High Dose Insulin Therapy in Patients undergoing Coronary Artery Bypass Grafting (CABG)

Turki B. Albacker, MD

Division of Surgical Research
McGill University, Montreal
February 2007

A thesis submitted to the faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Your file Votre référence
ISBN: 978-0-494-38382-7
Our file Notre référence
ISBN: 978-0-494-38382-7

Preface:

This thesis is a step forward in evaluating insulin therapy and defining its role in cardiac surgery first described as Glucose-Insulin-Potassium (GIK) solution 40 years ago.

Chapter (I) includes a review of the literature on insulin therapy in cardiac surgery and illustrates the scientific bases and controversies in this therapy.

Chapter (II) entitled: ***"Myocardial Protection During Elective Coronary Artery Bypass Grafting Using High Dose Insulin Therapy"*** represents a manuscript that was presented in the following meetings:

- *Local meetings:*

  1) McGill cardiovascular research day, February 1/2007, Montreal, Canada.

  2) Fraser Gurd annual research day, McGill surgery department, May 31/ 2007, Montreal, Canada.

- *National meetings:*

  1) 11th Annual Terrence Donnelly research day for Canadian cardiac surgery residents, May 26/ 2007, Toronto, Canada.

- *International meetings:*

  1) 43rd Annual meeting of the Society of thoracic surgeons (STS), January 30/2007, San Diego, United States.

  A full manuscript was submitted to "The Annals of Thoracic Surgery" for review.

Chapter (III) entitled: ***"High Dose Insulin Therapy Attenuates Systemic Inflammatory Response in Patients Undergoing Elective Coronary Artery Bypass Grafting"*** represents a manuscript that was presented in the following meetings:

- *Local meetings:*

  1) Fraser Guard McGill Surgery department annual research day, May 3/2006, Montreal, Canada.

- *National meetings:*

  1) 10th Annual Terrence Donnelly research day for Canadian cardiac surgery residents, May 26/ 2007, Toronto, Canada.

  2) Young investigator forum, Canadian Society of Clinical Investigators (CSCI), September 28/2006, Ottawa, Canada.

  2) 59th annual meeting of Canadian Cardiovascular Society (CCS), October 21/ 2006, Vancouver, Canada.

- *International meetings:*

  1) American Heart Association (AHA), November 12/2006, Chicago, United states.

Abstracts from this work were published in the following journals:

1) Clinical and Investigative Medicine, Vol. 29, No. 4, August 2006.

2) The Canadian Journal of Cardiology, Vol. 22 supp D, October 2006

3) Circulation, Vol. 114 supp, No. 18, October 2006

A full manuscript was submitted to "the journal of thoracic and cardiovascular surgery" for review.

Acknowledgements:

The first acknowledgment in every thing I do in my life always goes to my great parents who taught me how to excel, do what I like and like what I do.

An extended gratefulness to my wife and my two children for the full support of my courier and long working hours.

The real credit for this work goes to my supervisor Dr.Kevin Lachapelle who was the leader, the planner and the facilitator for every step in my work. He was my role model for hardworking and commitment.

Special thanks to my collaborators Dr George Carvalho and Dr.Thomas Schricker, the pioneers of Insulin Clamp technique that we used in our study, for their time and effort that contributed to the success of this work.

Finally, with great appreciation, I would like to thank the following persons:

* Dr. Ray Chiu for his insightful thoughts and Suggestions.

* Dr.David Blanc, the head of Biochemistry lab, and his great team for the amount of work and support they devoted to our study.

* Luise Mazza, chief technician in McGill Nutrition lab, for her technical help in collecting and processing the blood samples.

* Minh Doung, chief technician in McGill cardiac surgery research lab, who was always supportive and available to help and who taught me the "ELISA" technique.

Table of contents:

Chapter I

Introduction

History of insulin therapy in cardiac surgery:

The first use of insulin in cardiac surgery was in the form of Glucose-Insulin-Potassium solution (GIK) and was described in 1969. Braimbridge and associates (1) reported that GIK was successfully used to treat patients with low cardiac output not responding to isoprenaline, digoxin or pacemaking after triple-valve replacement. At that time, myocardial protection strategies were limited. The technique was more or less abandoned with the introduction of St. Thomas' cardioplegia and hypothermic cardiopulmonary bypass techniques in the mid-1970s and the subsequent availability of inotropic agents like dopamine and dobutamine and synthetic opioid anesthetic agents with minimal cardiodepressant and vasodilatory effects.

In the 1980s, GIK therapy was "rediscovered" in the setting of cardiac surgery for several reasons, including an increase in the number of patients with unstable coronary syndromes (i.e., severe myocardial ischemia preoperatively) requiring emergent coronary artery bypass grafting (CABG), the introduction of warm cardioplegia and cardiopulmonary bypass techniques (possibly allowing improved metabolic stimulation of normal myocardial enzymatic function), and more in general because it appeared that the limits of adequate cardioprotection had been reached specially with

older and sicker patients are being referred for cardiac surgery. During the past 10 years, there was a renewed interest in the role of GIK therapy in patients undergoing CABG, especially after extra corporeal circulation (ECC) and in the intensive care period. In 1995, Svedjeholm (2) used GIK successfully in an uncontrolled study in cardiac surgical patients with heart failure. They reported almost full recovery of hemodynamic performance in most patients at 6 hours after bypass.

In 1997, Lazar et al (3) described reduced inotropic requirement, improved cardiac index, and shorter duration of intensive care time and total hospital stay associated with GIK therapy in a randomized placebo controlled study in patients with unstable angina during urgent CABG. Also in 1997, Taegtmeyer et al (4) reported a retrospective analysis of cardiac surgical patients with impaired left ventricular function randomly treated with GIK or placebo. They concluded that "aggressive" therapy of postischemic dysfunctional myocardium appeared to be beneficial when pharmacologic and mechanic measures failed to improve cardiac function.

In 2001, van den Berghe et al (5) demonstrated that intensive insulin therapy reduced morbidity and mortality in critically ill patients. They showed that especially the subgroup of cardiac surgical patients receiving continuous intravenous insulin therapy, to maintain blood glucose levels

between 4.4 and 6.1 mmol/L starting upon arrival in the intensive care unit until they were discharged to the ward, benefited significantly. This pivotal study for the first time showed that "metabolic modulation" could significantly improve mortality in cardiac surgical patients.

In 2000 and 2004, Lazar et al (6), (7) Showed that GIK reduces perioperative morbidity in diabetic patients undergoing CABG. This important finding was recently confirmed in a study involving more than 140 000 patients undergoing CABG. Diabetes mellitus was a significant risk factor for short-term morbidity and mortality in patients undergoing CABG (8). The results of the studies that used GIK techniques in cardiac surgical patients are summarized in Table (1-1). This approach appears to have a beneficial effect on postoperative morbidity and mortality and this finding should be sufficient reason to further develop effective and safe techniques for the intravenous delivery of insulin therapy and to unravel its mode of action.

Rationale for the use of insulin therapy in cardiac surgery:

In cardiac surgery, GIK has been applied in several studies suggesting similar effects, i.e., a reduction in mortality and improvement of postoperative recovery (9), (10), (11), (12). However, the results of these studies were also questioned due to the low number of patients recruited,

inadequate study design, differences in protocols and inclusion criteria, as well as a lack of randomization.

Insulin therapy in diabetic cardiac surgery patients:

The role of diabetes mellitus in the metabolic treatment of heart disease and specifically insulin in cardiac surgery is not clear. In patients with diabetes mellitus type II insulin sensitivity is impaired to varying degrees (12). Therefore infusion of insulin may require adjustments in the protocol or may not even be effective at all. There is ample evidence that insulin therapy is a formidable treatment for diabetic patients with acute myocardial infarction (2). as well as for those patients undergoing cardiac surgery (11). It is conceivable that the changes in insulin sensitivity observed in the entire organism are also present in the heart. The heart has been described as a specific target of diabetes mellitus and mechanisms have been set forth to explain a "diabetic cardiomyopathy" (13). Jagasia and associates (14) demonstrated that patients with diabetes showed lower glucose extraction from the coronary arteries but that the total amount of glucose uptake was the same as in healthy persons. Assuming that insulin utilizes one or more of the metabolic mechanisms, the observation by Jagasia and associates could explain why diabetic patients respond well to insulin therapy. Taking this new evidence and the results of clinical trials on

diabetic patients into account it appears reasonable to apply insulin therapy in diabetic patients. The question whether the insulin dosage requires adjustment must remain open at this time.

Insulin therapy in non-diabetic cardiac surgery patients:

In the growing cohort of elderly cardiac surgical patients with a history of severe, chronic coronary artery disease (CAD), chronic heart failure, and reduced contractile reserve preoperatively. These patients frequently require prolonged episodes of extracorporeal circulation (ECC) for complicated coronary revascularization. Hemodynamic abnormalities and acute heart failure frequently develop in these patients after ECC. Serious cardiovascular complications usually begin in the period after ECC. At that time, acute ventricular failure may develop, a condition probably caused by postischemic dysfunction or myocardial stunning. This phenomenon may thus be superimposed on pre-existing impaired ventricular function preoperatively. The standard therapy consists of large doses of inotropic agents, nitroglycerin, peripheral vasopressors, intra Aortic balloon pumping or a combination of these. The use of adrenoceptor-stimulating therapy at high infusion rates is especially associated with a number of undesirable side effects, including tachycardia and increased oxygen requirement of the postischemic and dysfunctional myocardium, and is often

only effective for a limited period of time. The latter may be due to acute receptor down-regulation, an increase in plasma lipids in the presence of high endogenous and exogenous catecholamine levels, insulin resistance, and a reduction in myocardial glucose uptake and utilization. Previous studies showed that adrenergic stimulation suppresses myocardial glucose uptake and by raising the level of circulating free fatty acid and that this inhibition can be reversed by insulin on the basis of substrate availability and competition. These findings may have clinical importance in patients requiring long-term treatment with exogenous catecholamines and its metabolic consequences which is associated with adverse outcome after CABG and AMI(15), (16).

Mechanism of action of insulin on the heart:

Insulin was initially used as a polarizing agent to promote electrical stability (1), (17). Recent evidence suggests an effect of insulin on the cardiac membrane potential through a newly described ion channel (18). This influence may lead to a "membrane stabilizing" effect and could explain its reducing effects on atrial fibrillation. The mechanistic focus shifted over the years towards insulin induced changes in metabolism (19), (20), (21), (22),(23).

Metabolic effects of insulin:

- *Increasing cellular glycogen content:*

In the late 1970s and early 1980s many studies attributed the effects of insulin to its potential to increase preischemic glycogen content. As glycogen serves as fuel for anaerobic adenosine triphosphate (ATP) production, the preoperative application of insulin was supposed to increase recovery of contractile function by increasing preischemic glycogen content (24), (25). There has been a long-standing debate on the role of preischemic glycogen content in improving ischemia tolerance(26), (27), (28). Only recently has experimental work on the isolated working rat heart demonstrated that glycogen turnover rather than glycogen content may be associated with ischemia tolerance (29). From a clinical standpoint this hypothesis would not apply to those studies where insulin was administered during or even after ischemia.

- *Decreasing free fatty acid level:*

High concentrations of free fatty acids in the serum may have negative effects on postischemic contractile function for three main reasons (30), (31). First, high concentrations of free fatty acids in the cytosol may result in the accumulation of acyl-carnitine, which in turn may cause membrane damage. Second, incomplete oxidation of free fatty acids during periods of oxygen

deprivation may cause peroxide formation, which may also impair cellular function. Finally, the production of ATP from fatty acids is associated with relatively high oxygen consumption compared with ATP production from glucose oxidation(32), (33). Since ATP generation in heart muscle is governed by substrate competition, reducing free fatty acids in the serum may shift ATP production from fatty acid to glucose oxidation and therefore render the heart more oxygen efficient. This mechanism may apply particularly to the setting of cardiac surgery in which free fatty acids are liberated by the administration of heparin (34). Insulin has the potential to decrease serum free fatty acid levels by inhibiting hormone-sensitive lipase in adipose tissue and by directly inhibiting free fatty acid oxidation in the mitochondrion through the activation of acetyl-CoA-carboxylase(35).

- *Increasing glycolytic ATP production:*

A third metabolic mechanism discussed is the stimulation of glycolysis by insulin and glucose. This mechanism may apply during low-flow ischemia in particular. It has been suggested that increasing glycolysis results in cytosolic ATP production (36) which may be used in the maintenance of basic cell function such as ion pumps on the plasma membrane(37). While this mechanism has been considered a key principle in insulin action, it has also been the center of a debate on using GIK.

Increasing glycolysis through insulin would result in greater ATP production. The increase in anaerobic glycolysis also increases lactate production and results in acidosis(38). Neely and Grotyohann (34) suggested that reduction in anaerobic glycolytic activity would reduce ischemic injury and result in improved postischemic function, concluding that reducing intracellular acidosis may be beneficial. Many potential users of GIK have shied away from using this solution owing to this controversy. Others have tested Neely's hypothesis under similar or different conditions and were unable to repeat the results (39), (40).

Uncertainty still remains concerning this issue. However, because insulin as an exogenously applied drug must be delivered to the site of action through the blood stream, low-flow ischemia or reperfusion must be present rather than total ischemia. It is likely that if protons and lactate accumulate, this low flow will be able to discard the waste products.

- *Anaplerosis:*

The Taegtmeyer group suggested an anaplerotic mechanism to explain the effects of insulin on recovery of myocardial function (41), (42). The term Anaplerosis was coined by Kornberg (43) in 1966 and refers to the replenishment of ischemically depleted substrates of the glyoxylate cycle in bacteria. A similar depletion of substrate occurs in the citric acid cycle of the

mammalian heart during ischemia. This substrate depletion can impair the Krebs cycle's normal turnover rate and thus significantly interfere with energy production in the heart muscle during the recovery phase after ischemia. The same group demonstrated that anaplerotic pathways are surprisingly active in the myocardium(44).

- ***Other mechanisms:***

Insulin increases the activation of sympathetic nerves by hyperinsulinemia, which may then result in increased contractile function (45). Insulin is also a known vasodilator and thereby results in a decrease in peripheral vascular resistance (46;47). This decrease in peripheral vascular resistance would then improve cardiac output. The association of a decrease in peripheral vascular resistance by insulin and an increase in cardiac output has been demonstrated in several GIK studies (17), (48). In addition, recent evidence from animal studies suggests that insulin has the potential to reduce the inflammatory response that is associated with AMI and CABG (49). Finally insulin has been demonstrated to activate plasminogen activator inhibitor 1 (50), the significance of which in the setting of acute application in cardiac surgery or acute myocardial infarction is still unclear (51).

Controversies in insulin therapy in cardiac surgery:

Table (1-1) summarizes Clinical GIK studies in cardiac surgery (52).

uthor	Number of Patients		Exclusion Criteria	Operation	Time Point	Glucose	Insulin (IU)	K+ (mval)	Applicatio Mode
	Control	GIK							
ensson roup I oup II oup III	6	18[a]	VL, DM	CABG	Postop	1000 mL G40%	0.35/kg 1.5/kg 7.5/kg	100/L	Insulin as bolus after OP; AA.
olley	53	49	EF < 40%, MI, VA, VL	CABG	Periop	1000 mL G5%	20	20	Cold solution with 1200 mL/h in aortic root during XC
irard	40	40	———	CABG &/or HVR	Periop	1000 mL G50%	80	100	With 1.66 mL/kg/h from IA until XC.
stbacka	16	16	EF < 50%, EMD	CABG	Periop	0.6g/kg/h	0.12/kg/h	0.1/kg/h	From IA until start o CPB.
rodin	7	7	———	CABG	Periop	0.5g/kg/h	1.35/kg/h	0.25/kg/h	From IA until XC.
stbacka	20	20	EF < 40%, age > 70y, EMD	CABG	Periop & postop	0.2g/kg/h	0.12/kg/h	0.15/kg/h	From AC until CPB, then reduction t 30% until morning after OP.
azar	15	15	RF, LF, DM, hyperkalemia, VA, VL	CABG	Periop & postop	1000 mL G30%	50	80	From IA until CPB, & from en of over 12 with 1 mL/kg/h

›sogul	15	15	———	MVR	Preop	1000 mL G20%	10	45	Over 12 h-period on day before operation.
Rao ·oup I oup II	10 17	12 17	E, EF < 20%, DM, MI, emergency cases	CABG	Periop	7.56 g/L 15.1 g/L	10/L	d.n.a.	With every cardioplegi infusion
,azar	20	20	PCDM, RF, LF, no DM	CABG	Periop & postop	500 mL G5%	80	40	From IA until CPB, & from enc of XC over 12 h with 30 mL/h.
zabó	10	10	EF < 40%, LF, AP, age > 80y, no DM II	CABG	Postop	G30%	L/kg/h → 25	D.n.a.	Insulin for h after OP; AA; →into HLM.

Table (1-1) key:

AA = rates for glucose & potassium infusion were adjusted accordingly;

AC = aortic cannulation; AP = unstable angina pectoris;

CABG = coronary aortic bypass grafting; CPB = cardiopulmonary bypass;

d.n.a. = Data not available; DM = diabetes mellitus; E = female sex;

EF = ejection fraction; EMD = endocrine or metabolic disease;

GIK = glucose-insulin-potassium influsion; HLM = heart-lung-machine;

HVR = heart valve replacement; IA = induction of anesthesia;

LF = liver failure; MI = recent myocardial infarction;

MVR = mitral valve replacement; OP = operation;

PCDM = poorly controlled DM or metabolic disturbance other than diabetes or late complications of DM; $\rightarrow$ = Bolus.

RF = renal failure; VA = ventricular aneurysm; VL = valvular lesion.

XC = clamping of the aorta; [a] This study had three different GIK groups.

The question that arises is: why did GIK not become daily routine in cardiac surgery in spite of predominantly positive results from all these clinical studies? This is mainly due to the controversies in the different protocols used in cardiac surgery that is summarized below:

Mechanism of action:

From a mechanistic perspective, several of the positive insulin effects are not yet fully understood and the question as to how insulin deliver their actions remains unanswered. Despite the heterogeneity of studies in the context of cardiac surgery including various starting points, methods, and study designs, a majority of studies draws positive conclusions with regard to the insulin effects, leaving room for the assumption that insulin effect is probably multifactorial including unknown, non-metabolic, positive inotropic effects (52).

Optimum insulin dose:

While the mechanisms stated above may be more or less convincing, they all encounter serious limitations by conflicting experimental or clinical evidence. The major concerns involving all the metabolic mechanisms proposed is the presence of insulin resistance after ischemia and especially after cardiac surgery(53). Such insulin resistance may explain why protocols

supplying low doses of insulin have failed to demonstrate significant effects (54). As in the diabetic heart, it is not yet clear whether such whole-body insulin resistance translates to the heart. It is no secret that large amounts of insulin can be administered after cardiac surgery without significantly affecting serum glucose (55) and the beneficial effects of insulin were most prevalent when glucose and insulin had been given in high dosages (17), (56), (57). Additionally, the use of glucose-insulin-potassium in a titrated manner may cause severe disturbances in glucose homeostasis oscillating between hyper- and hypoglycemia. Therefore, the use of fixed high dose insulin therapy to maintain glucose homeostasis is a convincing concept.

Timing of insulin therapy:

The therapeutic potential of insulin administration early after ischemia is supported by experimental studies in pigs and rats, reporting a direct, nonmetabolic effect of insulin on postischemic contractile function (58) and a reduction in infarct size during reperfusion (59), (60). In addition, other smaller studies demonstrated significant effects when insulin was given postoperatively (17). Administration of insulin during reperfusion (i.e., postoperatively) appears critical. Several investigators have addressed the issue of timing in animal models, again with conflicting evidence (48), (51). Some studies revealed greater relative improvement by insulin when the

calculations were limited to the studies using insulin perioperatively as well as postoperatively (19.5% vs. 11.4%) (11), (14), (61). Lazar and associates (51) demonstrated that a perioperative protocol was superior compared with a postoperative one in pigs. While the subsequent clinical studies demonstrated benefits of two perioperative protocols (one for diabetic patients and one for nondiabetic patients), a strictly postoperative protocol has not been compared. A large randomized clinical trial of insulin application in cardiac surgery is a recent study by Rao and associates (62) where insulin was given in low concentrations into the cardioplegic solution. The authors were unable to demonstrate a reduction in postoperative low output syndrome or mortality. However, in a smaller study the same investigators demonstrated improved recovery of function by the same treatment in one group of that investigation (63). That could be attributed to the delay in starting the insulin therapy until the ischaemic insult was inflicted and may be as well due to the localized administration of insulin in the heart, which excludes the other beneficial systemic effects of insulin. The potential efficacy of insulin given during cold cardioplegia may also be challenged, because substrate metabolism should be minimal. Based on these observations and the results of this meta-analysis it appears most reasonable at this time to apply insulin as early as possible before the ischaemic insult.

With regard to the data available, it is currently difficult to make a recommendation on the best duration of insulin treatment. Although prolonged administration of insulin may reduce morbidity and mortality in patients requiring longer intensive care unit stays (13), (64), it may interfere with patient transfer in those recovering uneventfully. However, these questions can only be fully resolved when tested by large, randomized trials and when the underlying mechanisms are discovered.

Insulin therapy study endpoints:

GIK has been widely applied under various experimental and clinical conditions. 11 randomized GIK studies in cardiac surgery used recovery of contractile function as a primary endpoint (51). Myocardial infarction and the presence of low output syndrome were also used as study endpoints in a large trial (60). Insulin has also been shown to be effective in reducing the incidence of postoperative atrial fibrillation. It has been questioned whether these are the only important aspects to assess with respect to recovery of patients after cardiac surgery(65).

Summary:

Although the beneficial effects of insulin infusion in the treatment of cardiac failure and its use in cardiac surgery have been described for more than 40 years, this technique is still a matter of controversy—even though a plethora of theoretical and clinical studies exists. Despite positive results from experimental studies, GIK did not obtain ample acceptance in the past. However, interest towards this technique has started to shift after the positive effects of insulin on ischemic myocardium have been shown. These effects go far beyond a simple metabolic benefit; they include the better recovery of myocardial tissue after ischemia via still unknown mechanisms (64), (66), (67). Studies on the effects of insulin infusion in the last four decades are extremely heterogeneous and can only be compared with difficulties. Several of them are based on a low number of cases and often leave questions unanswered with respect to design and methods. Twenty-five of the 38 studies report positive results with regard to insulin infusions, 11 studies report neutral or negative results, and two studies argue neither for nor against insulin infusions, as both studies were primarily not aimed at insulin effects but on other endpoints. Three studies reporting neutral or negative results were performed on patients undergoing off-pump surgery. Due to the nature of this operative technique, it is questionable, whether

insulin can exert beneficial effects in patients without myocardial infarction at all(68). After excluding these three studies and the two studies that were not aimed at GIK insulin effects, the 'positive ratio' rises from 66% to 76% (25 of 33), which is in symphony with the results of other papers, e.g., Bothe and coworkers (56). Although the majority of studies come to a positive conclusion and recommend the use of insulin infusion in cardiac surgery, mechanisms of action for the beneficial effects as well as adequate measures for their assessment are still being discussed. Thus, none of the studies alone provide enough evidence for an ample use of insulin. However, a clear trend in favor of its use exists among the majority of studies. Due to the inconclusive and unsatisfying situation, insulin therapy has not become part of daily clinical routine especially in light of today's standards in evidence-based decision making. Uncertainty about the underlying mechanisms in an era of evidence-based decisions is one of the reasons preventing a major breakthrough in insulin therapy.

In view of the increasing number of older patients at higher risk, the demand to further improve the outcome of cardiac surgery has renewed the interest in the insulin therapy concept. The more recent evidence suggests that the entire potential of the insulin therapy has not yet been fully disclosed.

How did we address these controversies in our work?!

There were a number of novel approaches that we considered in our work:

1) We think that metabolic effects of insulin should be studied in any novel insulin therapy as well as considering other mechanisms, which was anti-inflammatory properties in our project.
2) We realized that if we want to study insulin effects then we have to develop a way of controlling insulin infusion. That makes high dose insulin therapy that we developed different that GIK solution used in previous studies as will be explained later.
3) We used the " before insult "principle which necessitates using insulin therapy prior to the induction of damage that we expect insulin to abolish or attenuate.
4) As an end point, we studied different end points that are specific to the mechanism of action being investigated.

Difference between GIK and high dose insulin therapy:

GIK therapy:	*High dose insulin therapy:*
1) Cocktail of glucose, insulin and potassium mixed together.	1) Insulin, glucose and potassium are given separately.
2) The whole solution is titrated to maintain different glycemic ranges according to the treatment protocol.	2) Insulin is infused at a fixed dose and glucose is titrated to different glycemic ranges according to the treatment protocol with potassium infusion or boluses as needed.
3) Difficult to control the differential amount of infusion of each component separately.	3) Easy to control all the components of the therapy.

Reference List

(1) (Braimbridge MV, Clement AJ, Hedley Brown A, Sabar E, Mendel D. Triple starr valve replacement. Br Med J. 1969;3:683-688)

(2) (Svedjeholm R, Huljebrant I, Håkanson E, Vanhanen I. Glutamate and high dose Glucose-Insulin-Potassium (GIK) in the treatment of severe cardiac failure after cardiac operations. Ann Thorac Surg. 1995;59:S23-S30).

(3) (Lazar HL, Phillipides G, Fitzgerald C. Glucose-insulinpotassium solutions enhance recovery after urgent coronary artery bypass grafting. J Thorac Cardiovasc Surg.1997;113:354-362)

(4) (Taegtmeyer H, Goodwin GW, Doenst T, Frasier OH. Substrate metabolism as a determinant for postischemic functional recovery of the heart. Am J Cardiol. 1997; 80:3A)

(5) (Van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in critically ill patients. N Eng J Med. 2001;345:1359)

(6) (Lazar H, Chipkin S, Philippides G, Bao Y, Apstein C. Glucose-Insulin-Potassium solutions improve outcomes in diabetics who have coronary artery operations. Ann Thorac Surg. 2000;70:145-150)

(7) (Lazar HL, Chipkin SR, Fitzgerald CA. Tight glycemic control in diabetic coronary artery bypass graft patients improves perioperative outcomes and decreases recurrent ischemic events. Circulation. 2004;09:1497-1502).

(8) (Carson JL, Scholz PM, Chen AY, Peterson ED, Gold J, Schneider SH. Diabetes mellitus increases short-term mortality and morbidity in patients undergoing coronary bypass graft surgery. J Am Coll Cardiol. 2002;40:418-423)

(9) (Lolley DM, Myers WO, Ray JF, Sautter RD, Tewksbury DA. Clinical experience with preoperative myocardial nutrition management. J Cardiovasc Surg. 1985;26:236-243),

(10) (Gradinac S, Coleman GM, Taegtmeyer H, Sweeney MS, Frazier OH. Improved cardiac function with glucose-insulin-potassium after coronary bypass surgery. Ann Thorac Surg. 1989;48:484-489),

(11) (Girard C, Quentin P, Bouvier H, et al. Glucose and insulin supply before cardiopulmonary bypass in cardiac surgery: a double-blind study. Ann Thorac Surg. 1992;54:259-263).

(12) (DeFronzo R., Ferranini E. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991;14:173-194)

(13) (Zarich S., Nesto R. Diabetic cardiomyopathy. Am Heart J 1989;118:1000-1012)

(14) Jagasia D, Whiting JM, Concato J, Pfau S, McNulty PH. Effect of Non-Insulin-Dependent Diabetes Mellitus on Myocardial Insulin Responsiveness in Patients With Ischemic Heart Disease. Circulation 2001; 103(13):1734-1739.

(15) (Capes SE, Hunt D, Malmberg K, Gerstein HC. Stress hyperglycemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. Lancet. 2000;335:773-778)

(16) (Zindrou D, Taylor KN, Bagger JP. Admission plasma glucose: independent risk factor in non-diabetic women after coronary artery bypass grafting. Diabetes Care. 2001;24: 1634-1639)

(17) (Fishleder BL. Estudios experimentales a nivelcelular del tratamiento polarizante. Arch Inst Cardiol Mex. 1964;34:681)

(18) Zhang YH, Hancox JC. A Novel, Voltage-Dependent Nonselective Cation Current Activated by Insulin in Guinea Pig Isolated Ventricular Myocytes. Circ Res 2003; 92(7):765-768.

(19) (Lolley DM, Ray JF III, Myers WO, Sheldon G, Sautter RD. Reduction of intraoperative myocardial infarction by means of exogenous anaerobic substrate enhancement: prospective randomized study. Ann Thorac Surg 1978;26:515-24),

(20) (Salerno T, Wasan SM, Charrette EJP. Glucose substrate in myocardial protection. J Thor Cardiovasc Surg. 1980;79:59-62),

(21) (Haider W, Benzer H, Schütz W, Wolner E. Improvement of cardiac preservation by preoperative high insulin supply. J Thorac Cardiovasc Surg. 1984;88:294-300)

(22) (Opie LH, Tansey M, Kennelly BM. Proposed metabolic vicious circle in patients with large myocardial infarcts and high plasma-free-fatty-acid concentrations. Lancet. 1977;2:890-892)

(23) (Taegtmeyer H. Is there a rationale for glucose-insulin-potassium therapy in acute myocardial infarction? Cardiol Rev. 1995;3:307-313)

(24) (Taegtmeyer H., Villalobos D.H. Metabolic support for the postischaemic heart. Lancet 1995;345:1552-1555)

(25) (Oldfield G.S., Commerford P.J., Opie L.H. Effects of preoperative glucose-insulin-potassium on myocardial glycogen levels and on complications of mitral valve replacement. J Thorac Cardiovasc Surg 1986;91:874-878)

(26) (Neely J.R., Grotyohann L.W. Role of glycolytic products in damage to myocardium: dissociation of adenosine triphosphate levels and recovery of function of reperfused canine myocardium. Circ Res 1984;55:816-824)

(27) (Lagerstrom C.F., Walker W.E., Taegtmeyer H. Failure of glycogen depletion to improve left ventricular function of the rabbit heart after hypothermic ischemic arrest. Circ Res 1988;63:81-86)

(28) (Taegtmeyer H. Energy metabolism of the heart: from basic concepts to clinical applications. Curr Prob Cardiol 1994;19:57-116).

(29) Goodwin GW, Arteaga JR, Taegtmeyer H. Glycogen Turnover in the Isolated Working Rat Heart. J Biol Chem 1995; 270(16):9234-9240.

(30) Lopaschuk GD, Wambolt RB, Barr RL. An imbalance between glycolysis and glucose oxidation is a possible explanation for the detrimental effects of high levels of fatty acids during aerobic reperfusion of ischemic hearts. J Pharmacol Exp Ther 1993; 264(1):135-144.

(31) (Lopaschuk G.D., Belke D.D., Gamble J., et al. Regulation of fatty acid oxidation in the mammalian heart in health and disease. Biochim Biophys Acta 1994;1213:263-276)

(32) (Lopaschuk GD. Optimizing cardiac energy metabolism: how can fatty acid and carbohydrate metabolism be manipulated? Cor Artery Dis 2001;12:S8-11)

(33) (Goodwin G.W., Ahmad F., Doenst T., et al. Energy provision from glycogen, glucose and fatty acids upon adrenergic stimulation of isolated working rat heart. Am J Physiol 1998;274:H1239-1247)

(34) (Wittnich C., Dewar M.L., Chiu R. Myocardial protection: heparin-induced free fatty acid elevation during cardiopulmonary bypass and its prevention. J Surg Res 1984;36:527-531)

(35) (Gamble J., Lopaschuk G.D. Insulin inihibition of 5'adenosine monophosphate-activated protein kinase in the heart results in activation of acetyl coenzyme A carboxylase and inhibition of fatty acid metabolism. Metabolism 1997;46:1270-1274)

(36) (Neely J.R., Morgan H.E. Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. Ann Rev Physiol 1974;36:413-439)

(37) (Weiss J., Hiltbrand B. Functional compartmentation of glycolytic versus oxidative metabolism in isolated rabbit heart. J Clin Invest 1985;75:436-447)

(38) (Neely J.R., Whitmer J.T., Rovetto M.J. Effect of coronary blood flow on glycolytic flux and intracellular pH in isolated rat hearts. Circ Res 1975;37:733-741)

(39) Goodwin GW, Arteaga JR, Taegtmeyer H. Glycogen Turnover in the Isolated Working Rat Heart. J Biol Chem 1995; 270(16):9234-9240.

(40) (Jonassen A.K., Aasum E., Riemersma R.A., et al. Glucose-insulin-potassium reduces infarct size when administered during reperfusion. Cardiovasc Drug Ther 2000;14:615-623)

(41) (Russell R, Taegtmeyer H. Anaplerosis improves fuel oxidation and function in isolated working rat hearts. Circulation 1990;82:III213)

(42) Apstein CS, Taegtmeyer H. Glucose-Insulin-Potassium in Acute Myocardial Infarction : The Time Has Come for a Large, Prospective Trial. Circulation 1997; 96(4):1074-1077.

(43) (Kornberg H.L. Anaplerotic sequences and their role in metabolism. Essays Biochem 1966;2:1-31.)

(44) Russell RR, III, Taegtmeyer H. Pyruvate carboxylation prevents the decline in contractile function of rat hearts oxidizing acetoacetate. Am J Physiol Heart Circ Physiol 1991; 261(6):H1756-H1762.

(45) (Rowe J.W., Young J.B., Minaker K.L., et al. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. Diabetes 1981;30:219-225)

(46) Abramson DI, Schkloven N, Margolis MN, Mirsky IA. INFLUENCE OF MASSIVE DOSES OF INSULIN ON PERIPHERAL BLOOD FLOW IN MAN. Am J Physiol 1939; 128(1):124-132.

(47) Baron AD, Brechtel G. Insulin differentially regulates systemic and skeletal muscle vascular resistance. Am J Physiol Endocrinol Metab 1993; 265(1):E61-E67.

(48) (Szabo Z., Arnqvist H., Hakanson E., et al. Effects of high-dose glucose-insulin-potassium on myocardial metabolism after coronary surgery in patients with type II diabetes. Clin Sci (Lond) 2001;101:37-43)

(49) (Jeschke MG, Klein D, Bolder U, et al. Insulin attenuates the systemic inflammatory response in endotoxemic rats. Endocrinology. 2004;145:4084-4093)

(50) Nordt TK, Sawa H, Fujii S, Sobel BE. Induction of Plasminogen Activator Inhibitor Type-1 (PAI-1) by Proinsulin and Insulin In Vivo. Circulation 1995; 91(3):764-770.

(51) Doenst T, Bothe W, Beyersdorf F. Therapy with insulin in cardiac surgery: controversies and possible solutions. Ann Thorac Surg 2003; 75(2):721S-7728.

(52) Bothe W, Olschewski M, Beyersdorf F, Doenst T. Glucose-Insulin-Potassium in Cardiac Surgery: A Meta-Analysis. Ann Thorac Surg 2004; 78(5):1650-1657.

(53) (Degens H, Soop M, Höök P, et al. Post-operative effects on insulin resistance and specific tension of single human skeletal muscle fibres. 1999;97:449-55)

(54) (Ceremuzynski L., Budaj A., Czepiel A., et al. Low-dose glucose-insulin-potassium is ineffective in acute myocardial infarction: results of a randomized multicenter Pol-GIK trial. Cardiovasc Drug Ther 1999;13:191-200.)

(55) (Svensson S., Berglin W.-O.E.E., Ekroth R., et al. Hemodynamic effects of a single large dose of insulin in open heart surgery. Cardiovasc Res 1984;18:697-701)

(56) Diaz R, Paolasso EA, Piegas LS, Tajer CD, Moreno MG, Corvalan R et al. Metabolic Modulation of Acute Myocardial Infarction : The ECLA Glucose-Insulin-Potassium Pilot Trial. Circulation 1998; 98(21):2227-2234.

(57) (Rogers W.J., Stanley A.W., Breing J.B., et al. Reduction of hospital mortality rate of acute myocardial infarction with glucose-insulin-potassium infusion. Am Heart J 1976;92:441-454.)

(58) Doenst T, Richwine RT, Bray MS, Goodwin GW, Frazier OH, Taegtmeyer H. Insulin improves functional and metabolic recovery of reperfused working rat heart. Ann Thorac Surg 1999; 67(6):1682-1688.

(59) Jonassen AK, Sack MN, Mjos OD, Yellon DM. Myocardial Protection by Insulin at Reperfusion Requires Early Administration and Is Mediated via Akt and p70s6 Kinase Cell-Survival Signaling. Circ Res 2001; 89(12):1191-1198.

(60) Lazar HL, Zhang X, Rivers S, Bernard S, Shemin RJ. Limiting Ischemic Myocardial Damage Using Glucose-Insulin-Potassium Solutions. Ann Thorac Surg 1995; 60(2):411-416.

(61) (Wistbacka JO, Nuutinen LS, Lepojarvi MV, Nissinen J, Karlqvist KE, Ruokonen A. Perioperative glucose-insulin-potassium infusion in elective coronary surgery: minor benefit in connection with blood cardioplegia? Infusionsther Transfusionsmed. 1994;21:160-166)

(62) Rao V, Christakis GT, Weisel RD, Ivanov J, Borger MA, Cohen G. The Insulin Cardioplegia Trial: Myocardial protection for urgent coronary artery bypass grafting. J Thorac Cardiovasc Surg 2002; 123(5):928-935.

(63) Rao V, Borger MA, Weisel RD, Ivanov J, Christakis GT, Cohen G et al. INSULIN CARDIOPLEGIA FOR ELECTIVE CORONARY BYPASS SURGERY. J Thorac Cardiovasc Surg 2000; 119(6):1176-1184.

(64) Furnary AP, Gao G, Grunkemeier GL, Wu Y, Zerr KJ, Bookin SO et al. Continuous insulin infusion reduces mortality in patients with diabetes

undergoing coronary artery bypass grafting _. J Thorac Cardiovasc Surg 2003; 125(5):1007-1021.

(65) (Lazar HL. The insulin cardioplegia trial. J Thorac Cardiovasc Surg. 2002;123:822-844)

(66) (Wallin M, Barr G, oWall A, Lindahl SG, Brismar K. The influence of glucose-insulin-potassium (GIK) on the GH/IGF-1/IGFBP-1 axis during elective coronary artery bypass surgery. J Cardiothorac Vasc Anesth 2003;17(4):470-7)

(67) (Rassias AJ, Givan AL, Marrin CA, Whalen K, Pahl J, Yeager MP. Insulin increases neutrophil count and phagocytic capacity after cardiac surgery.Anesth Analg 2002;94(5):1113-9),

(68) (Lell WA, Nielsen VG, McGiffin DC, Schmidt Jr FE, Kirklin JK, Stanley Jr AW. Glucose-insulin-potassium infusion for myocardial protection during off-pump coronary artery surgery. Ann Thorac Surg 2002;73(4):1246-51)

Chapter II

Myocardial Protection

During Elective Coronary Artery Bypass Grafting (CABG)

Using High Dose Insulin Therapy

Abstract:

Background: Coronary artery bypass grafting (CABG) with cardioplegic cardiac arrest and cardiopulmonary bypass (CPB) is associated with myocardial injury. The aim of this study was to investigate whether High dose insulin therapy has a myocardial protective effect by enhancing early metabolic recovery of the arrested heart during revascularization.

Methods: A total of 44 patients undergoing elective CABG were randomized to either receive intraoperative titrated insulin infusion according to insulin sliding scale (n=22) or intraoperative fixed high dose systemic insulin infusion at 5mU/kg/min (n=22). Blood samples were collected simultaneously from the radial artery and the coronary sinus before starting CPB, 5 minutes and 10 minutes after release of the aortic cross clamp to determine lactate, Oxygen saturation and hemoglobin concentration. Lactate extraction/ Release percentage and myocardial oxygen extraction were calculated and compared between the two groups. Change in Cardiac indices were determined immediately postoperatively as a measure of functional recovery and Troponin level was measured 4 hours post operatively as an indicator of myocardial protection.

Results: Operative characteristics including CPB and cross clamp time were similar between the two groups. Arterial O_2 content was similar in both

groups. However, High dose insulin therapy group had early extraction of lactate, higher oxygen extraction and better improvement of cardiac indices immediately postoperatively compared to the standard group that had persistent release of lactate and higher Troponin level 4 hours post operatively.

Conclusion: High dose insulin therapy promotes early metabolic recovery of the heart during elective CABG leading to better myocardial protection and functional recovery.

Introduction:

Coronary artery bypass grafting (CABG), performed with the aid of cardioplegia and cardiopulmonary bypass (CPB), requires a period of cardiac arrest. During this time myocardial ischemia and necrosis may occur which is an important determinant of functional and clinical outcome (1). Reducing the period of arrest during which the myocardium is subjected to ischemia and necrosis would be one method of limiting myocardial injury, but more complex and time consuming procedures are now being performed in an older population of patients with reduced cardiac reserve. Different myocardial protection strategies have thus been introduced and compared (2). One of these strategies is the metabolic preconditioning of the ischemic myocardium. The normal heart obtains approximately two-thirds of its energy from free fatty acid (FFA) oxidation. During partial reduction in flow, Ischemia stimulates the glycolytic pathway; however, there is continued oxidation of fatty acids and a greater mitochondrial NADH-to-NAD^+ ratio, which operate to inhibit flux through pyruvate dehydrogenase (PDH) pathway. Thus there is greater pyruvate formation from glycolysis but a decreased ability to oxidize pyruvate and NADH in the mitochondria, which drives pyruvate conversion to lactate, accumulation of lactate and H^+, and contractile dysfunction (3). During reperfusion, persistent lactate release

suggests a delayed recovery of aerobic myocardial metabolism and may be related to intraoperative misadventure or inadequate myocardial protection. Therefore, myocardial lactate release was advocated as an alternative end-point in clinical trials evaluating perioperative myocardial protection (4).

Glucose-insulin-potassium (GIK) was commonly used as an adjuvant therapy in cardiac surgery because of its potentially beneficial effects on myocardial metabolism and contractile function. However, controversy exists as to the exact aims of therapy, the relative concentration of insulin and glucose required, the degree of blood glucose control achievable, and the optimum timing and delivery technique (5) which lead to inconsistency in demonstrating beneficial effects of this therapy. One of the obstacles with this therapy in cardiac surgery is CPB and surgery induced insulin resistance which alters glucose metabolism and makes the balance between optimal glucose and insulin delivery complex, often leading easily to elevated blood glucose levels during the therapy (6). Due to the inconsistent beneficial effects of GIK and associated hyperglycemia it has been advocated to use higher doses of insulin in future studies (5).

The aim of this study was to investigate whether High dose insulin therapy has a myocardial protective effect by enhancing early metabolic recovery of the arrested heart during revascularization.

Methods:

Study design and Patient enrollment

After obtaining approval from the research ethics committee in our institution, an informed consent was obtained from all participants. All patients referred for elective CABG between Nov 2005 and Nov 2006 were assessed for eligibility. Among 93 patients assessed, 49 patients were excluded due to the presence of one or more of the exclusion criteria which included: 1) Emergency CABG, 2) Redo CABG, 3) Combined CABG and any other cardiac procedure, 4) Any deviation from the protocol. The other 44 patients were randomized using a computerized randomization tables into group I, the standard of care, and group II, the high dose insulin therapy.

Treatment protocols

The study included both diabetic and non-diabetic patients. In diabetic patients on oral hypoglycemic agents, administration of oral hypoglycemic agents was discontinued 24 hours prior to surgery. Diabetic patients on insulin had their daily dose of insulin held the evening before surgery. A subcutaneous insulin sliding scale was started preoperatively in diabetic patients according to the protocol specified below, (Table 2-1). Intraoperatively, group I received the standard of care using an intravenous

insulin infusion according to insulin sliding scale starting at blood glucose level of 10 mmol/L as specified below, (Table 2-2). Blood glucose levels were checked every 30 minutes with the Accu-chek® glucose monitor (Roche Diagnostics, Switzerland). As previously described (7), Group II received a fixed high dose of intravenous insulin infusion at 5mU/kg/min. Dextrose 20% infused in the same group at a rate adjusted to maintain a BG of 4-6mmol/L. Additional boluses of insulin were given if the blood glucose remains > 6.0 mmol/L according to the sliding scale specified below, (Table 2-3). Arterial blood glucose was measured every 5 to 10 minutes throughout the procedure. In both groups the protocol was started immediately on arrival to the operating room and stopped just before being transferred to the intensive care unit (ICU).

Anesthetic management

Preoperative sedation with 1 to 3 mg of lorazepam p.o. and oxygen was administered to patients on call to the operating room. All patients received prophylactic perioperative antibiotics (cefazolin 2 g before incision and 2 g post-CPB or vancomycin 1 g before incision and 500 mg post-CPB if allergic to penicillin). The same anesthesiologist administered standardized total intravenous anesthesia using sufentanil, midazolam and pancuronium. Immediately prior to CPB, heparin 400 IU/Kg was

administered intravenously followed by additional doses, if necessary, to maintain an activating clotting time >500 seconds. Protamine was administered as 1 mg/100IU of the heparin dose after complete separation from CPB.

Surgical procedure

All patients had CABG by the same surgeon with the use of CPB, which was conducted with a roller pump, and a membrane oxygenator primed with a solution consisting of 1 L Ringer's lactate, 5000 IU heparin, 750 ml pentaspan, and 44-mEq bicarbonate. During CPB, pump flow was set at 2.4 times the body surface area and mean arterial pressure maintained between 50 and 60 mmHg. Temperature was allowed to drift with active rewarming at the end of CPB. Cardioplegia solution (Plegisol, Hospira, INC., Lake Forest, IL, USA) was free of glucose and consisted of high dose (100 mEq/L) and low dose (40 mEq/L) potassium used at the discretion of the cardiac surgeon. A single clamp technique was used and cardioplegia was given in an anterograde fashion with blood in a ratio of 1:4. Blood cardioplegia was also administered with each successive distal vein graft anastemoses. All patients had left internal mammary artery harvested and anastomosed to the left anterior descending artery. The rest of grafts were constructed using the great saphenous vein. After rewarming and suturing of

all anastemoses prior to the removal of the aortic cross-clamp "a hot shot" of 1 L of warm cardioplegia was administered. After total release of the aortic cross-clamp, epicardial atrial and/or ventricular pacing wires were placed. After an appropriate test dose of protamine, aortic and venous cannulas were removed and the surgery proceeded with closure of the pericardium and sternum.

Lactic acid (Lac) balance and myocardial Oxygen extraction:

Simultaneous blood samples were collected from arterial blood and coronary sinus, by using a retrograde perfusion cannnula, just before commencing CPB, 5 and 10 minutes after the release of the aortic cross clamp. These samples were used to determine lactate concentration, hemoglobin concentration (Hb) and oxygen saturation (O2 Sat). Lactate balance was calculated as follows: (lactate balance = Arterial – Coronary sinus lactate concentration). A negative value indicates lactate release and a positive value indicates lactate uptake and their values were expressed as a ratio of lactate balance to the arterial lactate concentration at the same time point. Oxygen content in both arterial and coronary sinus blood was calculated using the following formula: (Oxygen content = 1.38 * Hb * O2 Sat). Arterial – Coronary sinus Oxygen content difference was calculated and its ratio to arterial Oxygen content represented Oxygen extraction.

Functional recovery and postoperative Troponin level:

Cardiac index (C.I) was calculated pre- and immediately postoperatively to determine the percentage change in C.I as an indicator of functional recovery. Central venous pressure (CVP) and pulmonary capillary wedge pressure (PCWP) measurements were compared at the same time points to ensure similar preload filling status. Troponin Concentrations were measured 4 hours postoperatively as an indicator of myocardial protection.

Statistical analysis

Statistical analysis was performed using NCSS statistical software (2004). Continuous variables were compared using either the two-sample ***t***-test or the Wilcoxon rank sum test as appropriate by the distribution of data. Categorical variables were compared using chi-square test or Fisher's exact test depending on the number of items in each group. The statistical analysis was done using "per-protocol" method and statistical significance was deemed present when $P< 0.05$.

Results:

Demographic characteristics

Among 44 patients enrolled in the study, 22 patients were randomized to the control group (standard) and 22 patients to the high dose insulin

therapy group (clamp). Patients in the clamp group were slightly younger (59±3 years Vs 65±2 years in standard group, P=0.05) and there were more patients with left main coronary artery disease in the clamp group (45% Vs 14% in the standard group, P=0.02). Prevalence of Diabetes was similar in both groups (P= 0.20). The rest of demographic data is shown below, (table 2-4).

Intraoperative characteristics

The CPB time was not statistically different between the two groups (86 ± 6 minutes in the standard group Vs. 78 ± 5 minutes in the clamp group, P=0.30) as well as the aortic cross clamp time (71 ± 5 minutes in the standard group Vs. 65 ± 5 minutes in the clamp group, P=0.33). The mean number of grafts was 3.4 in the standard group compared to 3.1 in the Clamp group. The median average blood glucose level intraoperatively was 7.5 mmol/L (95%CL= 6.0 – 8.1) in the standard group while it was 4.9 mmol/L (95%CL= 4.6 - 5.0) in the clamp group (P=0.00). Also the standard group had lower median average intraoperative blood insulin level than the clamp group [117 pmol/L (95%CL= 44 - 169) Vs. 3142 pmol/L (95%CL= 2664-3873) consequently, P=0.00]. Both groups had similar blood levels of glucose (median = 5.9 mmol/L in both groups, P=0.40) and insulin

[standard = 77 pmol/L (95%CL= 50 - 98) Vs. 99 pmol/L (95%CL= 44- 110) in the clamp group] on arrival to the operating room.

Lactic acid balance and myocardial Oxygen extraction:

The myocardial lactate balance started to change from release to uptake in the clamp group after 5 minutes of aortic cross clamp removal and reperfusion with 0% balance at 5 minutes and 3% at 10-15 minutes while in the standard group the myocardium continued to release Lac > 10 minutes as indicated below, (Table 2-5), (Figure 2-1).

Myocardial oxygen extraction was lower post ischemia than its pre-ischemic level in both groups. However the oxygen extraction in the clamp group started to increase gradually during reperfusion (Figure 2-2), and it was significantly higher than the standard group after 10 minutes of reperfusion, (Table 2-6).

Functional recovery and post operative Troponin level:

Preoperative cardiac indices were comparable between the two groups (2.1 ± 0.1 in the standard group Vs. 1.9 ± 0.1 in the clamp group, P=0.19). Under the same preload filling status [(CVP = 8 ± 1 in both groups, P=1.0), (Pulmonary capillary wedge pressure = 11± 1 in the standard group Vs 10 ±

1 in the clamp group)], patients in the clamp group had higher immediate postoperative cardiac indices [median=3.4 (95%CL= 2.9-3.6) Vs 2.7 (95%CL= 2.2-3.2) in the standard group, P=0.02] with 71% increase from preoperative C.I compared to 34% improvement in the standard group (P=0.02), (Figure 2-3).

Troponin concentrations were measured in the arterial blood immediately and 4 hours postoperatively which was thought to represent the ischemic insult that occurred intraoperatively and it was significantly higher in the standard compared to the clamp group [7.5 (95%CL= 4.09-16.7) Vs 4.3 (95%CL= 3.0-6.0) in the clamp group, P=0.05], (Figure 2-4).

Postoperative characteristics

The median length of intubation was 15 hours in the standard group compared to 13 hours in the clamp group (P=0.12). There was no difference in the length of stay in the intensive care unit (24 hours in both groups, P=0.94) or in hospital stay (standard=6 days, clamp=5.5 days, P=0.48). There was no difference in the incidence of postoperative complications between the two groups. In group I: There was 1 perioperative MI, 1 re-exploration for bleeding, 1 superficial wound infection and 3 patients had new onset atrial fibrillation. In group II: There was no perioperative MI, no

patients were re-explored for bleeding, 1 superficial wound infection, 6 patients had new onset atrial fibrillation and 2 patients had respiratory failure. Due to the low number of perioperative complications no statistical comparison could have been made.

Discussion:

In this study we demonstrated that administration of intraoperative high dose insulin therapy have prepared the heart metabolically for ischemia and led to earlier shift to aerobic metabolism during reperfusion as indicated by earlier lactate uptake. This strategy resulted into better myocardial protection which was confirmed by lower post operative Troponin level and consequently higher postoperative cardiac indices. Similar results were shown by Haider and his colleagues (8) in patients who underwent mitral valve replacement in which the preventive application of high doses of insulin lead to an augmented myocardial adenosine triphosphate provision and a maintained cellular energy charge during coronary ischemia. As a result, ischemic tolerance was enhanced and myocardial protection was improved.

Effect of ischemia on cardiac metabolism:

Moderate ischemia, as observed during cardiac surgery, results in high levels of circulating free fatty acids and severely inhibited glucose oxidation.

Fatty acid oxidation, therefore, continues despite significant reductions in blood flow but incompletely. On the other hand, glucose oxidation is inhibited through the activation of Pyruvate kinase. Glycolysis is not inhibited to the same degree as oxidation, leading to an uncoupling of glycolysis and glucose oxidation. This uncoupling results in the production of excessive protons, causing injury and contributing to the observed decrease in cardiac function. Furthermore, there is significant evidence that preserving and/or enhancing aerobic metabolism is a key in maintaining cardiac function after ischemia (9).

The findings from trials comparing different cardioplegia strategies indicated that early lactate uptake is consistent with a more rapid recovery of aerobic metabolism. Moreover, the higher lactate release during reperfusion could be an expression of a more elevated glycolytic activity and anaerobic production of adenosine triphosphate (ATP) (10).

Metabolic effects of insulin:

Although insulin was initially used as a polarizing agent to promote electrical stability, the mechanistic focus shifted over the years towards insulin-induced changes in metabolism (8). Different metabolic effects of insulin have been proposed to cause beneficial effects in cardiac surgery: 1)

It enhances ischemia tolerance by increasing tissue-protective glycogen (9). Intramyocardial glycogen stores provide an immediate source of glycosyl units for aerobic and anaerobic glucose oxidation in the early stages of no-flow ischemia before substrate supply by enhanced glucose extraction from residual blood flow occurs (11), 2) Stimulates glycolysis and hence ATP synthesis, 3) increases the activity of pyruvate dehydrogenase (PDH), leading to improved myocardial uptake of lactate and pyruvate and an earlier return to aerobic metabolism (12), 4) Reduction of free fatty acid levels in plasma., 5) Increases Glucose uptake and oxidation, which becomes the main substrate for oxidative metabolism of the heart when the concentrations of glucose and insulin are high (13). The last two effects may be attenuated during cardiac surgery with the use of CPB due to induced insulin resistance that could be overcome by using high dose insulin therapy (12).

Other than these metabolic effects, insulin also: 1) Protects during reperfusion by insulin-mediated anti-apoptotic paths (14), 2) Increases coronary flow and cardiac efficiency in vivo (15) and in rat heart in vitro (16).

Cardiac metabolism and functional recovery:

The functional recovery at reperfusion is mainly determined by the extent of irreversible ischemic damage. After brief episodes of ischemia (up

to 20 to 30 minutes), oxidative metabolism rapidly returns, well before contractile activity is restored (17). This functional recovery could be related directly or indirectly to the above mentioned metabolic effects of insulin. The association between preserved myocardial glycogen and improved postischemic ventricular function has been described previously(18). Improved contractile recovery during reperfusion is more associated with the stimulation of glucose (19) and pyruvate oxidation (20) through activation of PDH, as opposed to the nonoxidative metabolism of glycolytic end products that forms alanine and lactate in the cytosol. This balance between oxidative and nonoxidative pyruvate metabolism is central to the recovery of pH and cytosolic redox state that are critical to the recovery of postischemic contractile function (21). Weiss and colleagues (22) demonstrated that ATP produced by oxidative phosphorylation was preferentially utilized for myocardial contractile function. Therefore, it is possible that persistent anaerobic lactate release during reperfusion may lead to inadequate ventricular function in the early postoperative period. Although it has been shown that mortality could be significantly lowered by achieving normoglycemia, to increase myocardial systolic function: elevated insulin levels and prevention of hyperglycemia are required concomitantly (23).

Postoperative Troponin level:

Intraoperative net troponin release has functional significance, as it is closely related to ischaemic time and reflects delayed recovery of left ventricular function and oxidative metabolism; therefore, its measurement may contribute to the perioperative assessment of myocardial injury sustained during coronary artery surgery (2). In a meta-analysis conducted by Bothe and his colleagues, despite the theoretical protective effects of GIK, no cardio protective effect of GIK was observed when Troponin level was used as a marker of myocardial protection (5). That supports our hypothesis that high dose insulin therapy is essential for myocardial protection.

Timing of therapy:

In our study, the high dose insulin therapy was started as soon the patient was brought to the operating rooms, which resulted in higher lactate uptake in the clamp group even before onset of ischemia and consequently lead to earlier recovery post ischemia and reperfusion. This is in agreement with the previous clinical and experimental studies that suggested that interventions aimed at decreasing ischemic damage prior to cardioplegic

arrest and reperfusion will result in the best recovery of myocardial function. It was also shown that substrate enhancement prior to cardioplegic arrest in acutely ischemic myocardium may limit myocardial necrosis (5). Although theoretically, and based on strong experimental evidence, insulin was advocated to be given early to the ischaemic myocardium prior to reperfusion, glucose and/or insulin when present prior to induction of ischemia gave better protection from fatty-acid induced regional ischaemia in the isolated rat heart (12).

Study limitations:

One study limitation relates to the use of concentration differences between arterial and coronary sinus blood to evaluate myocardial metabolism. When this measure is positive, it reflects myocardial uptake and, when it is negative it indicates a release from the myocardium. However, the actual uptake or release is also determined by blood flow. In this study, coronary blood flow was not measured. Great caution should therefore be employed in the interpretation of data.

Conclusion:

High dose insulin therapy promotes early metabolic recovery of the heart during elective CABG leading to better myocardial protection and functional recovery. In order for this therapy to exert its beneficial cardio-protective effects, it has to be administered systematically, in high doses and prior to the onset of ischemic insult. Our study is a metabolic study and a large-scale clinical outcome study is warranted to investigate the effect of this therapy on perioperative morbidity and mortality.

Reference List

(1) (Namay DL, Hammermeister KE, Zia MS, et al. Effect of perioperative myocardial infarction on late survival in patients undergoing coronary artery surgery. Circulation 1982;65:1066-1071)

(2) Koh TW, Hooper J, Kemp M, Ferdinand FD, Gibson DG, Pepper JR. Intraoperative release of troponin T in coronary venous and arterial blood and its relation to recovery of left ventricular function and oxidative metabolism following coronary artery surgery. Heart 1998; 80(4):341-348.

(3) (Randle, PJ. Fuel selection in animals. Biochem Soc Trans 14: 799-806, 1986).

(4) Rao V, Ivanov J, Weisel RD, Cohen G, Borger MA, Mickle DAG. Lactate release during reperfusion predicts low cardiac output syndrome after coronary bypass surgery. Ann Thorac Surg 2001; 71(6):1925-1930.

(5) Bothe W, Olschewski M, Beyersdorf F, Doenst T. Glucose-Insulin-Potassium in Cardiac Surgery: A Meta-Analysis. Ann Thorac Surg 2004; 78(5):1650-1657.

(6) Bruemmer-Smith S, Avidan MS, Harris B, Sudan S, Sherwood R, Desai JB et al. Glucose, insulin and potassium for heart protection during cardiac surgery. Br J Anaesth 2002; 88(4):489-495.

(7) Carvalho G, Moore A, Qizilbash B, Lachapelle K, Schricker T. Maintenance of Normoglycemia During Cardiac Surgery. Anesth Analg 2004;99:319-24.

(8) Haider W, Benzer H, Schutz W, Wolner E. Improvement of cardiac preservation by preoperative high insulin supply. J Thorac Cardiovasc Surg 1984; 88(2):294-300.

(9) Keith M, Errett L. Myocardial Metabolism and Improved OutcomesAfter High Risk Heart Surgery. Semin Cardiothorac Vasc Anesth 2005; 9(2):167-171.

(10) Mezzetti A, Calafiore AM, Lapenna D, Deslauriers R, Tian G, Salerno TA et al. Intermittent antegrade warm cardioplegia reduces oxidative stress and improves metabolism of the ischemic-reperfused human myocardium, , . J Thorac Cardiovasc Surg 1995; 109(4):787-795.

(11) (Depre C, Vanoverschelde JL, Taegtmeyer H: Glucose for the heart. Circulation 99:578-588, 1999)

(12) (Svensson S., Svedjeholm R., Ekroth R., et al. Trauma metabolism and the heart. Uptake of substrates and effects of insulin early after cardiac operations. J Thorac Cardiovasc Surg 1990;99:1063-1073)

(13) (Taegtmeyer H, Hems R, Krebs HA. Utilization of energy providing substrates in the isolated working rat heart. Biochem J. 1980;186:701-711)

(14) Jonassen AK, Sack MN, Mjos OD, Yellon DM. Myocardial Protection by Insulin at Reperfusion Requires Early Administration and Is Mediated via Akt and p70s6 Kinase Cell-Survival Signaling. Circ Res 2001; 89(12):1191-1198.

(15) McNulty, PH, Pfau S, and Deckelbaum LI. Effect of plasma insulin level on myocardial blood flow and its mechanism of action. Am J Cardiol 85: 161-165, 2000

(16) Doenst T, Richwine RT, Bray MS, Goodwin GW, Frazier OH, Taegtmeyer H. Insulin improves functional and metabolic recovery of reperfused working rat heart. Ann Thorac Surg 1999; 67(6):1682-1688.

(17) Gorge G, Chatelain P, Schaper J, Lerch R. Effect of increasing degrees of ischemic injury on myocardial oxidative metabolism early after reperfusion in isolated rat hearts. Circ Res 1991; 68(6):1681-1692.

(18) Iyengar SR, Charrette EJ, Iyengar CK, et al: Myocardial glycogen in prevention of perioperative ischemic injury of the heart: A preliminary report. Can J Surg 19:246-251

(19) Mallet RT, Hartman DA, Bunger R. Glucose requirement for postischemic recovery of perfused working heart. Eur J Biochem 1990; 188(2):481-493.

(20) Opie LH. Myocardial ischemia-metabolic pathways and implications of increased glycolysis. Cardiovasc Drugs Ther. 1990;4(suppl):777-790

(21) Lewandowski ED, White LT. Pyruvate Dehydrogenase Influences Postischemic Heart Function. Circulation 1995; 91(7):2071-2079.

(22) Weiss J., Hiltbrand B. Functional compartmentation of glycolytic versus oxidative metabolism in isolated rabbit heart. J Clin Invest 1985;75:436

(23) Ellger B, Debaveye Y, Vanhorebeek I, Langouche L, Giulietti A, Van Etten E et al. Survival Benefits of Intensive Insulin Therapy in Critical Illness: Impact of Maintaining Normoglycemia Versus Glycemia-Independent Actions of Insulin. Diabetes 2006; 55(4):1096-1105.

Table 2-1: The preoperative subcutaneous insulin sliding scale for diabetic patients

- Blood glucose measurement every 6 hours.
- If blood glucose

	Action
< 4.0 mmol/L	Give 25 ml of Dextrose 50% and repeat blood glucose measurement after 10 minutes
4.0 – 6.0 mmol/L	No Insulin
6.1 – 8.0 mmol/L	2 units insulin IV bolus
8.1 – 10.0 mmol/L	4 units insulin IV bolus
10.1 – 12.0 mmol/L	6 units insulin IV bolus
12.1 – 14.0 mmol/L	8 units insulin IV bolus
14.1 – 16.0 mmol/L	10 units insulin IV bolus
16.1 – 18.0 mmol/L	12 units insulin IV bolus
18.1 – 20.0 mmol/L	14 units insulin IV bolus
> 20.0 mmol/L	14 units insulin IV bolus and call MD on-call

Table 2-2: The intraoperative intravenous insulin sliding scale for the standard group

- If baseline blood glucose level > 10 mmol/L, start with a bolus of 2 units followed by insulin infusion at 2 units/hour.
- Repeat Blood glucose measurement every 30 minutes.
- If blood glucose Action

>10 mmol/L	Increase infusion by 2U/h
> 6 and <10 mmol/L	Maintain current infusion rate
<6.0 mmol/L	Stop insulin infusion
< 4.0 mmol/L	Stop insulin infusion and administer 25 ml of Dextrose 50%

- Maximum insulin infusion = 20 units/hour.

Table 2-3: **Additional intraoperative intravenous insulin boluses for the high dose insulin therapy group**

• If blood glucose	Action
6.1 – 8.0 mmol/L	2 units insulin IV bolus
8.1 – 10.0 mmol/L	4 units insulin IV bolus
10.1 – 12.0 mmol/L	6 units insulin IV bolus
12.1 – 14.0 mmol/L	8 units insulin IV bolus
> 14.0 mmol/L	10 units insulin IV bolus

Table 2-4: **Demographic characteristics**

	Standard (N = 22)	*High dose insulin N = 22)*	*P value*
Age (years)	65 ± 2 *	59 ± 3	0.05
Female	6 (27 %)	2 (9%)	0.12
D.M	9 (41 %)	5 (23 %)	0.20
HTN	19(86 %)	16 (73 %)	0.26
Hypercholestrolemia	20(91 %)	19 (86 %)	0.63
Smoker	8 (36 %)	13 (59%)	0.30
NYHA class			
II	6 (27 %)	4 (18 %)	
III	14(64 %)	13 (59 %)	0.38
IV	2 (9 %)	5 (23 %)	
Ejection Fraction	47 ± 3 *	49 ± 3	0.77
Left main disease	3 (14 %)	10(45%)	0.02
Recent MI	3 (14%)	4 (18 %)	0.68
Old MI	6 (27 %)	2 (9 %)	0.12
Atrial fibrillation	2 (9%)	1 (5 %)	0.55
Peripheral vascular disease	1 (5 %)	1 (5 %)	1.00
Chronic lung disease	2(9 %)	1 (5 %)	0.55
Peptic ulcer disease	2 (9%)	3 (14 %)	0.63

* mean ± SEM

Table 2-5: **Coronary sinus Lactic acid balance**

	Standard (N = 22) Mean ± SEM	*High dose insulin (N = 22) Mean ± SEM*	*P value*
A – CS lactate difference (mmol/L)			
Before Aortic X-Clamp	0.11 ± 0.06	0.48 ± 0.05	0.00
post Aortic clamp removal:			
0 min	-1.58 ± 0.03	-0.10 ± 0.03	0.17
5 min	-0.1 (-0.20-0.00) *	0.00 (-0.10-0.10) *	0.02
10-15 min	-0.05 ± 0.07	0.01 ± 0.13	0.65
Lactate Extraction/Excretion %			
Before Aortic X-Clamp	7 ± 7	34 ± 3	0.00
Post Aortic clamp removal:			
0 min	-1 ± 3	-9 ± 4	0.17
5 min	-9 (-14- 0) *	0 (-4- 8) *	0.02
10-15 min	-7 ± 6	3 ± 8	0.72

A = Arterial, CS = Coronary Sinus, Lac = Lactic Acid, * Median (95% CL)

Table 2-6: **Arterial and Coronary sinus Oxygen content**

	Standard (N = 22) Mean ± SEM	*High dose insulin (N = 22) Mean ± SEM*	*P value*
Arterial Oxygen content (ml O2/ml blood)			
Before Aortic X-Clamp	18 (16-19) *	18 (17-19) *	0.41
Post Aortic clamp removal:			
0 min	11 ± 0	11 ± 0	0.71
5 min	11 ± 0	11 ± 0	0.61
10-15 min	11 ± 0	12 ± 1	0.48
CS Oxygen content (ml O2/ml blood)			
Before Aortic X-Clamp	8 ± 0	8 ± 1	0.80
Post Aortic clamp removal:			
0 min	8 ± 1	9 ± 0	0.55
5 min	7 ± 1	7 ± 0	0.90
10-15 min	7 ± 1	5 ± 0	0.02
Oxygen Extraction %			
Before Aortic X-Clamp	60 (51-63) *	66 (43-67) *	0.45
Post Aortic clamp removal:			
0 min	13 6-26) *	12 (9-21) *	0.86
5 min	41 ± 4	39 ± 4	0.63
10-15 min	42 ± 5	60 ± 4	0.01

CS = Coronary sinus, * Median (95% CL)

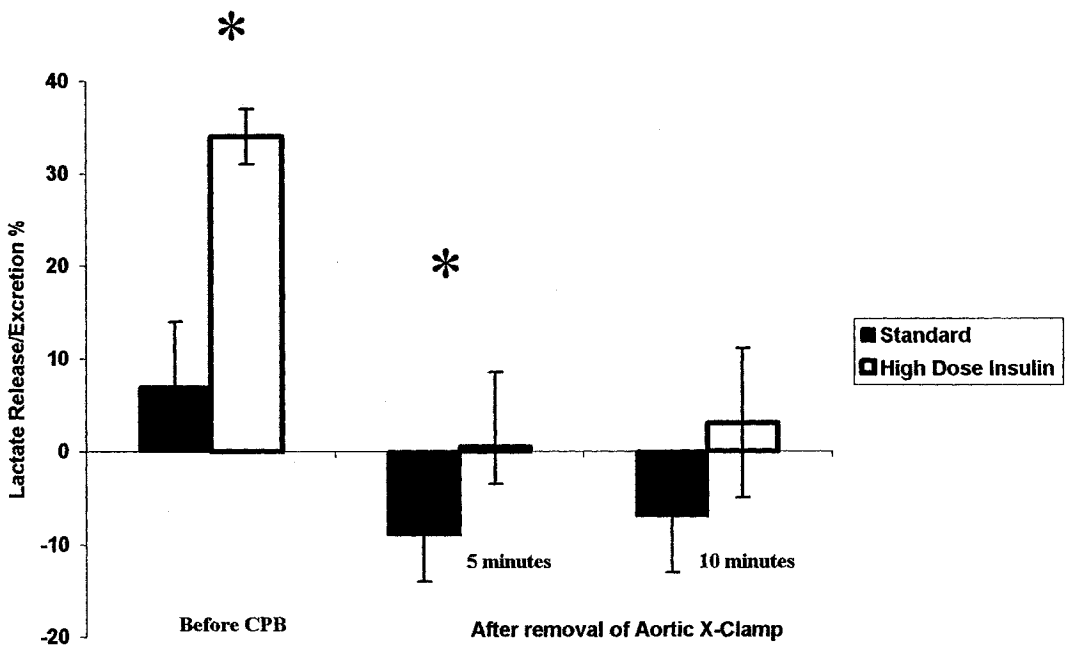

Figure 2-1: **Myocardial Lactic acid Extraction / Excretion %:**
* $p < 0.05$

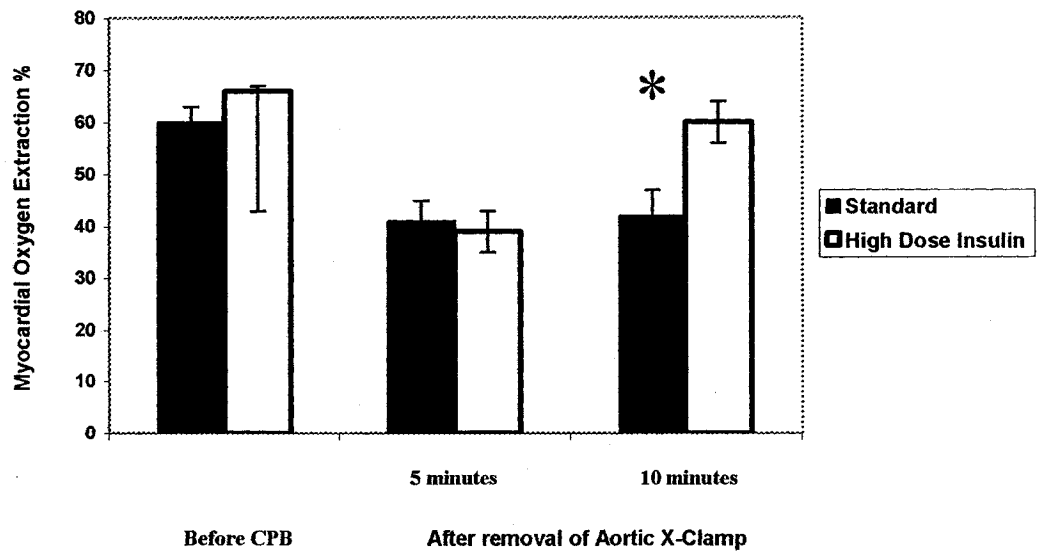

*Figure 2-2: Myocardial oxygen Extraction %, * $p < 0.05$*

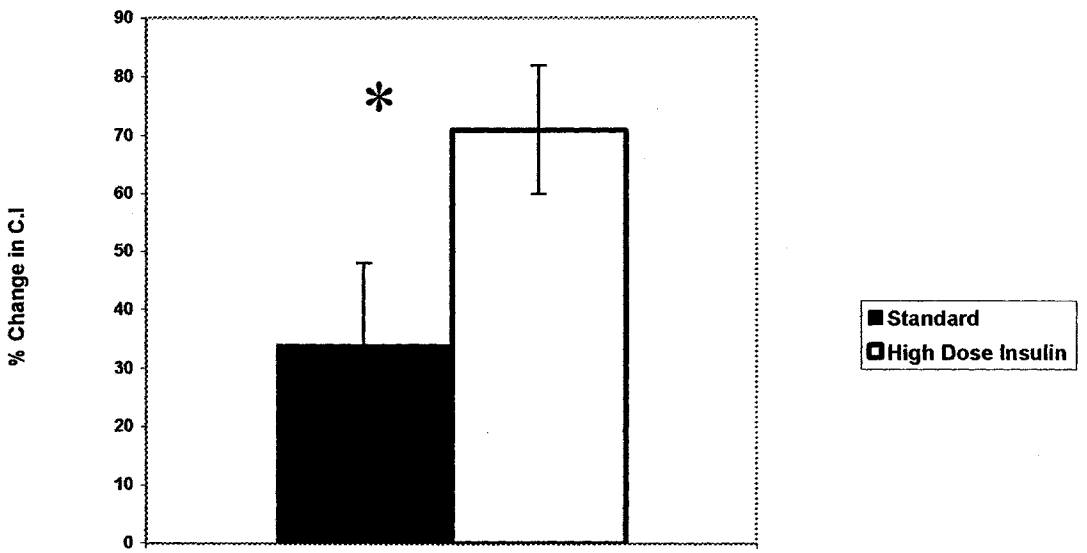

Figure 2-3: **Perioperative Cardiac Index (C.I) change:** * $p < 0.05$

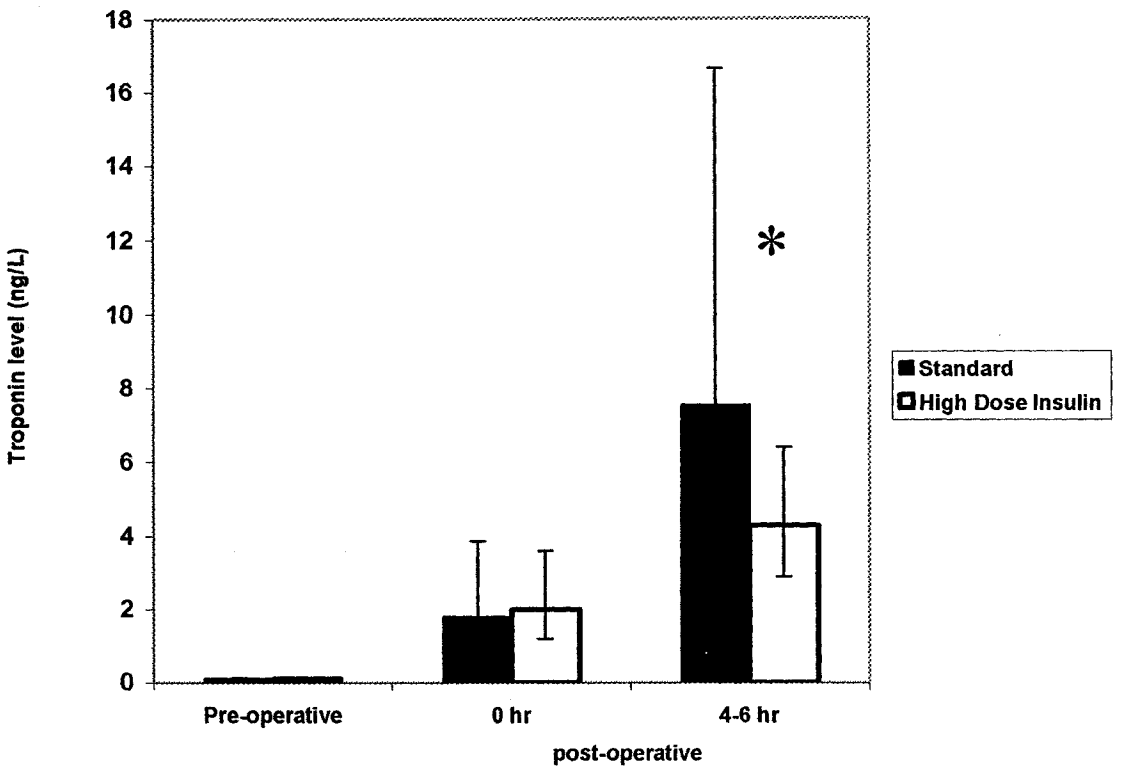

Figure 2-4: **Perioperative Troponin level:**
* $p < 0.05$

Chapter III

High Dose Insulin Therapy Attenuates Systemic Inflammatory Response in Patients Undergoing Elective Coronary Artery Bypass Grafting

Abstract:

Background:

Cardiac surgery with cardiopulmonary bypass (CPB) induces an acute phase reaction that has been implicated in the pathogenesis of several postoperative complications. Studies have shown that pro-inflammatory cytokines are increased by acute hyperglycemia. Recent evidence suggests that insulin has anti-inflammatory properties. Therefore our hypothesis is that high dose insulin therapy will attenuate the systemic inflammatory response to CPB in coronary artery bypass grafting (CABG) patients while maintaining normoglycemia.

Methods:

A total of 50 patients who presented for elective CABG were randomized into two groups: group I, the standard of care (n=25) receiving insulin sliding scale starting at blood glucose concentrations >10mmol/L, and group II, the high dose insulin therapy group (n=25) receiving insulin infusion at 5mU/kg/min starting from arrival at the operating room until the end of the surgical procedure with dextrose 20% infused at a rate adjusted to maintain a BG of 4-6mmol/L. Blood samples were collected preoperatively, on arrival to the intensive care unit (ICU), 6, 24 and 48 hours postoperatively to determine white blood cells count (WBC), high sensitivity

C reactive protein (hsCRP), Tumor necrosis factor α (TNFα), interlukin 6 and 8 (IL6, IL8), C1 esterase inhibitor (C1EI) and complement factor 3 and 4 (C3, C4).

Results:

Biometric, demographic and operative characteristics were similar in both groups. There were lower levels of IL6 [150 pg/dl (95%CL=127-340) Vs. 248 pg/dl (95%CL=194-476), p=0.02], IL-8 [49 pg/dl (95%CL=33-63) Vs. 74 pg/dl (95%CL=43-116), p=0.05] and TNFα [2.2 pg/dl (95%CL=1.9-3.7) Vs. 3.0 pg/dl (95%CL=2.3-6.6), p=0.04] in group II in the early postoperative period and higher levels of C3 (1.02±0.05 Vs. 0.88±0.04, p=0.04) at 48 hours postoperatively in group II. There was no difference in the levels of WBC, hsCRP nor C1EI between the two groups.

Conclusion:

High dose insulin therapy blunts the early postoperative surge in inflammatory response to CPB as reflected by decreased levels of IL6, IL8 and TNFα and increased level of C3.

Introduction:

Organ dysfunction after cardiac surgery is largely attributed to the systemic inflammatory response syndrome (SIRS) due to high levels of cytokines released following surgical trauma and when blood is exposed to artificial surfaces during cardiopulmonary bypass (CPB) (1). Furthermore, acute hyperglycemia frequently develops in patients undergoing coronary artery bypass grafting (CABG), usually following CPB (2), and it has been demonstrated in both rats and humans that pro-inflammatory cytokine concentrations are increased by acute hyperglycemia (3). Prevention of the inflammatory response after cardiac surgery has been attempted using different strategies such as Methylprednisolone, aprotinin, leukocyte depletion and heparin-bonded circuits, all with muted success.(4)

Recent evidence from animal studies suggests that insulin has anti-inflammatory properties (5). Most of the previous studies on insulin therapy in cardiac surgery patients focused on its metabolic effects (i.e. increasing glycogen content, decreasing free fatty acid levels ...etc) (6) with inconsistent support of its beneficial effects. The results of these studies were questioned due to inadequate study design, differences in protocols and inclusion criteria, lack of randomization, the time point of insulin application and the primary endpoints assessed (7). Interestingly; there was a trend

toward more beneficial effects when glucose and insulin were given in high dosages (6). We have previously described an insulin clamp technique, which demonstrated that high dose insulin therapy is safe and effective in maintaining perioperative normoglycemia in CABG patients (8).

The objective of this study was to investigate the role of high dose insulin therapy in elective coronary artery bypass grafting (CABG), and we hypothesized that intraoperative implementation of fixed high dose insulin therapy reduces systemic inflammatory response.

Methods:

Study design and Patient enrollment

After obtaining approval from the research ethics committee in our institution, an informed consent was obtained from all participants. All patients referred for elective CABG between July 2005 and July 2006 were assessed for eligibility. Among 111 patients assessed, 58 patients were excluded due to the presence of one or more of the exclusion criteria which included: 1) Emergency CABG, 2) Redo CABG, 3) Combined CABG and any other cardiac procedure, 4) Preoperative use of steroids or non-steroidal anti-inflammatory drugs (NSAID) and 5) Any deviation from the protocol.

The other 53 patients were randomized using a computerized randomization tables into group I, the standard of care, and group II, the fixed high dose insulin therapy. (Figure 3-1)

Treatment protocols

In diabetic patients on oral hypoglycemic agents, administration of oral hypoglycemic agents was discontinued 24 hours prior to surgery. Diabetic patients on insulin had their daily dose of insulin held the evening before surgery. A subcutaneous insulin sliding scale was started in diabetic patients according to the protocol specified previously (Table 2-1). Intraoperatively, group I received the standard of care using a titrated insulin infusion according to intravenous insulin sliding scale starting at blood glucose level of 10 mmol/L as specified previously (Table 2-2). Blood glucose levels were checked every 30 minutes with the Accu-chek® glucose monitor (Roche Diagnostics, Switzerland). As previously described (8), Group II received a fixed dose of intravenous insulin infusion at 5mU/kg/min. Dextrose 20% infused in the same group at a rate adjusted to maintain a BG of 4-6mmol/L. Additional boluses of insulin were given if the blood glucose remains > 6.0 mmol/L according to the sliding scale specified previously (Table 2-3). Arterial blood glucose was measured every 5 to 10 minutes throughout the procedure In both groups the protocol was started

immediately on arrival to the operating room and stopped just before being transferred to the intensive care unite (ICU).

Anesthetic management

Preoperative sedation with 1 to 3 mg of lorazepam p.o. and oxygen was administered to patients on call to the operating room. All patients received prophylactic perioperative antibiotics (cefazolin 2 g before incision and 2 g post-CPB or vancomycin 1 g before incision and 500 mg post-CPB if allergic to penicillin). The same anesthesiologist administered standardized total intravenous anesthesia using sufentanil, midazolam and pancuronium. Immediately prior to CPB, heparin 400 IU/Kg was administered intravenously followed by additional doses, if necessary, to maintain an activating clotting time >500 seconds. Protamine was administered as 1 mg/100IU of the heparin dose after complete separation from CPB.

Surgical procedure

All patients had CABG by the same surgeon with the use of CPB, which was conducted with a roller pump, and a membrane oxygenator primed with a solution consisting of 1 L Ringer's lactate, 5000 IU heparin, 750 ml pentaspan, and 44 mEq bicarbonate. During CPB, pump flow was set at 2.4 times the body surface area and mean arterial pressure maintained

between 50 and 60 mmHg. Temperature was allowed to drift with active rewarming at the end of CPB. Cardioplegia solution was free of glucose and consisted of high dose (100 mEq/L) and low dose (40 mEq/L) potassium used at the discretion of the cardiac surgeon. A single clamp technique was used and cardioplegia was given in an anterograde fashion with blood in a ratio of 1:4. Blood cardioplegia was also administered with each successive distal vein graft anastemoses. All patients had left internal mammary artery harvested and anastomosed to the left anterior descending artery. The rest of grafts were constructed using the great saphenous vein. After rewarming and suturing of all anastemoses prior to the removal of the aortic cross-clamp "a hot shot" of 1 L of warm cardioplegia was administered. After total release of the aortic cross-clamp, epicardial atrial and/or ventricular pacing wires were placed. After an appropriate test dose of protamine, aortic and venous cannulas were removed and the surgery proceeded with closure of the pericardium and sternum.

Inflammatory markers and sampling protocol

A number of inflammatory markers were studied including interlukin 6 and 8 (IL-6,8), Tumor necrosis factor α (TNFα), C1 esterase inhibitor (C1EI), Complement factor 3 and 4 (C3, C4) and high sensitivity C reactive protein (hsCRP). Theses markers were collected preoperatively (On arrival

to the operating room) and 0, 6, 24 and 48 hours post operatively. Additionally the levels of IL-6, IL8 and TNFα were measured intraoperatively at the following time points: prior to opening the pericardium, before starting CPB and immediately after separation from CPB.

Low systemic vascular resistance (SVR) state

Given the limitations of the clinical criteria to define systemic inflammatory response syndrome (SIRS) in cardiac surgery patients, we used the indexed systemic vascular resistance (SVRi) described by Kristof et al(9) calculated by the following formula: SVRi = (MAP – CVP) x 80/ CI. These parameters were obtained pre operatively as well as 0, 4, 8, 12 and 16 hr post operatively. Low SVR state was defined as SVRi < 1800 dyne.sec.cm^5/m^2 at two consecutive hours. The duration and the total amount of vasopressor agents used in those patients to maintain a mean blood pressure > 60 mmHg were collected. The temperature and white blood cell count (WBC) were measured at 0, 6, 24 and 48 hours corresponding to the same time points at which the samples for the inflammatory markers were collected.

Statistical analysis

Statistical analysis was performed using NCSS statistical software (2004). Continuous variables were compared using either the two-sample ***t***-test or the Wilcoxon rank sum test as appropriate by the distribution of data. Categorical variables were compared using chi-square test or Fisher's exact test depending on the number of items in each group. Statistical significance for grouped data over time was ascertained using two-way analysis of variance (ANOVA). The statistical analysis was done using "per-protocol" method and statistical significance was deemed present when $P< 0.05$.

Results:

Demographic characteristics

Among 53 patients enrolled in the study, 27 patients were randomized to the control group (standard) and 26 patients to the high dose insulin therapy group (clamp). Two patients were excluded from the standard group intraoperatively; the first one was done off CPB due to calcification of the ascending aorta and in the second patients a non standard cardioplegia (miniplegia) was used. One patient was excluded from the clamp group postoperatively when he developed extensive mesenteric ischemia, which

would have biased the level of inflammatory markers. Demographic data is shown below (table 3-1). There was similar distribution of age, sex and premorbid conditions in both groups. The prevalence of DM was 44% in the standard group and 40% in the high dose insulin group. There were 24% of patients with left main (LM) coronary artery disease in the standard group compared to 28% in the clamp group (P=89%). 4 patients in each group (16%) had recent MI (within 1 month) and 5 patients in each group (20%) had old MI (> 1 month).

Intraoperative characteristics

The CPB time tended to be longer in the standard groups but with out statistically significant difference (92 ± 5 minutes in the standard group Vs. 79 ± 5 minutes in the clamp group, P=0.06). There was no difference in the mean aortic cross clamp time between both groups as well (76 ± 4 minutes in the standard group Vs. 67 ± 4 minutes in the clamp group, P=0.13). The mean number of grafts was 3.5 in the standard group compared to 3 in the Clamp group. The median average blood glucose level intraoperatively was 7.3 mmol/L (95%CL= 6.1 - 7.6) in the standard group while it was 4.9 mmol/L (95%CL= 4.5 - 5.1) in the clamp group (P=0.00). Also the standard group had lower median average intraoperative blood insulin level than the clamp group [91 pmol/L (95%CL= 44 - 159) Vs. 3720 pmol/L (95%CL=

2871- 4016) consequently, P=0.000]. Both groups had similar blood levels of Glucose and insulin on arrival to the operating room. However the clamp group had significantly lower blood glucose levels and higher blood insulin levels (Figure 3-2) at all time points intraoperatively.

Inflammatory markers

There was no significant elevation of IL-6, IL-8 or TNFα in either group intraoperatively but started to rise significantly after separation from CPB. The peak level of IL-6 and IL-8 was reached 6 hours postoperatively while the peak level of TNFα was immediately post operatively. (Figure 3) In the clamp group, there was significant reduction of the median peak level of IL-6 [150 pg/dl (95%CL=127-340) Vs. 248 pg/dl (95%CL=194-476), p=0.02], IL-8 [49 pg/dl (95%CL=33-63) Vs. 74 pg/dl (95%CL=43-116), p=0.05] and TNFα [2.2 pg/dl (95%CL=1.9-3.7) Vs. 3.0 pg/dl (95%CL=2.3-6.6), p=0.04]. (Table 3-2) the levels of these markers decreased back close to its baseline level 24-48 hours postoperatively. The standard group had lower mean level of C3 at 48 hours post operatively compared to the clamp group (standard C3=0.88±0.04, clamp C3=1.02±0.05, p=0.04) but no difference in C4 levels between the two groups at the same time point (standard C4=0.18±0.01, clamp C4=0.21±0.01, p=0.06). Both groups had similar levels of C1EI pre and postoperatively. (Figure 3-4) (Table 3-3) Max level

of hsCRP was 48 hours postoperatively [Standard=159 mg/l (95%CL=123-175), Clamp=182 mg/l (95%CL=136-241), p=0.31], but we would not be able to know whether that was the peak level or not because collection of samples was not carried out beyond our study end point for the other inflammatory markers. There was no difference in the postoperative temperature or WBC between the two groups at all time points.

Low SVR state

Calculated SVRi as indicated in the method section above was used to study the SVR status post operatively. The lowest SVR status occurred 4 hours postoperatively with a median SVRi = 1920 dyne.sec.cm^5/m^2 (95%CL= 1628-2222) in the standard group compared to 1976 dyne.sec.cm^5/m^2 (95%CL= 1725-2163) in the Clamp group, P=0.86. There was tendency toward lower SVR status in the standard group at 8 and 12 hours postoperatively but did not reach statistical significance. In the standard group 10 out of 25 patients (40%) had low SVR state (SVRi < 1800) but it occurred in only 5 out of 25 patients (20%) in the clamp group (P=0.10) (Figure 3-5).

Postoperative vasopressors requirement

Norepinephrine was the only vasopressor agents used in all the study patients. 19 patients (76%) in the standard group required vasopressor

support postoperatively which was required for >8 hours in 14 of them (56%). In the Clamp group, 15 patients (60%) required postoperative vasopressor support and it was required >8hr in 11 patients (44%). Median duration of vasopressor support was = 10 hours (95%CL= 3-13) in the standard group compared to 7 hours (95%CL= 0-12) in the Clamp group, P=0.33. The mean total amount of vasopressor agent used was = 92 ± 37 μg/minute in the standard group compared to 59 ± 19 μg/minute in the clamp group (P=0.44) (Figure 3-5).

Postoperative characteristics

The median length of intubation was 15.5 hours in the standard group compared to 14 hours in the clamp group (P=0.26). There was no difference in the length of stay in the intensive care unit (standard=26.5 hours, clamp=26 hours, P=0.65) or in the hospital stay (6 days in both groups, P=0.41). There was no difference in the incidence of postoperative complications between the two groups. In group I: There were 2 perioperative MI, 1 re-exploration for bleeding and 1 superficial wound infection. In group II: one patient had pulmonary embolism and another patient had a superficial wound infection complicated by bacteremia 5 days postoperatively.

Discussion:

CPB triggers an inflammatory response involving proinflammatory cytokines leading clinically to a post perfusion syndrome characterized by fever and fluid accumulation in the interstitium (10). In addition, low systemic vascular resistance can develop (11). We showed in our study that the systemic inflammatory response post CABG indicated by the levels of inflammatory cytokines could be attenuated using high dose insulin therapy in both diabetic and non-diabetic patients under going CABG.

Insulin therapy in cardiac surgery patients

Doenst and coworkers provided evidence that hyperglycemia is an independent predictor of perioperative morbidity and mortality in both diabetic and non-diabetic patients (12). Tight glycemic control in diabetic CABG patients improves perioperative outcomes, enhances survival, and decreases the incidence of ischemic events and wound complications (13). Reviewing the literature revealed that the beneficial effects of insulin were most prevalent when glucose and insulin had been given in high dosages. This statement is based on observations where studies applying low dose GIK after myocardial infarction did not report any beneficial effects(14),

whereas studies using high doses demonstrated impressive reductions in mortality (15).

Mechanism of action of insulin in cardiac surgery patients

Insulin was initially used as a polarizing agent to promote electrical stability in the form of Glucose-Insulin-Potassium (GIK) solution (16). The mechanistic focus shifted over the years towards GIK-induced changes in metabolism but there is convincing evidence that the beneficial effects of insulin in cardiac surgery are multifactorial (6) and go far beyond simple metabolic benefits(17). Yet, the exact underlying mechanisms remain still unknown. In our protocol: we implemented a new strategy of insulin therapy that is different from GIK solution, which titrates insulin infusion to the blood glucose level, by fixing the insulin infusion at a high concentrations to stress the importance of hyperinsulinemia while maintaining normoglycemia.

Anti-inflammatory effect of insulin in CABG patients

We demonstrated in our study two important aspects of insulin therapy in CABG patients: first, high dose insulin therapy has an anti-inflammatory effect post CABG which may contribute to the above-mentioned beneficial effects of insulin therapy. These anti-inflammatory

properties were demonstrated previously on animal studies and in trauma patients (5). Visser and colleagues showed that insulin applied as hyperinsulinaemic-normoglycaemic clamp reduces postoperative CRP levels(18) but they failed to show a difference in the level of other inflammatory markers such as IL-6, IL-8 and TNFα which was likely due to the sampling protocol that missed the peak levels of these markers at 0-6 hours postoperatively as we have shown in our study and supported by other studies as well (19). Second, Given the early postoperative peak of these inflammatory markers, this stresses the importance of starting this high dose insulin therapy protocol as early as possible preoperatively or immediately intraoperatively.

The significance of increased levels of inflammatory markers post CABG

Increased levels of pro-inflammatory cytokines have generally been associated with negative outcomes after cardiac surgery. Recent data allow us to better understand these effects. IL-6 plays an important role during the acute-phase response. It stimulates the release of immune-competent proteins from the liverlike C-reactive protein and, together with TNF-α and IL-1, causes activation of T cells. IL-8 is a proinflammatory cytokine released by cells which has an important influence on the chemotactile

activation of T cells and the endothelial barrier function of the endothelium and has been recognized as a relevant mediator of organ dysfunction based on its major role of recruitment and activation of leukocytes, seen in adult respiratory distress syndrome (20). IL-8 also seems to participate in myocardial ischemic injury; indeed, a monoclonal antibody directed against IL-8 reduces myocardial ischemia-reperfusion injury(21) and the levels of IL-8 correlate positively with the levels of cardiac Troponin I, suggesting a role for IL-8 in myocardial injury after cardiac surgery(22). TNF-α and IL-1β synergistically depress human myocardial contractile function (23).

Complement activation results in cell injury by promoting neutrophil adhesion to the endothelium and by directly lysing cellular membranes (24). The magnitude of this inflammatory response on CPB, particularly the level of C3a, has been shown to correlate with postoperative organ dysfunction and adverse clinical outcomes(25). In our study, we included a relatively healthy cohort with a low risk profile and we demonstrated a trend toward improved clinical markers of SIRS (i.e. higher SVR status and lower vasopressors requirement). However, our study demonstrates a point of principle and a large clinical outcome trial is needed to confirm the clinical outcome of this novel therapy which may even be more evident in high risk

groups (i.e. patients with chronic renal failure, congestive heart failure ...etc).

Conclusion:

High dose insulin therapy blunts the early postoperative surge in inflammatory response to CPB as reflected by decreased levels of IL6, IL8 and TNFα. Early institution of this high dose regimen intraoperatively is essential to achieve its anti-inflammatory effect. Whether the high dose insulin therapy has a direct anti-inflammatory effect or an indirect effect through its tight glycemic control still to be elucidated.

Reference List

(1) Wan S, LeClerc JL, Vincent JL. Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies. Chest 1997; 112(3):676-692.

(2) Chaney MA, Nikolov MP, Blakeman BP, Bakhos M. Attempting to Maintain Normoglycemia During Cardiopulmonary Bypass with Insulin May Initiate Postoperative Hypoglycemia. Anesth Analg 1999; 89(5):1091.

(3) Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M et al. Inflammatory Cytokine Concentrations Are Acutely Increased by Hyperglycemia in Humans: Role of Oxidative Stress. Circulation 2002; 106(16):2067-2072.

(4) Gott JP, Cooper WA, Schmidt FEJ, Brown WM, III, Wright CE, Merlino JD et al. Modifying risk for extracorporeal circulation: trial of four anti-inflammatory strategies. Ann Thorac Surg 1998; 66(3):747-754.

(5) Jeschke MG, Klein D, Bolder U, Einspanier R. Insulin Attenuates the Systemic Inflammatory Response in Endotoxemic Rats. Endocrinology 2004; 145(9):4084-4093.

(6) Doenst T, Bothe W, Beyersdorf F. Therapy with insulin in cardiac surgery: controversies and possible solutions. Ann Thorac Surg 2003; 75(2):721S-7728.

(7) Bothe W, Olschewski M, Beyersdorf F, Doenst T. Glucose-Insulin-Potassium in Cardiac Surgery: A Meta-Analysis. Ann Thorac Surg 2004; 78(5):1650-1657.

(8) Carvalho G, Moore A, Qizilbash B, Lachapelle K, Schricker T. Maintenance of Normoglycemia During Cardiac Surgery. Anesth Analg 2004; 99(2):319-324.

(9) Kristof AS, Magder S. Low systemic vascular resistance state in patients undergoing cardiopulmonary bypass. Crit Care Med 1999; 27:11211-7.

(10) Olthof C.G., Jansen P.G., de Vries J.P., et al. Interstitial fluid volume during cardiac surgery measured by means of a non-invasive conductivity technique. Acta Anaesthesiol Scand 1995;39:508-512

(11) Christakis G.T., Fremes S.E., Koch J.-P., et al. Determinants of low systemic vascular resistance during cardiopulmonary bypass. Ann Thorac Surg 1994;58:1040-1049

(12) Doenst T, Wiseysundera D, Karkootik K, et al. Hyperglycemia during cardiopulmonary bypass is an independent risk factor for mortality in patients undergoing cardiac surgery. J Thorac Cardiovasc Surg 2005;130:1144-1150

(13) Lazar HL, Chipkin SR, Fitzgerald CA, Bao Y, Cabral H, Apstein CS. Tight Glycemic Control in Diabetic Coronary Artery Bypass Graft Patients Improves Perioperative Outcomes and Decreases Recurrent Ischemic Events. Circulation 2004; 109(12):1497-1502.

(14) Ceremuzynski L., Budaj A., Czepiel A., et al. Low-dose glucose-insulin-potassium is ineffective in acute myocardial infarction: results of a randomized multicenter Pol-GIK trial. Cardiovasc Drug Ther 1999;13:191-200

(15) Rogers WJ, Stanley AW, Breing JB, Prather JW, McDaniel HG, Moraski RE, Mantle JA, Russell RO, Rackley CE. Reduction of hospital mortality rate of acute myocardial infarction with glucose-insulin-potassium infusion. Am Heart J. 1976;92:441–454

(16) Sodi-Pallares D, Testelli MR, Fishleder BL, et al. Effects of intravenous infusion of a potassium-glucose-insulin solution on the electrocardiographic signs of myocardial infarction. Am J Cardiol. 1962;9:166-181

(17) Schipke JD, Friebe R, Gams E. Forty years of glucose-insulin-potassium (GIK) in cardiac surgery: a review of randomized, controlled trials. Eur J Cardiothorac Surg 2006; 29(4):479-485.

(18) Visser L, Zuurbier CJ, Hoek FJ, Opmeer BC, de Jonge E, de Mol BAJM et al. Glucose, insulin and potassium applied as perioperative hyperinsulinaemic normoglycaemic clamp: effects on inflammatory response during coronary artery surgery. Br J Anaesth 2005; 95(4):448-457.

(19) Struber M, Cremer JT, Gohrbandt B, Hagl C, Jankowski M, Volker B et al. Human cytokine responses to coronary artery bypass grafting with and without cardiopulmonary bypass. Ann Thorac Surg 1999; 68(4):1330-1335.

(20) Patrick D.A., Moore F.A., Moore E.E., Barnet C.C., Sillimann C.C., Jr The inflammatory profile of inteleukin-6, interleukin-8, and soluble intercellular adhesion molecule-1 in post-injury multiple organ failure. Am J Surg 1996;172:425-431

(21) Boyle E.M., Kovacich J.C., Hebert C.A., et al. Inhibition of interleukin-8 blocks myocardial ischemia-reperfusion injury. J Thorac Cardiovasc Surg 1998;116:114-121

(22) Wan S., Izzat M.B., Lee T.W., et al. Avoiding cardiopulmonary bypass in multivessel CABG reduces cytokine response and myocardial injury. Ann Thorac Surg 1999;68:52-57

(23) B.S. Cain, D.R. Meldrum, C.A. Dinarello, X. Meng, K.S. Joo, A. Banerjee and A.H. Harken , Tumor necrosis factor-alpha and inteleukin-1beta synergistically depress human myocardial function. Crit Care Med 27 (1999), pp. 1309-1318

(24) Lazar HL, Bokesch PM, van Lenta F, Fitzgerald C, Emmett C, Marsh HC, Jr. et al. Soluble Human Complement Receptor 1 Limits Ischemic Damage in Cardiac Surgery Patients at High Risk Requiring Cardiopulmonary Bypass. Circulation 2004; 110(11_suppl_1):II-274.

(25) Kirklin JK, Westaby S, Blackstone EH, Kirklin JW, Chenoweth DE, Pacifico AD. Complement and the damaging effects of cardiopulmonary bypass. J Thorac Cardiovasc Surg 1983; 86(6):845-857.

Table 3-1: Demographic characteristics

	Standard (N = 25)	*High dose insulin (N = 25)*	*P value*
Age (years)	68 ± 2 *	62 ± 2	0.06
Female	9 (36 %)	6 (24%)	0.49
D.M	11 (44 %)	10 (40 %)	0.91
HTN	22(88 %)	19 (76 %)	0.30
Hypercholestrolemia	20(80 %)	19 (76 %)	0.56
Smoker	9 (36 %)	10 (40 %)	0.81
NYHA class			
I	2 (8 %)	0 (0 %)	
II	7 (28 %)	5 (20 %)	0.34
III	14(56 %)	15 (60 %)	
IV	2 (8 %)	5 (20 %)	
Ejection Fraction	55 (50 – 60) †	55 (40 – 60)	0.90
Left main disease	6 (24 %)	7(28 %)	0.81
Recent MI	4 (16%)	4 (16 %)	0.99
Old MI	5 (20 %)	5 (20 %)	0.99
Atrial fibrillation	2 (8%)	1 (4 %)	0.53
Peripheral vascular disease	1 (4 %)	2 (8 %)	0.57
CVA or TIA	2(8 %)	0 (0%)	0.14
Asthma	2(8 %)	0 (0 %)	0.14
COPD	2 (8 %)	2 (8 %)	0.99
Peptic ulcer disease	3 (12 %)	3 (12 %)	0.99

* mean ± SEM, ‡ median (95% CL)

Table 3-2: **Perioperative levels of IL-6, IL-8, TNFα and hsCRP**

	Standard (N = 25) Median (95% CL)	*High dose insulin (N = 25) Median (95% CL)*	*P value*
IL-6			
Preoperative	9 (8-13)	11 (10-13)	0.29
Pre-pericadiotomy	11 (8-15)	11 (9-15)	0.55
Pre-CPB	11 (9-15)	12 (11-16)	0.16
Post-CPB	51 (28-68)	35 (27-49)	0.48
Postoperative			
0 hours	113 (75-161)	102 (62-116)	0.46
6 hours	248 (194-476)	150 (127-340)	0.02
24 hours	130 (72-167)	116 (72-159)	0.90
48 hours	86 (59-113)	110 (79-132)	0.22
IL-8			
Preoperative	15 (13-18)	13 (12-17)	0.22
Pre-pericadiotomy	15 (13-26)	13 (11-14)	0.01
Pre-CPB	11 (10-13)	9 (9-11)	0.08
Post-CPB	23 (15-41)	20 (14-28)	0.27
Postoperative			
0 hours	54 (29-157)	39 (29-65)	0.25
6 hours	74 (43-116)	49 (33-63)	0.05
24 hours	33 (28-54)	33 (25-44)	0.33
48 hours	29 (21-39)	28 (20-33)	0.58
TNFα			
Preoperative	1.8 (1.5-2.6)	1.6 (1.5-2.4)	0.97
Pre-pericadiotomy	1.8 (1.6-2.1)	1.9 (1.6-2.4)	0.80
Pre-CPB	1.9 (1.7-2.2)	1.9 (1.5-2.4)	0.95
Post-CPB	2.2 (1.7-2.8)	1.8 (1.5-2.3)	0.08
Postoperative			
0 hours		2.2 (1.9-3.7)	0.04
6 hours	3.0 (2.3-6.6)	2.1 (1.6-2.9)	0.55
24 hours	2.3 (1.7-3.7)	2.0 (1.5-2.7)	0.82
48 hours	1.9 (1.7-2.5)	2.1 (1.8-2.9)	0.73
	2.1 (1.7-2.6)		

hsCRP			
Preoperative	3.3 (1.2-4.1)	2.2 (1.9-5.0)	0.85
Postoperative			
0 hours	1.0 (0.3-2.1)	1.2 (0.4-3.2)	0.35
6 hours	10.6 (6.9-12.6)	10.1 (7.0-16.7)	0.98
24 hours	117.2 ± 5.6 *	114.7 ± 7.4	0.79
48 hours	159.0 (122.7-174.6)	182.1 (135.8-241.0)	0.31

* mean ± SEM

Table 3-3: **Perioperative levels of C3, C4 and CIEI**

	Standard (N = 25) Median (95% CL)	*High dose insulin (N = 25) Median (95% CL)*	*P value*
C3			
Preoperative	1.11 ± 0.07 *	1.29 ± 0.06	0.06
Postoperative			
0 hours	0.63 (0.55-0.80)	0.64 (0.53-0.71)	0.62
6 hours	0.65 ± 0.04 *	0.63 ± 0.04	0.79
24 hours	0.74 ± 0.04 *	0.78 ± 0.04	0.40
48 hours	0.88 ± 0.03 *	1.02 ± 0.05	0.04
C4			
Preoperative	0.23 (0.20-0.25)	0.27 (0.22-0.31)	0.07
Postoperative			
0 hours	0.13 (0.11-0.15)	0.14 (0.12-0.17)	0.53
6 hours	0.13 ± 0.01 *	0.14 ± 0.01 *	0.19
24 hours	0.14 ± 0.01 *	0.16 ± 0.01	0.15
48 hours	0.18 ± 0.01 *	0.21 ± 0.01	0.06
C1EI			
Preoperative	0.28 ± 0.01 *	0.28 ± 0.01 *	0.55
Postoperative			
0 hours	0.16 ± 0.01 *	0.16 ± 0.01 *	0.97
6 hours	0.19 ± 0.01 *	0.20 ± 0.01 *	0.44
24 hours	0.24 ± 0.01 *	0.25 ± 0.01 *	0.71
48 hours	0.31 ± 0.01 *	0.31 ± 0.01 *	0.84

* mean ± SEM

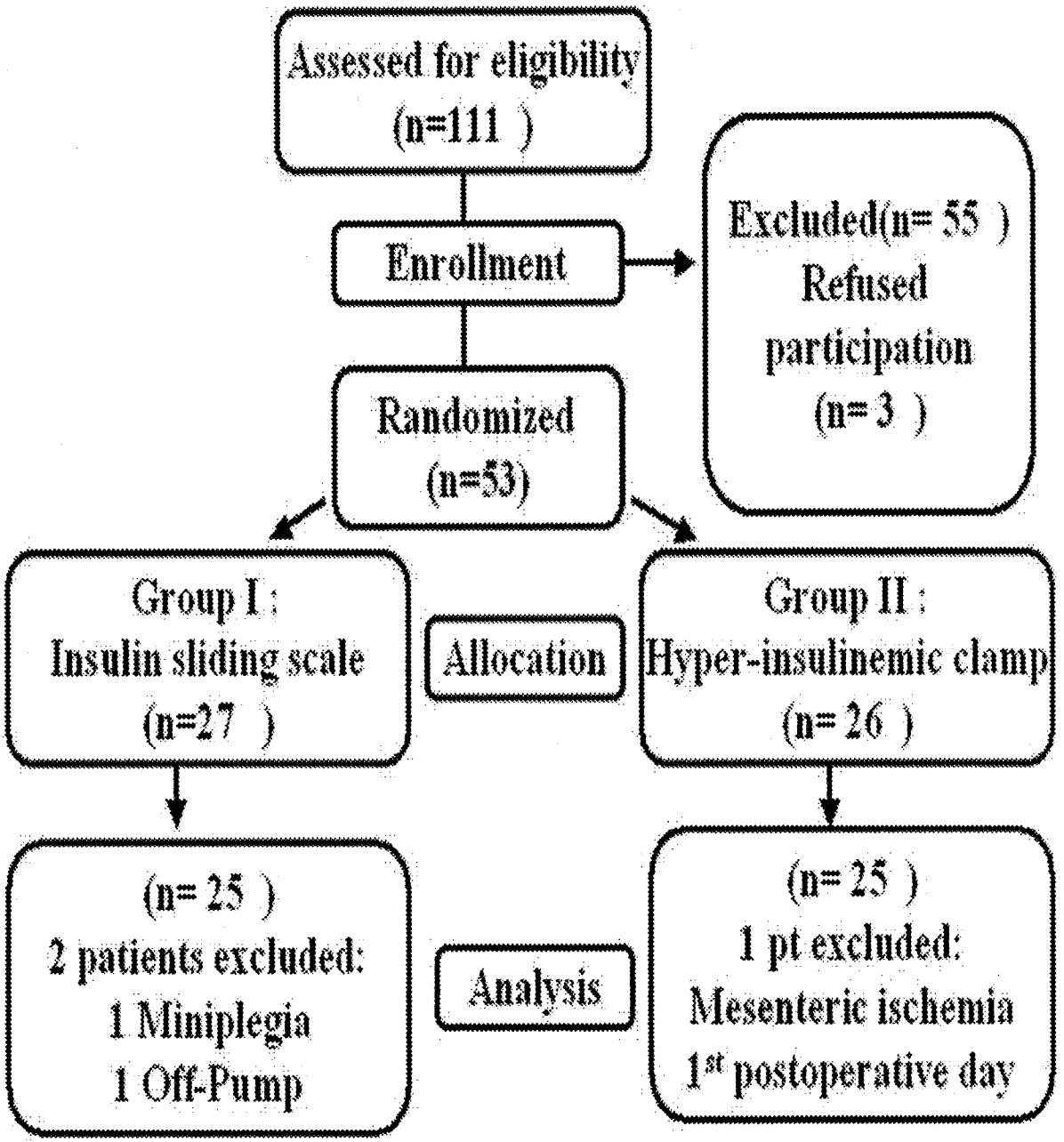

Figure 3-1: **Patients distribution flow chart**

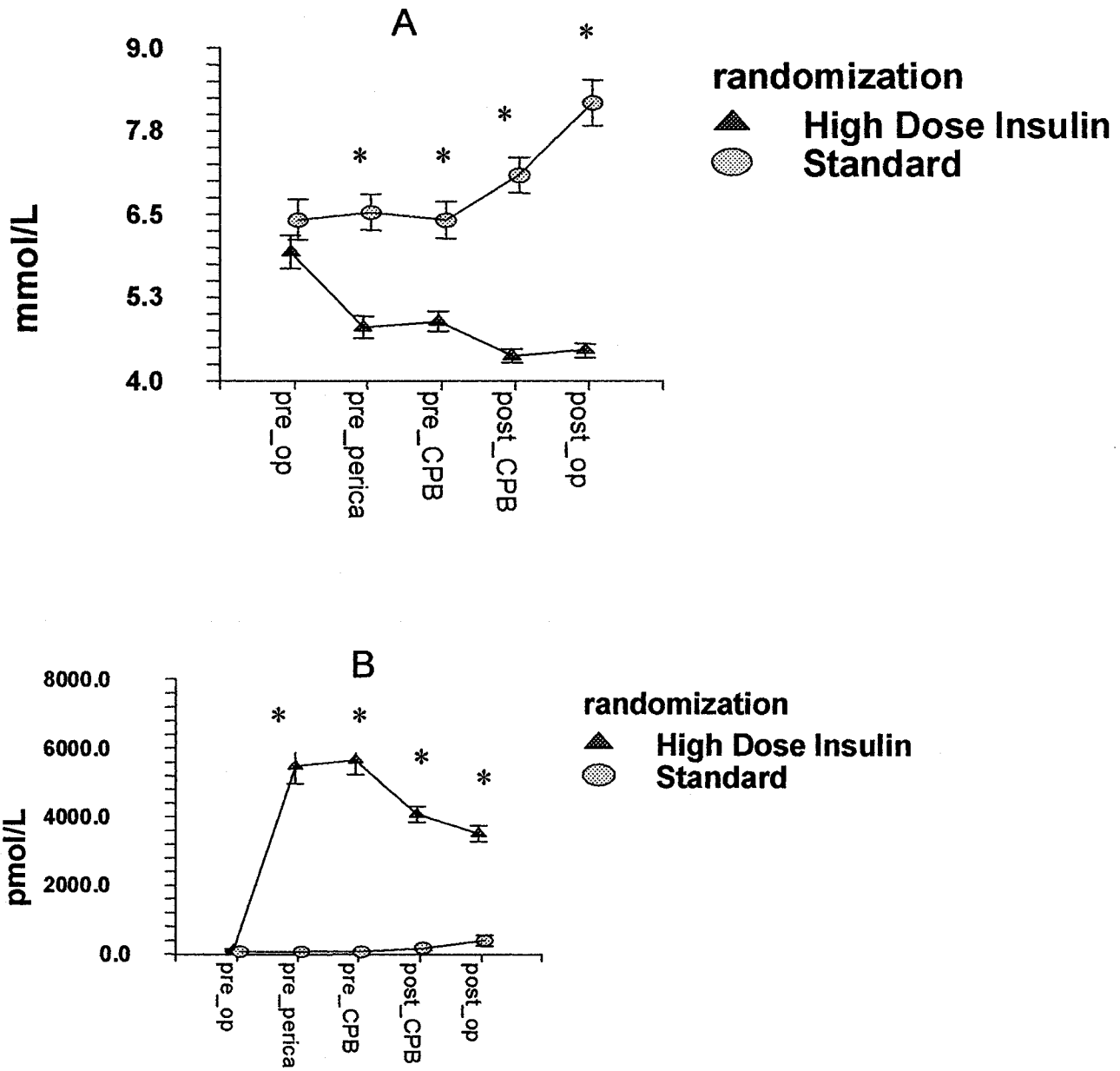

Figure 3-2: **Intraoperative blood glucose and insulin levels:**
A) Intraoperative blood glucose level, B) Intraoperative blood insulin level, * $p < 0.05$

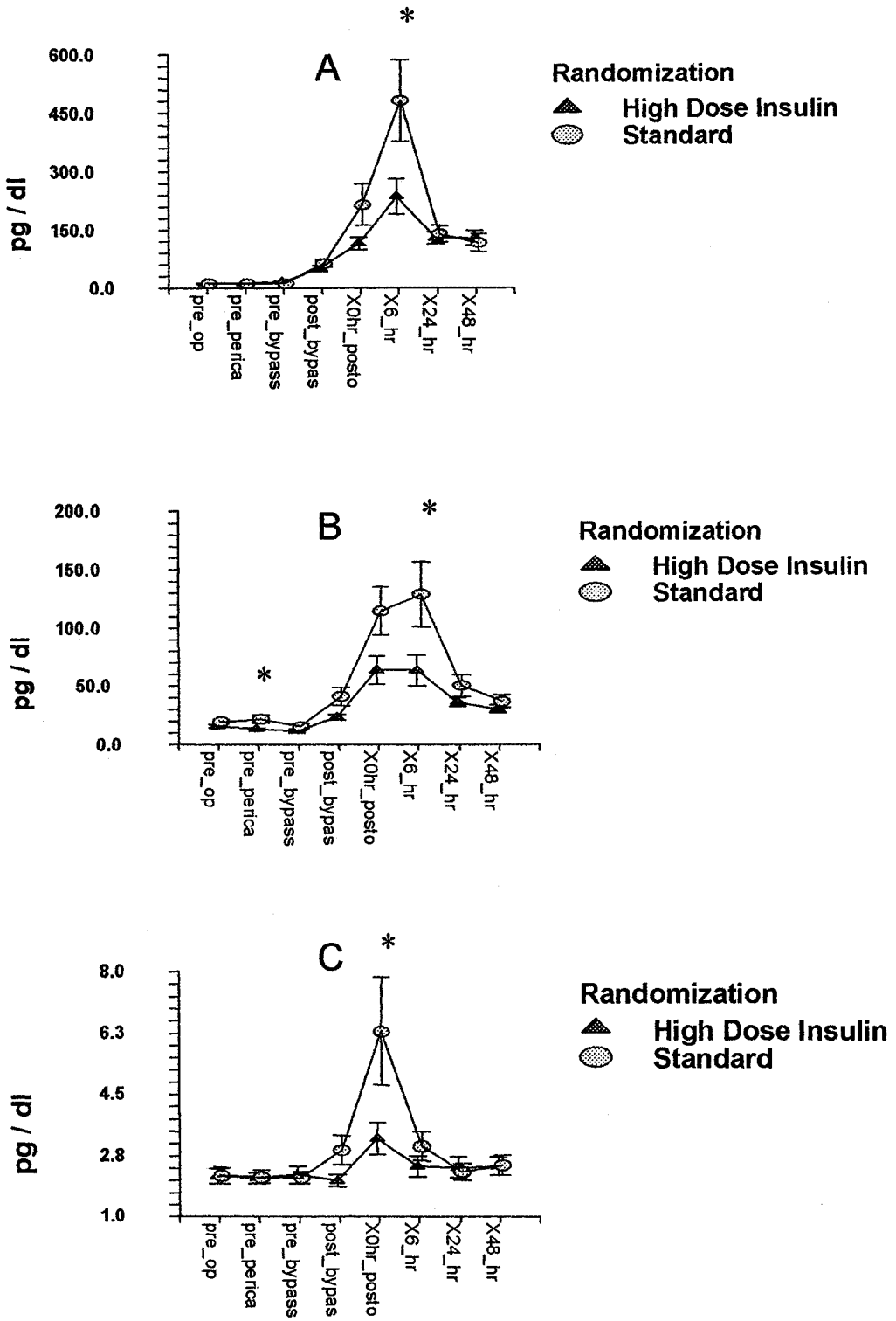

Figure 3-3: **Perioperative level of IL-6, IL-8 and TNFα:**
A) IL-6, B) IL-8, C) TNFα, * $p < 0.05$

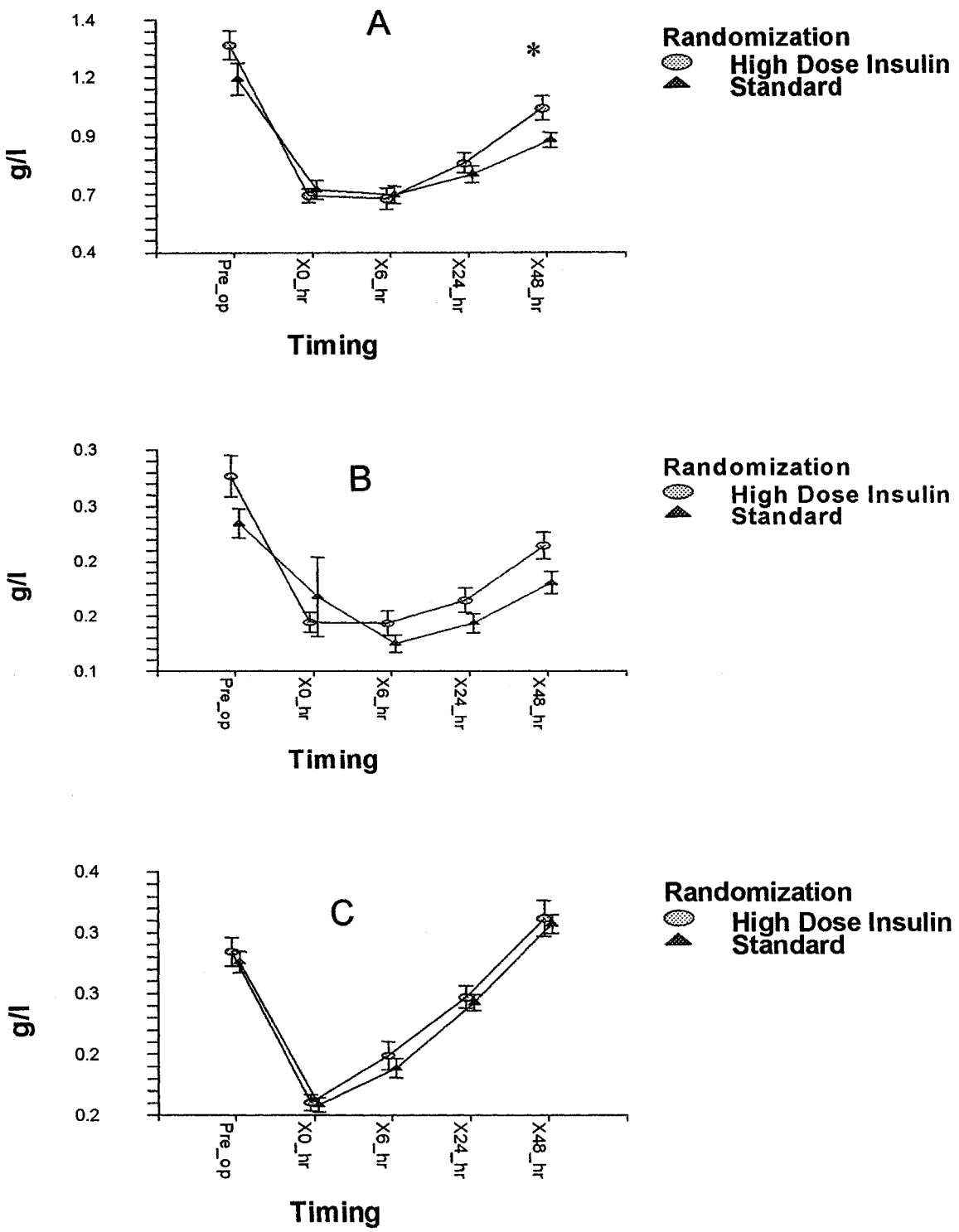

Figure 3-4: **Perioperative level of C3, C4 and C1EI**:C3, B) C4, C) C1esterase inhibitor (C1EI), * $p < 0.05$

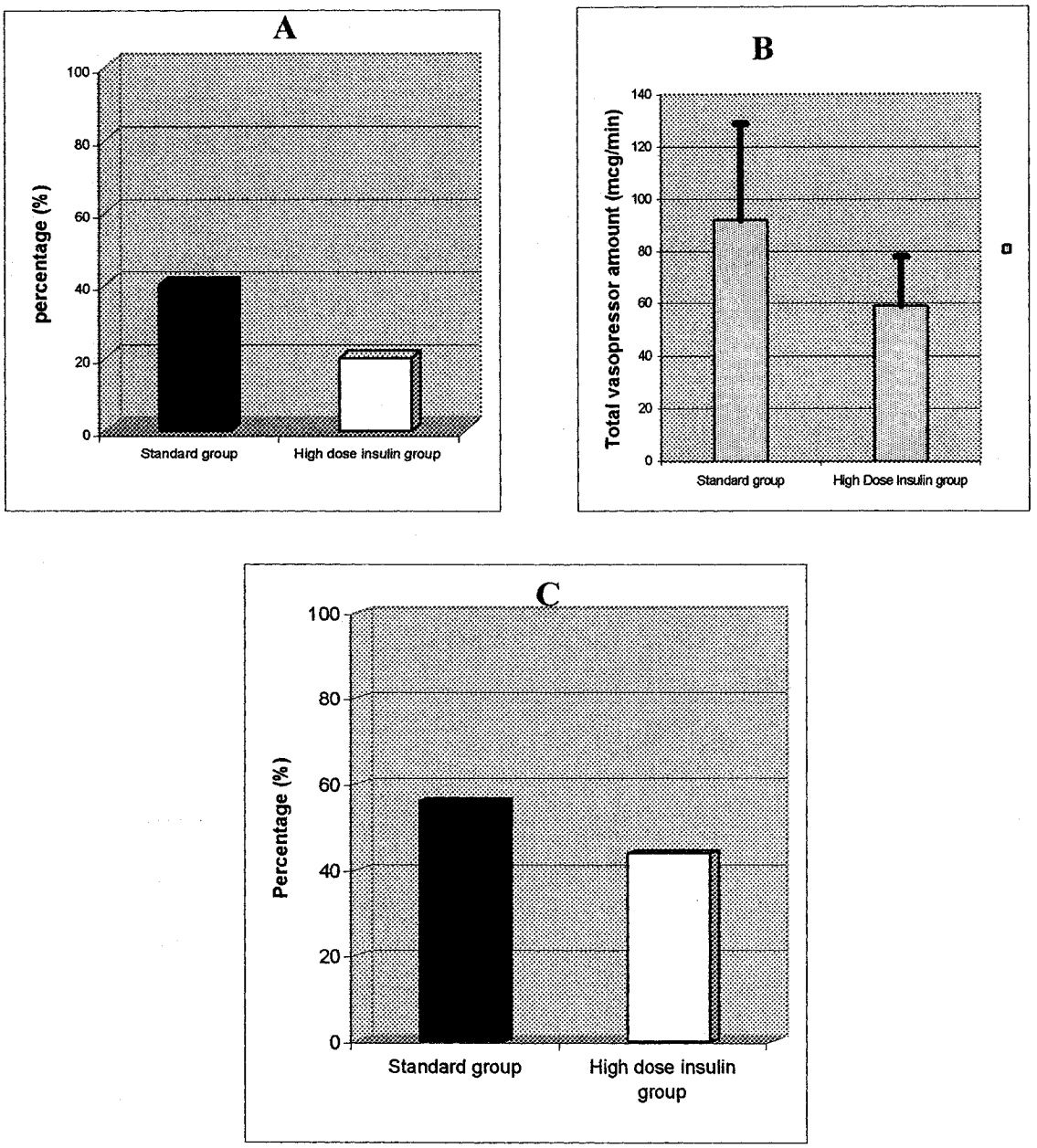

Figure 3-5: Postoperative SVR status and vasopressor support:

A) Percentage of patients with low SVR state, B) Total amount of postoperative vasopressor requirement, C) Percentage of patients who required postoperative vasopressor support > 8 hours

Future Directions

During our work on insulin therapy in cardiac surgery patients, we identified a number of questions that need to be answered by future studies and represent potential directions in the advancement of our knowledge about insulin therapy in cardiac surgery in particular and surgical patients in general.

a. The tight relation between insulin and glucose infusion in the control of normoglycemia makes it very difficult to manipulate one with out changing the level of the other. Our study suggested a novel way of fixing the insulin at high dose to allow titrating only the glucose. However, how high should the insulin infusion be fixed at and what target of normoglycemia should we aim for still not defined yet.

b. Previous studies have given us some insight about the positive clinical effect of insulin therapy and it was shown that these effects are more prominent in diabetic patients and patients with poor ventricular function. However, whether the effect of high dose insulin therapy will be more pronounced in a

specific group of patients was not proven yet by any randomized controlled trial.

c. We showed in our work that high dose insulin therapy have both anti-inflammatory and metabolic enhancement effects. That leaves us with the important question whether these anti-inflammatory properties preserve the endothelial and myocardial cellular function leading to better enhancement of cellular metabolism and consequently improved myocardial protection and function or these metabolic effects are a primary mechanism.

d. Our study brings the attention to the beneficial effects of high dose insulin therapy from mechanistic point of view. However, the ultimate goal is to achieve better clinical outcomes in cardiac surgery patients in terms of perioperative morbidity and mortality and improved long-term survival and function. These effects need to be further studied by a large-scale clinical outcome study.