there may be yet-unidentified substances less than 10 kD in diameter that may prove to have some effect on heart function, and these substances may have potentially been removed. The fourth group, in which dialysate glucose concentrations were equivalent to that of normal blood, was examined for this reason. Results obtained from this group demonstrated regional function not different from untreated controls, indicating that, with the exception of glucose, no substance removed from the blood by dialysis was crucial for supporting contractile function. The observed results of group 3 can thus be attributed to coronary hypoglycemia.

An important observation obtained from group 3 included a two-fold increase in oxygen consumption values at CPP 40 compared to the untreated group 1 with glucose available. Thus, when the myocardium was forced to oxidize fatty acids by elevating coronary arterial fatty acid concentrations and removing glucose, hypoperfusion did not result in significantly lower oxygen consumption, even though supply was greatly reduced. This was observed as flow was reduced by about 60%.

In addition, we have noted that there was no difference in regional contractile function between any group. Because of the increase in oxygen consumption, the analysis leads to the conclusion that the efficiency of oxygen utilization was reduced. This was observed by using a model for oxygen utilization efficiency (O_2UE) equal to [(heart rate * % segment shortening * maximum left ventricular pressure) / oxygen consumption]. As reported, this efficiency was indeed significantly reduced by forced fatty acid metabolism.

The effects of increased fatty acid utilization and decreased glucose usage on contractile function during hypoperfusion was the major focus of our investigation. Henderson et al. demonstrated reduced contractility in isolated rat papillary muscle when exogenous fatty acids were supplied during hypoxia and anoxia (7). Henderson et al. also found depressed contractility in isolated perfused rat hearts with elevated fatty acid concentrations (8). These isolated models differ from our protocol, as we utilized intact *in situ* dog hearts. Tune, using the same model as our protocol, found that insulin increased glucose uptake and maintained contractile function during moderate ischemia, but could not rule out other possible inotropic effects of the insulin therapy (28). Ichihara and Neely used isolated ischemic rat hearts exposed to excess fatty acids, and they found no depression of mechanical function compared to controls with normal fatty acid levels during ischemia (10). Pacold et al. failed to demonstrate

a decrease in ejection fraction in human subjects with coronary artery disease given Intralipid® and heparin administrations to elevate free fatty acids (24). It should be noted that Pacold et al.'s method was similar to our protocols involving fatty acid elevation, and this method has been well documented (8, 12, 13, 15, 29).

This study has successfully examined myocardial substrate selection during hypoperfusion in the intact canine model. The results thus obtained demonstrate the inability of glucose, a more oxygen efficient substrate, to maintain contractile function at levels above that of myocardium forced to consume fatty acids during moderate ischemia. The relatively small increase of the P/O ratio attained by glucose usage may play a role in the prevention of cellular damage during ischemia, but is not able to increase mechanical function during hypoperfusion. In addition, mechanical function was not further compromised when the moderately ischemic myocardium was deprived of glucose in the presence of elevated fatty acids. Therefore, although selective substrate utilization by the heart does occur during ischemia, blocking this selection does not alter the functional status of the working myocardium.

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Table 1. Hemodynamic variables of the four groups at normal and reduced coronary perfusion pressures

Group	CPP (mmHg)	AoP (mmHg)	HR (beats/min)	CBF (ml/min/g)	LVP_{max} (mmHg)
Untreated	100	119±5	154± 8	1.06±.07	111±2
Fatty Acid Elev.	100	115±6	143± 7	1.02±.05	108±6
Dialysis	100	106±6	154±11	1.03±.02	107±6
Dialysis w/ Glu.	100	102±6	150±15	0.98±.03	115±7
Untreated	60	120±2	155± 7	0.72±.06*	115±2
Fatty Acid Elev.	60	113±5	143± 7	0.78±.09*	108±5
Dialysis	60	105±6	156±11	0.71±.04*	103±6
Dialysis w/ Glu.	60	100±5	149±15	0.68±.05*	111±9
Untreated	50	122±3	145± 8	0.51±.08*	112±3
Fatty Acid Elev.	50	106±3	138± 8	0.57±.07*	102±5
Dialysis	50	108±5	162±13	0.55±.05*	104±6
Dialysis w/ Glu.	50	103±8	151±15	0.49±.05*	114±8
Untreated	40	113±3	154± 7	0.30±.04*	109±3
Fatty Acid Elev.	40	107±6	145± 8	0.28±.07*	102±5
Dialysis	40	101±7	167±13	0.37±.06*	99±7
Dialysis w/ Glu.	40	99±4	153±17	0.34±.08*	108±4

Values are mean ±SEM from untreated (group 1, n=16), fatty acid control (group 2, n=10), dialysis (group 3, n=10), and dialysis w/ glucose control (group 4, n=5) hearts. CPP = coronary perfusion pressure, AoP = mean aortic pressure, HR = heart rate, CBF = coronary blood flow, LVP_{max} = maximum left ventricular pressure. * indicates significant difference from CPP 100, same group.

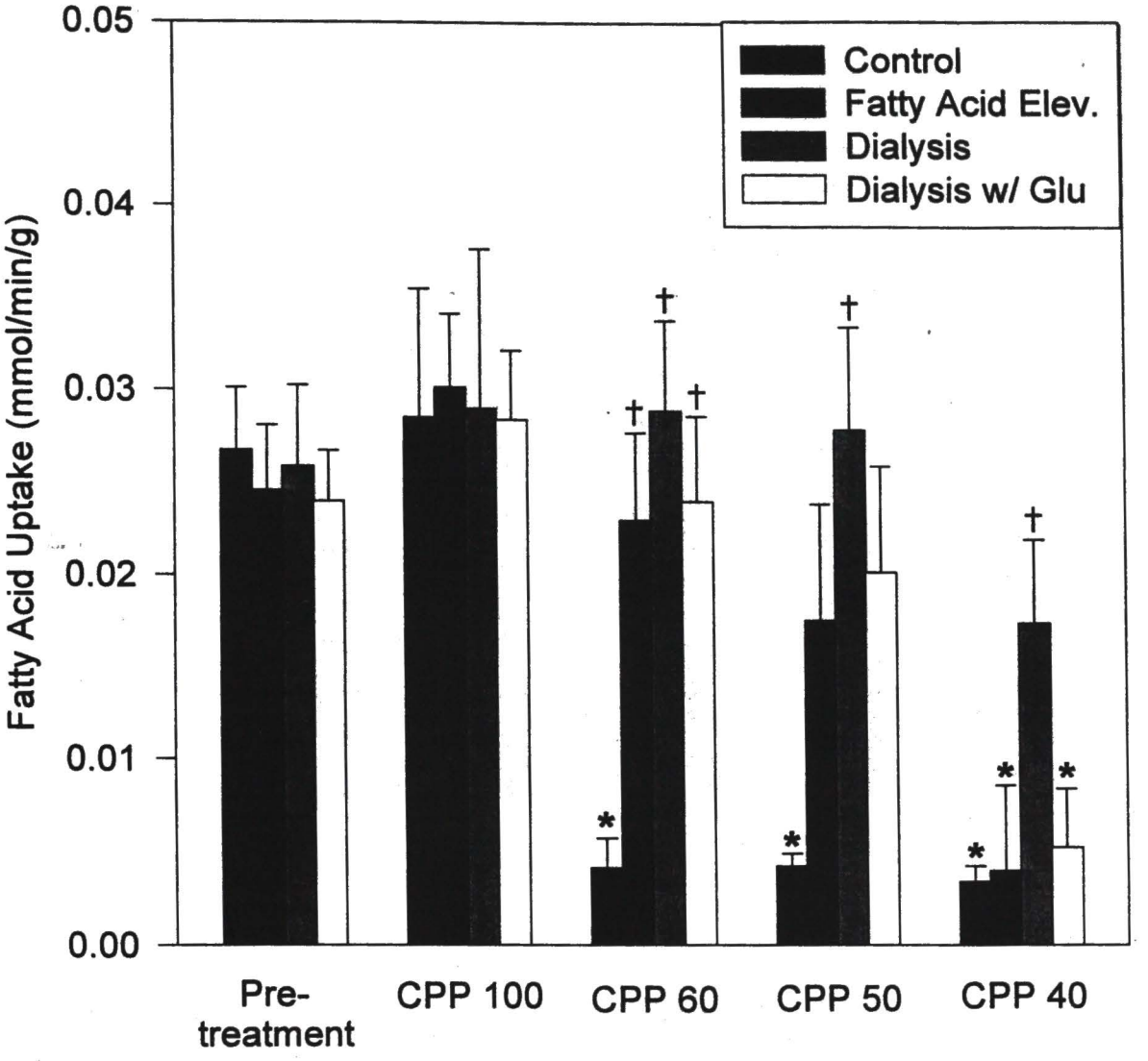

Figure 1. The effect of lowered perfusion pressure on myocardial fatty acid uptake. In this and the following figures, values are mean ± SE, n=16 for untreated control group 1, n=10 for elevated fatty acid group 2, n=10 for dialysis group 3, n=5 for dialysis w/ glucose group 4. Black bars: untreated control, Dark gray: elevated fatty acid, Light gray: dialysis, White bars: dialysis w/ glucose. * $p<0.05$ vs. treated CPP 100, same group. Pretreatment values were obtained at CPP 100. † $p<0.05$ vs. control group 1, same CPP.

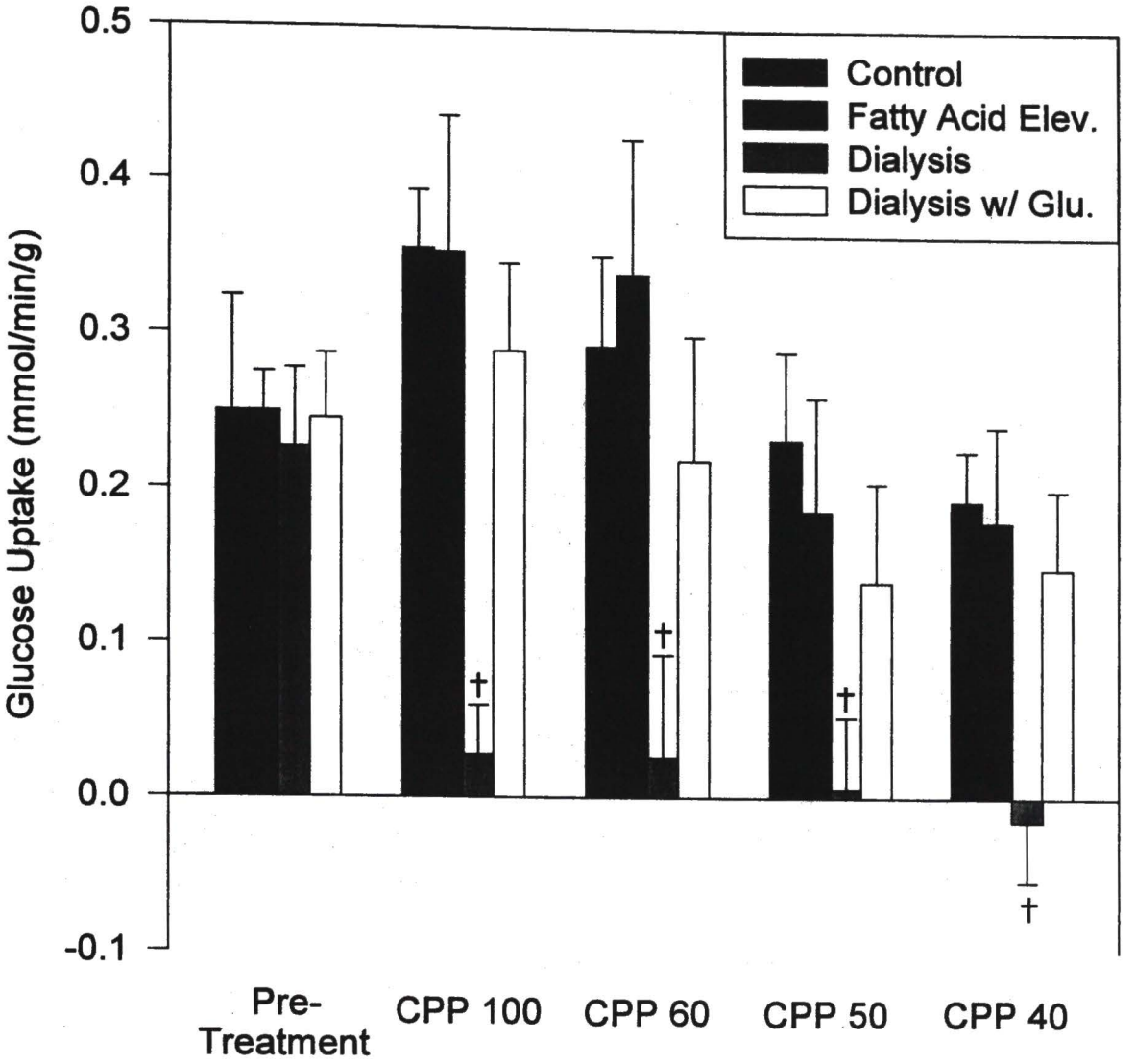

Figure 2. The effect of lowered perfusion pressure on myocardial glucose uptake. † $p<0.05$ vs. control group 1, same CPP.

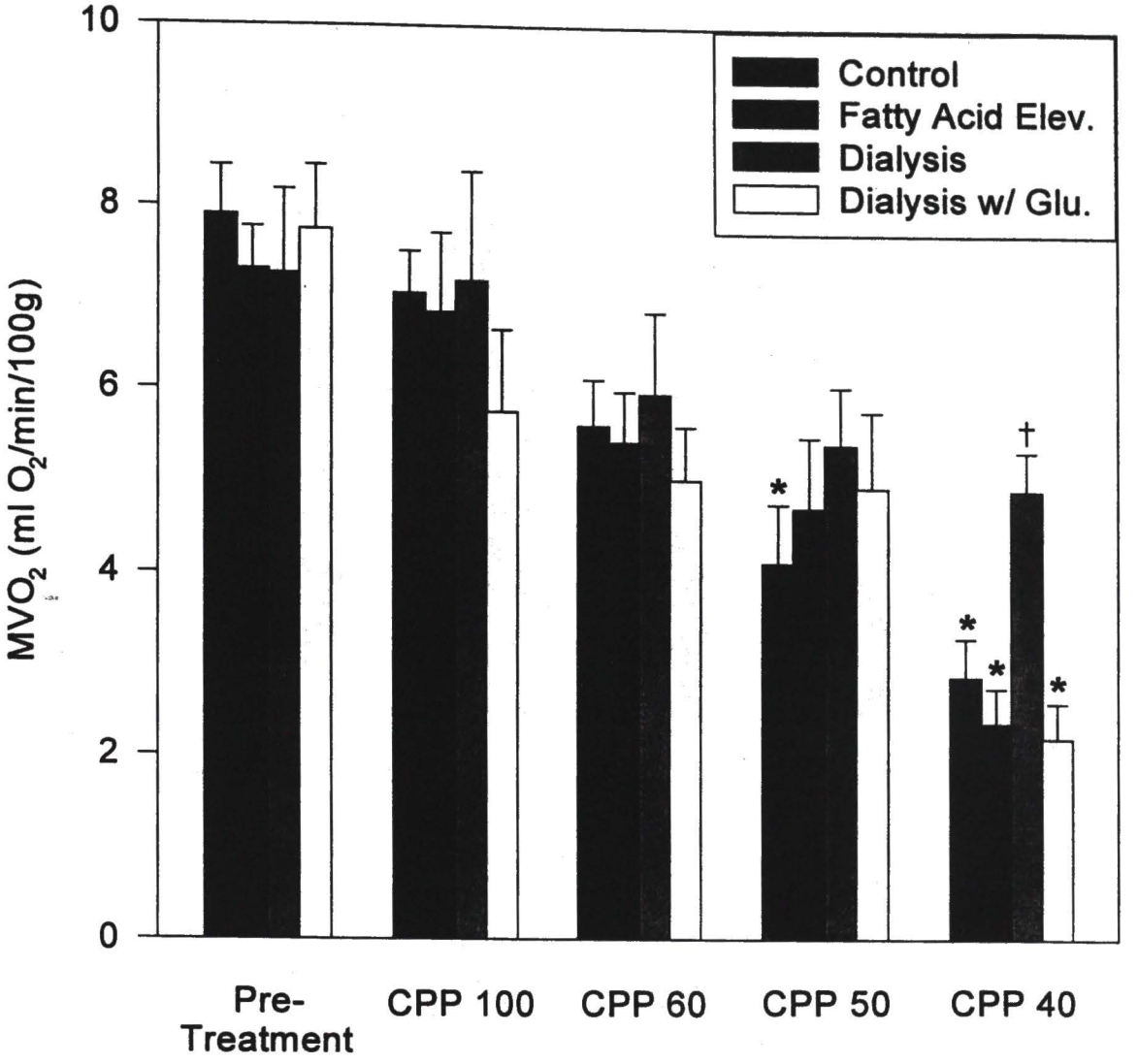

Figure 3. The effects of lowered perfusion pressure on myocardial oxygen consumption. * $p<0.05$ vs. CPP 100, same group. † $p<0.05$ vs. control group 1, same CPP.

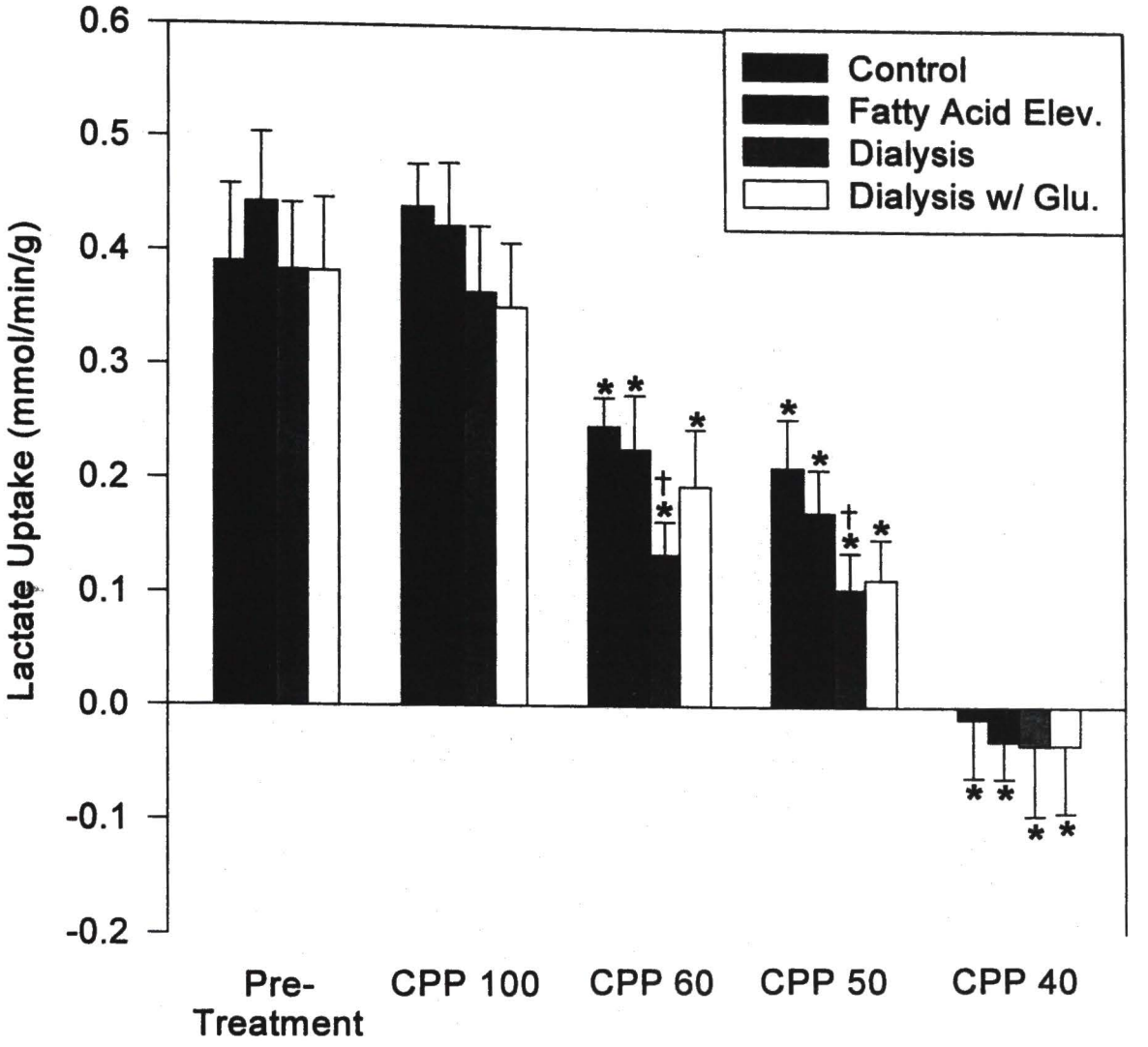

Figure 4. The effect of lowered perfusion pressure on myocardial lactate uptake.

* $p<0.05$ vs. CPP 100, same group. † $p<0.05$ vs. control group 1, same CPP.

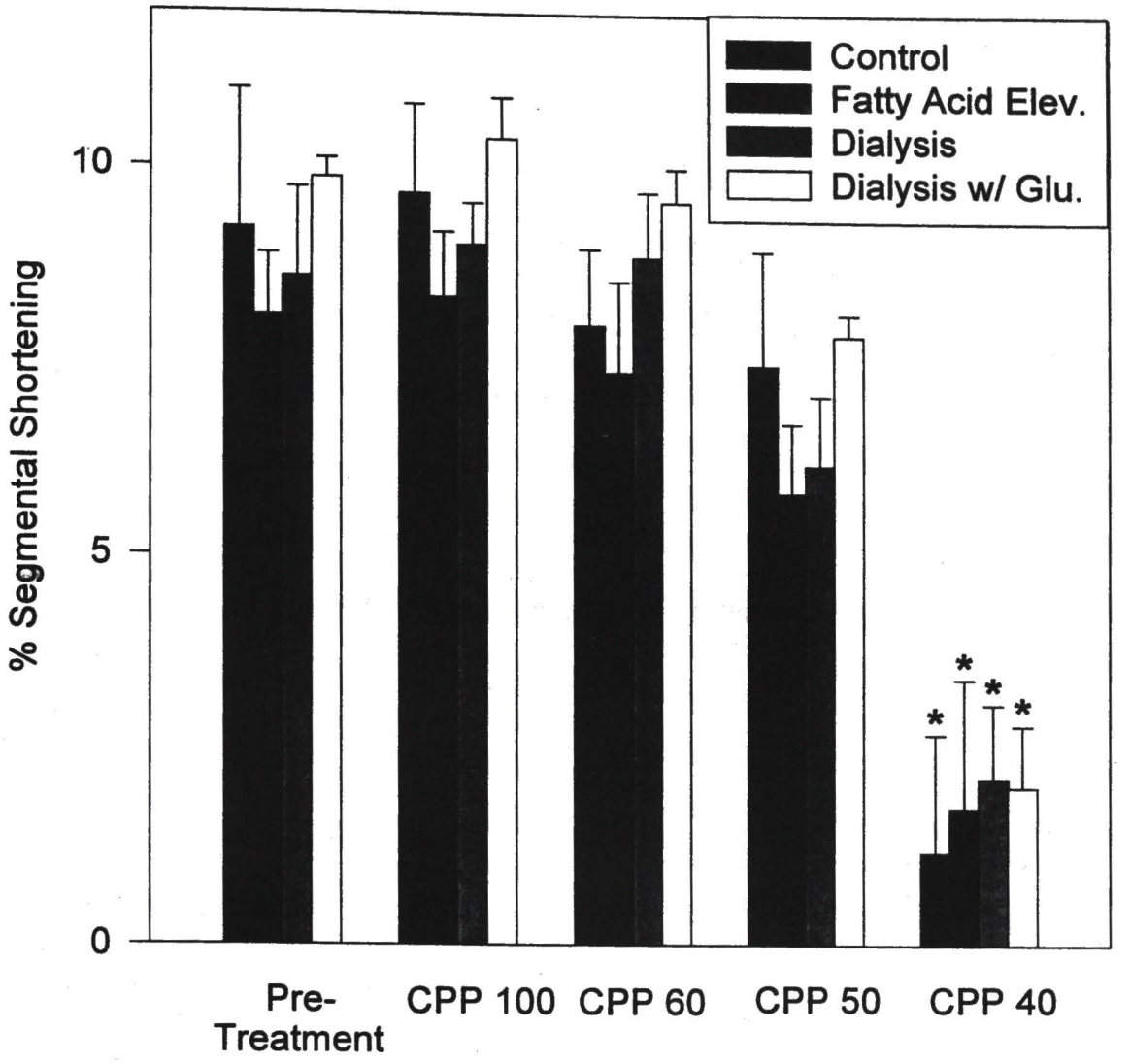

Figure 5. The effect of lowered perfusion pressure on % segmental shortening.

* $p<0.05$ vs. CPP 100, same group.

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CHAPTER III

CONCLUSION

The current study was designed to determine the possible detrimental effects of hypoglycemia on myocardial contractile function and oxygen utilization during coronary hypoperfusion. By using dialysis to remove glucose from the coronary perfusate blood and by elevating arterial plasma fatty acid concentrations, we are able to force the heart to utilize plasma free fatty acids for ATP generation. We found that forcing the heart to use fatty acids as a primary energy source during hypoperfusion, during a period when uptake values are normally depressed, did not affect contractile function. Oxygen consumption significantly increased as a result of the increased fatty acid utilization. These results have led us to the following conclusions:

1) Forced fatty acid metabolism during coronary hypoperfusion causes increased oxygen consumption compared to normal hearts. Increased fatty acid metabolism during hypoperfusion decreases the P/O ratio, and decreases the oxygen utilization efficiency of the heart compared to control hearts.

2) This increased oxygen cost does not result in diminished contractile function or force generation by the myocardium under these conditions. Although glucose is more oxygen efficient, it is unable to maintain an increased contractile function compared to an instance when glucose is not available. Thus, glucose supplementation or inhibition of fatty acid metabolism may not be beneficial to patients with partial blockage of a coronary artery.

CHAPTER V

PROPOSAL FOR FURTHER RESEARCH

This investigation has closely examined myocardial contractile function, force generation, and oxygen utilization during coronary hypoperfusion. It has brought to light some surprising new information. These conclusions may lead to further studies focusing on myocardial substrate selection. The following areas are suggested for further examination:

1) Document myocardial energetic status by measuring phosphorylation potential of the myocytes. Intracellular energetic potential may be significantly lower in animals without glucose available during hypoperfusion, even though contractile function was not.

2) Examine longer-term effects of hypoglycemia during hypoperfusion. Perfusion periods longer than 30 minutes may yield results that differ from those obtained by our protocol.

3) Examine the effects of hypoglycemia in the presence of fatty acid metabolic blockers. This would determine if there is another major substrate that may play an important role in hypoperfused myocardial metabolism in the absence of the two major contributors.

4) Determine if metabolic substrate selection plays a more important role in the hypoperfused right ventricle.

APPENDIX

ADDITIONAL MEASUREMENTS

Oxygen Utilization Efficiency

Figure 6 presents the results obtained from our analysis of oxygen utilization efficiency. These analyses were accomplished using the formula O_2UE = [Power Index] / [MVO_2]. The power index we used was defined as P.I. = % segment shortening * maximum left ventricular systolic pressure * heart rate. We could notice a tendency for an increase in O_2UE as CPP was reduced to 50 in groups 1, 2, and 4, although not significant. At CPP 40, values were not different from CPP 100 in these groups. O_2UE was significantly reduced at CPP 40 in group 3. This resulted from the increase in MVO_2 of group 3 hearts.

Contractile Function

In our protocol, regional heart function was analyzed from several angles. There was no difference between values for % segment shortening, as previously described. In addition to the piezoelectric crystals, a strain gauge (force transducer) was sewn into the mid-myocardial muscle layer of the LAD-perfused region to analyze force generation by the heart. The strain gauge data was analyzed in two ways. Loop area was compared between groups. This strain gauge loop was computer generated, using force generated on the y-axis and segment length on the x-axis. There was no significant difference in loop area between any group at CPP 40. We also examined the maximum left ventricular forces developed, and also observed no significant differences

between the four groups at CPP 40. Figure 7 presents maximum developed force by the LAD-perfused area. The conclusion thus attained is that no changes in % segment shortening nor force generation will occur by preventing glucose metabolism and accentuating fatty acid metabolism during moderate ischemia.

Dialysis System

The dialysis method we have devised and employed has proven to be an efficient and very adequate procedure for removing glucose from the coronary blood supply. It is appropriate to mention important considerations before utilizing this method. There is inevitably an imbalance in pressure across the dialyzing membrane. The dialysate solution composition results in a coronary perfusate blood containing ionic concentrations identical to systemic blood. The dialysate was measured to assure that it was isosmotic with the blood. Arterial blood pressure had a tendency to force water from the blood into the dialysate, resulting in a higher hematocrit and lower coronary blood flows. Using preliminary experimentation, however, we were able to devise a method to apply back pressure by constricting the outflow of dialysate from the ultrafiltration tube. This allowed glucose removal influenced only by the concentration gradient.

The efficiency of glucose removal was obviously impacted by the flow of blood through the filtration tube. At the lower CPP of 40 mmHg, concentration

equilibration had more time to occur. Thus, arterial blood glucose concentrations can be more efficiently removed at lower flow rates. The dialysate solution itself receives glucose down the glucose concentration gradient, and this will lessen the efficiency of glucose removal as dialysate glucose levels equilibrate with the blood. Increased dialysate flow rates will allow less time for this equilibration and will bring coronary arterial blood glucose concentrations closer to zero.

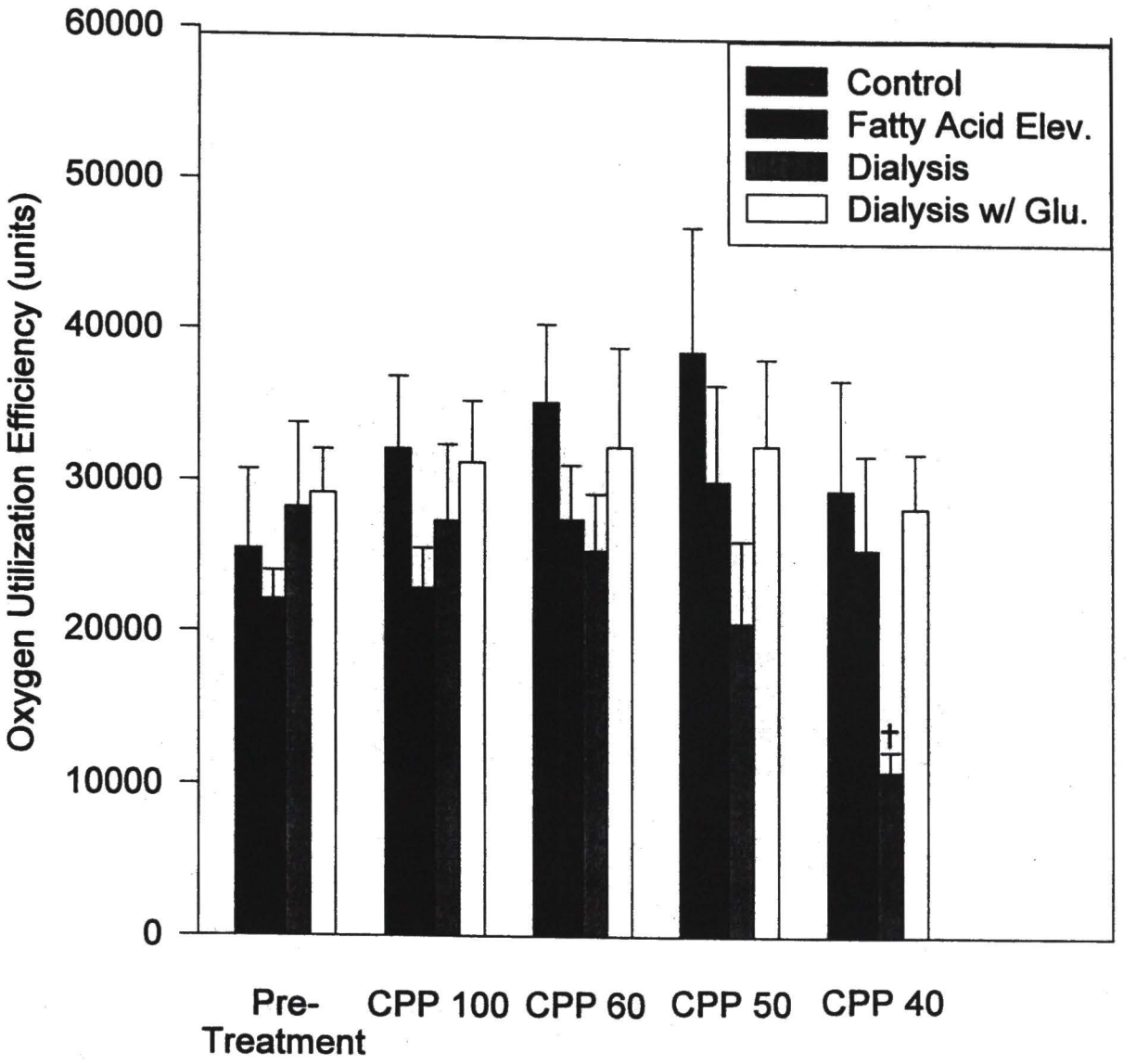

Figure 6. The effect of lowered perfusion pressure on oxygen utilization efficiency. † $p<0.05$ vs. control group 1, same CPP.

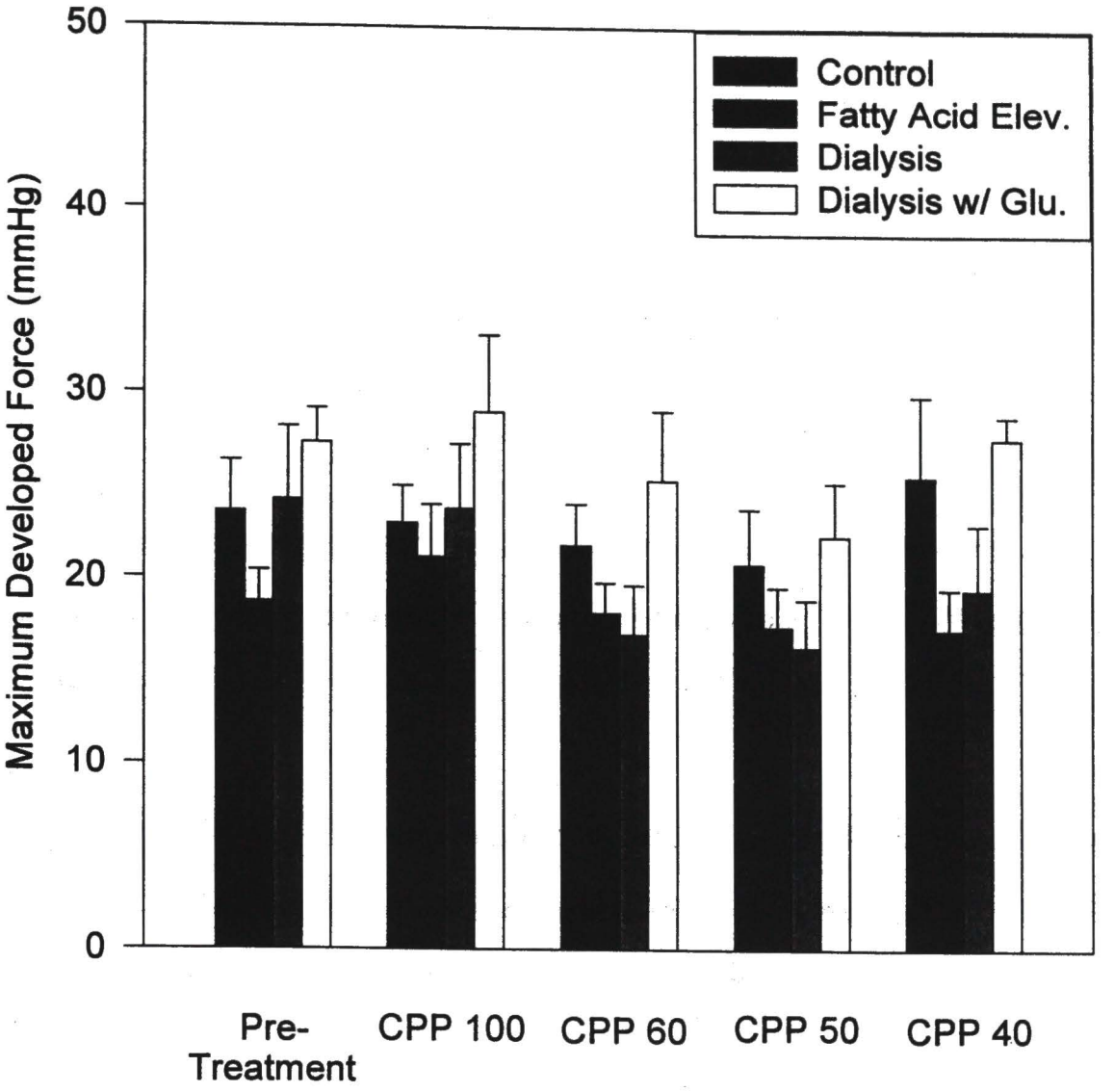

Figure 7. The effect of lowered perfusion pressure on maximum developed force.

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