The effect of insulin on whole body protein and glucose metabolism after cardiac surgery using stable isotope kinetics: a pilot study

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Abstract

High doses of insulin are required to prevent the hyperglycemic response to open heart surgery. Insulin has been shown to produce hypoaminoacidemia during coronary artery bypass graft (CABG) surgery. The present study investigated the effect of high-dose insulin therapy on whole body protein and glucose metabolism in patients undergoing CABG surgery using stable isotope tracers [6,6-$^{2}H_2$]glucose and L-[1-^{13}C]-leucine. Hyperinsulinemic-normoglycemic clamp was used to deliver high-dose insulin. The primary objective was to establish a protocol and to assess the feasibility of using stable isotope tracers after cardiac surgery while continuously infusing high-dose insulin. The effect of high-dose insulin on protein breakdown, synthesis and oxidation and glucose turnover was evaluated.

Fifteen patients were recruited to the study, seven in the control group receiving standard glycemic control and eight in the insulin group receiving the hyperinsulinemic-normoglycemic clamp. Isotopic plateaus of L-[1-^{13}C]leucine, [6,6-$^{2}H_2$]glucose and expired $^{13}CO_2$ were attained in all patients in both groups with a coefficient of variance <5%. Protein breakdown and synthesis both decreased in patients who received high-dose insulin, while protein oxidation remained the same, resulting in a negative protein balance regardless of treatment group. Endogenous glucose production was almost completely suppressed by the administration of high-dose insulin and 20% dextrose solution. The hyperinsulinemic-normoglycemic clamp is a metabolic intervention that when

used in the perioperative setting of cardiac surgery, has significant effects on whole body protein breakdown.

Résumé

Lors de chirurgies pour pontage aorto-coronarien, l'utilisation de hautes doses d'insuline est habituellement nécessaire pour prévenir l'augmentation de la glycémie en réponse au stress chirurgical. Il a été démontré que l'administration d'insuline induit une hypoaminoacidémie chez ces patients. La présente étude a pour but d'évaluer les effets de l'administration de hautes doses d'insuline sur le métabolisme des protéines et du glucose par l'usage d'isotopes stables pour ce type d'intervention chirurgicale. Les isotopes utilisés étaient le [6,6-$^{2}H_2$]glucose et la L-[1-^{13}C]leucine. L'objectif principal était de vérifier s'il est possible de faire le suivi postopératoire de ces marqueurs isotopiques en présence de hautes doses d'insuline, et ce afin d'établir un protocole. Les effets de ces hautes doses d'insuline sur la lyse, la synthèse et l'oxydation protéique ainsi que les effets sur le métabolisme du glucose ont été évalués.

Quinze sujets ont été recrutés, soit sept dans le groupe témoin recevant le traitement habituel de contrôle de glycémie, et les huit autres dans le groupe d'étude soumis à des hautes doses d'insuline. Les plateaux du [6,6-$^{2}H_2$]glucose, de la L-[1-^{13}C]leucine et du $^{13}CO_2$ ont été obtenus pour tous les candidats des deux groupes, avec un coefficient de variance < 5%. La lyse et la synthèse protéiques des patients recevant de hautes concentrations d'insuline ont toutes deux diminuées alors que l'oxydation, quant à elle, est restée la même. Un bilan de protéines négatif a été obtenu quelque soit le groupe de traitement. La production endogène de glucose a pratiquement été abolie par l'administration de grande quantité d'insuline et de dextrose 20%. Cette intervention métabolique,

lorsqu'utilisée dans le contexte de chirurgies cardiaques, a des effets significatifs sur le métabolisme global des protéines et du glucose des patients.

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AA: amino acids

BCAA: branched chain AA

ATP: adenosine triphosphate

CABG: coronary artery bypass graft

CAD: coronary artery disease

CI: cardiac index

CPB: cardiopulmonary bypass

EAA: essential amino acids

FFA: free fatty acid

GIK: glucose-insulin-potassium

GS/MS: gas chromatography mass spectrometry

ICU: intensive care unit

IL: interleukin

IRMS: isotope ratio-mass spectrometry

IRS: insulin receptor substrate

KIC: ketoisocaproic acid

MAPK: ras-mitogen-activated protein kinase

MPE: molecules percent excess

NEAA: non-essential amino acids

NFkB: nuclear factor-kappaB

PI3K: phosphatidylinositol-3-kinase

Ra: rate of appearance

RQ: respiratory quotient

SBP: systolic blood pressure

TNF: tumour necrosis factor

VCO_2: CO_2 production

VO_2: O_2 consumption

Ub: ubiquitin

1. Introduction

Open heart surgery is associated with a catabolic response which is characterized by hyperglycemia and whole body protein loss (Wolfe, 2005). Given that even moderate increases in circulating blood glucose levels contribute to higher morbidity and mortality, many patients undergoing cardiac procedures receive insulin for blood glucose control (Estrada et al, 2003; Gandhi et al, 2005). The hyperinsulinemic-normoglycemic clamp technique is a precise method of providing insulin and glucose while maintaining normoglycemia in a reliable and secure manner (Carvalho et al, 2004; Sato et al, 2010). High-dose insulin has profound effects on glucose and protein metabolism. Although the effects of insulin on perioperative glucose homeostasis are well recognized, its influence on protein metabolism has received little attention in the medical literature.

It has been recently demonstrated in coronary artery bypass graft (CABG) surgery patients that high-dose insulin therapy causes hypoaminoacidemia which is most likely a consequence of decreased whole body proteolysis (Hatzakorzian et al, 2011). The aim of this pilot project was to investigate the effect of high-dose insulin therapy, in the absence of exogenous amino acids (AAs), on glucose and protein metabolism in patients undergoing CABG surgery as assessed by stable isotope tracers ([6,6-$^{2}H_2$]glucose, L-[1-^{13}C]-leucine). The primary objective of the study was to establish a protocol and to assess the feasibility of using stable isotope tracers after cardiac surgery while continuously infusing high-dose insulin. The second objective was to test the hypothesis that hypoaminoacidemia

in the presence of insulin administration is the result of a suppression of whole body protein breakdown.

2. Literature Review

2.1 Coronary artery bypass graft surgery

2.1.1 Coronary artery disease and cardiac surgery

Coronary artery disease (CAD) is due to the formation of plaques within the coronary arteries which can impede the flow of blood to the heart muscle, resulting in a mismatch between myocardial oxygen demand and supply and inducing myocardial ischemia. It is estimated that CAD is implicated in one fifth of all deaths in developed countries (Reddy, 2004). Medical management is crucial in all patients with CAD. CABG surgery together with percutaneous coronary intervention is a method of revascularization aimed at reducing cardiac events and death (Cassar et al, 2009). CABG surgery is one of the most commonly performed major surgeries; over four hundred thousand were performed in United States alone in 2004 according to the American Heart Association (Thom et al, 2006). It remains the best treatment for multivessel or left main stem CAD due its proven long-term outcome (Taggart, 2006; Serruys et al, 2009; Hlatky et al, 2009).

2.1.2 Perioperative outcome and CABG surgery

CABG surgery causes major stress to the body and has an operative mortality of 2-3%. There is an ongoing effort to influence outcomes from CABG surgery by perfecting old modalities and introducing new interventions. Surgical techniques improving cardiac protection during cardiopulmonary bypass (CPB) has been the main focus of these efforts. But in the last two decades there has been a

concentrated interest in the metabolic derangements caused by the surgical trauma and novel metabolic interventions (amino acids and insulin) that could benefit the myocardium and potentially improve outcome (Svedjehom et al, 1995). The effect of high-dose insulin therapy on the myocardium has been studied but its effect on whole body protein metabolism has received little attention.

2.2 Surgical tissue trauma

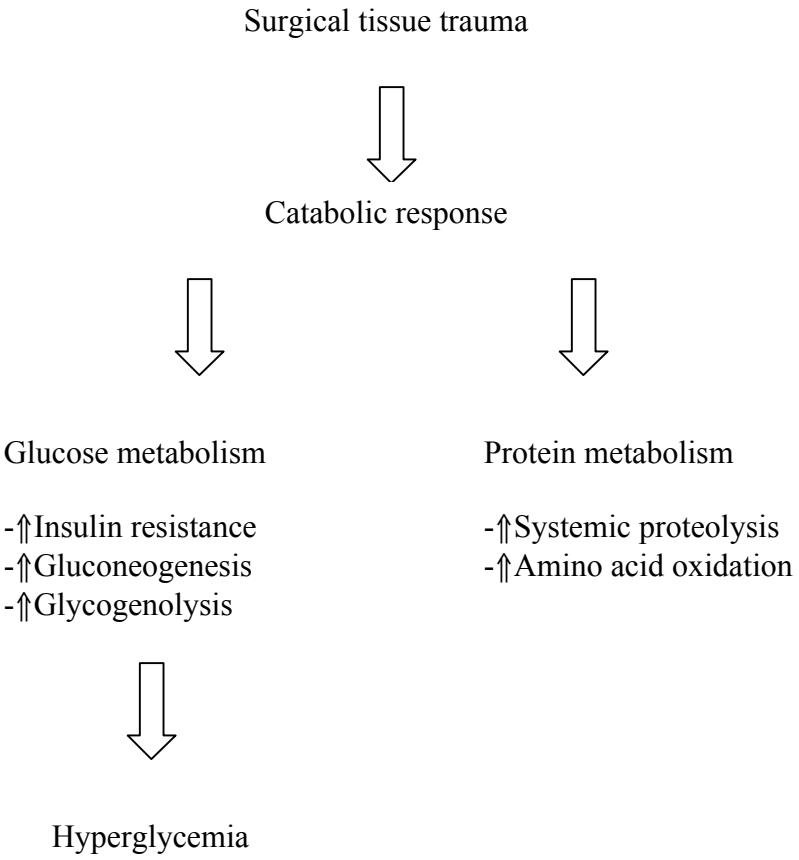
2.2.1 Metabolic stress response

Surgical tissue trauma provokes a catabolic response which is characterized by hyperglycemia, enhanced systemic proteolysis and increased AA oxidation, resulting in a negative nitrogen balance (Weissman, 1990; Wilmore, 2000, Desborough, 2000) (Figure 2.1). This response to stress was first described almost 80 years ago in four patients with lower limb injuries (Cuthbertson, 1932). Since then the stress response to trauma and surgery has been the focus of much attention in the medical literature.

This metabolic response to stress is mediated through neural pathways and the neuroendocrine axis. Various hormones (catecholamines, cortisol) and cytokines (interleukin IL-1, IL-6 and tumor necrosis factor (TNF)) are involved in the catabolic response to the surgical tissue trauma. The main goal of this response is restoration of adequate tissue perfusion, oxygenation, and release of substrates for the vital organs. Over the last few decades attempts have been made to find different ways to modulate this exaggerated neurohormonal stress response in order to improve delivery and utilization of substrates. Some strategies include

changing surgical techniques, enhancing anesthetic strategies (neuraxial blockade), and recently introducing new and innovative modalities, for instance the hyperinsulinemic-normoglycemic clamp technique.

Figure 2.1 Catabolic response to surgery



2.2.2 Surgical trauma and carbohydrate metabolism

Glucose is the most important energy source in the human body. Blood glucose concentrations increase in the perioperative period secondary to a rise in cortisol and catecholamine levels, which promotes hepatic gluconeogenesis (synthesis of glucose from non-carbohydrate sources such as AAs) and glycogenolysis (breakdown of glycogen to glucose) and an increase in peripheral insulin resistance (Desborough, 2000; Khan et al, 2001). This rise in blood glucose levels is directly related to the severity of the surgical stress. In patients undergoing cardiac surgery, blood glucose concentrations can increase up to 10-12 mmol/L for 24 hours. However hyperglycemia in cardiac surgical patients is associated with adverse perioperative outcomes (Estrada et al, 2003; Gandhi et al, 2005). The initial rise in blood glucose during the surgical procedure is due to breakdown of hepatic glycogen. As the surgical stress continues and glycogen stores in the liver get consumed and depleted, hepatic gluconeogenesis becomes a significant contributor to glucose blood. AAs derived from muscle protein breakdown are one of the essential substrates in hepatic gluconeogenesis along with lactate, pyruvate, and glycerol. As such, muscle proteolysis, the hallmark of the catabolic response to surgical tissue trauma, is directly related to glucose production (Schricker et al, 2000a).

2.2.3 Toxic effects of hyperglycemia

The acute toxicity of hyperglycemia in patients undergoing major stress is secondary to the deleterious effects of glycolysis and oxidative phosphorylation. Cells and tissues where glucose uptake is mediated by noninsulin dependent transport (GLUT-1, GLUT-2, GLUT-3) are more prone to the toxic effects of glucose (Tirone et al, 2001). As such, renal tubular, hepatic, endothelial and immune cells are at an increased risk of glucose toxicity as they do not rely on insulin for glucose uptake. High levels of glucose are associated with an increased production of mitochondrial superoxide which can interfere with cellular function and energy production (Brownlee, 2001). This in turn may cause end-organ dysfunction and increase mortality, especially in critically ill patients (Van den Berghe et al, 2001; 2004).

2.2.4 Surgical trauma and protein metabolism

Enhanced systemic proteolysis and negative whole body protein balance are the hallmarks of the catabolic response caused by surgical stress. Gluconeogenesis from glucoplastic AAs is stimulated leading to depletion of skeletal muscle protein. The human body contains roughly 12 kg of protein, each with its own particular AA sequence and function. Skeletal muscle contains 60% (7kg) of all proteins in the human body, primarily in the form of contractile proteins, and contributes to 30% of whole-body protein turnover (Welle, 1999). The main alteration in muscle protein metabolism in the catabolic state is an increase in protein breakdown (Biolo et al, 2002). There is also inhibition of muscle protein

synthesis after surgical trauma as demonstrated after open and laparoscopic cholecystectomy (Essen et al, 1992; 1995). Furthermore, Caso and colleagues showed that immediately after CABG surgery, muscle protein synthesis was approximately 30% lower than in the preoperative state (Caso et al, 2008). The loss of one third of the whole body protein mass can result in severe generalized muscle weakness, respiratory impairment, and decreased immune function (Welle, 1999).

Sepsis and major trauma are pathophysiologic conditions that may cause severe muscle protein loss (>50% in just two weeks) and have a significant impact on morbidity and mortality. Protein catabolism results in significant weight loss and muscle wasting in patients after major surgical interventions. Elderly patients are more at risk of requiring prolonged mechanical ventilation and long-term physical rehabilitation after a catabolic injury due to their reduced skeletal muscle mass (Windsor et al, 1988). By finding novel treatment modalities to reduce protein and muscle wasting during and after major operations like CABG surgery, we may reduce perioperative morbidity and mortality especially in the elderly patient population.

2.3 Amino acids

2.3.1 Amino acids and their role in whole body metabolism

AAs are vital to basic functioning of various organs in the body and play a major role in regulating different metabolic pathways and cellular gene expression. The

first few AAs were discovered in the early 19th century. Among the 300 AAs detected in nature, only 22 AAs serve as building blocks of proteins. AAs are molecules containing an amine group, a carboxylic acid group and one of twenty R-groups. The proteinogenic AAs are categorized as essential AAs (EAA) and non-essential AAs (NEAA). EAAs are those that are dependent on dietary intake as opposed to NEAA that can be produced in adequate amounts by the body (Wu, 2009). Skeletal muscle is the largest reservoir of AAs in the body.

AAs are linked together in varying sequences to form a vast variety of acute phase proteins and mediators (Prod'homme et al, 2004; Timmerman et al, 2008). AAs have many regulatory roles. They are used as substrates to generate energy for the brain, the immune system and the heart. Tyrosine and phenylalanine are the precursors for the synthesis of epinephrine, norepinephrine, and dopamine which play a central role in the catabolic response to stress. Certain AAs play an important regulatory role in the preservation of renal blood flow and in wound healing processes (Castellino et al, 1986; Mackay et al, 2003). AA infusion (arginine and glutamine) during cardiac surgery is associated with a reduction in renal vascular resistance and hence increased renal blood flow (Jeppsson et al, 1997; 2000). Branched chain AAs (BCAAs) leucine, isoleucine and valine are crucial to muscle protein anabolism and wound healing (Tom et al, 2006; Zhang et al, 2004)

2.3.2 The role of amino acids in cardiac metabolism and heart surgery

Human heart function requires the production of adenosine triphosphate (ATP) through the use of different substrates. Under normal physiological circumstances, the human heart generates 70% of its ATP requirements from the oxidation of free fatty acids (FFA), 20% from the oxidation of glucose and just 10% from lactic acid and AAs (Doenst et al, 2008). In certain conditions however, the role of AAs in ATP production may take precedence. BCAAs as well as glutamate, aspartate, arginine and ornithine, AAs that are associated with the malate-aspartate cycle, are involved in myocardial energy production especially during myocardial ischemia and the post-ischemic recovery phase (Safer, 1975; Svedjeholm et al, 1995; Rau et al, 1979). In animal studies, an infusion of aspartate and glutamate was shown to decrease the extent of myocardial infarction during coronary artery occlusion (Pisarenko et al, 1989; Engelman et al, 1991). Early after cardiac surgery, the uptake of glutamate and BCAAs has been shown to correlate with myocardial oxygen consumption, suggesting a direct link of these AAs to myocardial energy production (Svedjeholm et al, 1991a). Finally, Pisarenko and colleagues have demonstrated that the infusion of glutamate after CABG surgery improves heart metabolism and function (Pisarenko et al, 1986). Therefore, a metabolic intervention that enhances myocardial uptake of AAs could potentially improve cardiac performance and overall outcome after CABG surgery.

2.4 High-dose insulin and cardiac surgery

2.4.1 Insulin

Insulin is a peptide hormone composed of 51 amino acids secreted by pancreatic beta cells. It is stored in the body as a unit of six insulin molecules (hexamer), while the active form is its monomer. Insulin exerts various metabolic effects. Its main function is to regulate carbohydrate and fat metabolism in the body, primarily counteracting hyperglycemia. Insulin binds to insulin membrane receptors on different sites (muscle, liver and heart) and activates two main pathways through insulin receptor substrate (IRS) proteins. The first pathway, the phosphatidylinositol-3-kinase (PI3K) system, exerts metabolic effects such as promotion of protein synthesis, antiproteolysis, antilipolysis, glycogen synthesis and glucose uptake. The second pathway is the Ras-mitogen-activated protein kinase (MAPK) system which promotes cellular proliferation and anti-apoptosis (Taniguchi et al, 2006; Van den Berghe, 2004). Insulin also has many non-metabolic effects, mainly on the vascular system, as well as anti-inflammatory properties. Insulin decreases vascular tone by enhancing nitric oxide production in the endothelium (Steinberg et al, 1994). Its anti-inflammatory effects are mediated through inhibition of the production of inflammatory cytokines (TNF-α), nuclear factor-kappaB (NFkB) regulated pathways and the generation of superoxides (Dandona et al, 2005).

2.4.2 Rationale for using insulin in cardiac surgery

Cardiac surgery causes myocardial ischemia by several mechanisms: CPB and aortic cross-clamping decrease blood flow, while the neuroendocrine stress response including hyperglycemia and increased plasma concentrations of FFA, cortisol and catecholamines also affect heart perfusion. Elevated levels of FFA increase myocardial oxygen consumption, reduce myocardial function, and accumulate as toxic metabolites (Oliver al, 1994).

Although myocardial glucose uptake is essential for glycogen replenishment and ATP supply, insulin resistance, a typical feature of the endocrine response to open heart surgery, actually inhibits cellular glucose uptake (Svensson et al, 1989a). The administration of insulin together with glucose has been used to optimize myocardial metabolism and improve cardiac function in patients undergoing cardiac surgery (Lazar et al, 2000; 2004; Koskenkari et al, 2005). This treatment strategy reduces FFA levels, facilitates intracellular glycogen storage and thus is postulated to provide myocardial protection (Haider et al, 1984; Svedjeholm et al, 1991b). Insulin at supraphysiologic doses has vasodilative and inotropic properties (Svensson et al, 1989b; Lucchesi, 1972). It has been demonstrated that in order to obtain the maximum metabolic benefit of this intervention normoglycemia must be maintained (Gandhi et al, 2005).

The hyperinsulinemic-normoglycemic clamp technique is a precise method of simultaneously infusing insulin and glucose while maintaining normoglycemia in a reliable and safe manner (Carvalho et al, 2004; Sato et al, 2010). Blood glucose

is maintained between 4 to 6 mmol/L using a fixed infusion of insulin at 5 $mU \cdot Kg^{-1} \cdot min^{-1}$ together with 20% dextrose infused at variable rate. This treatment modality has been shown to have protective effect on the myocardium, improve left ventricular function, and blunt the inflammatory response after CABG surgery (Albacker et al, 2008; Carvalho et al, 2011; Sato et al, 2011).

2.4.3 The effect of insulin on protein turnover

Although the effects of insulin on perioperative glucose homeostasis are well recognized, its influence on protein metabolism has received little attention in the medical literature.

Major surgical trauma leads to a catabolic state characterized by an imbalance between protein breakdown and synthesis. The main alteration in protein metabolism appears to be an increase in protein breakdown (Wolfe, 2005). In healthy subjects insulin has long been recognized to exert anti-catabolic effects. It is well established that insulin decreases, in a dose-dependent manner, the plasma levels of BCAAs, the consequence of an overall decreased whole body proteolysis (Fukagawa et al, 1985; 1986; Tessari et al, 1986).

Protein degradation is mediated through the ubiquitin-proteasome proteolytic pathway (Wing, 2005). This pathway is involved in skeletal muscle proteolysis in stress induced conditions like diabetes, cancer and sepsis (Wing, 2008; Tiao et al, 1997; Llovera et al, 1994). It is also most likely responsible for activating proteolysis in major surgical tissue trauma. PIK3 pathway mediated by insulin

receptors seems to be involved in inhibiting ubiquitin (Ub) stimulated proteolysis. Thus, high dose insulin through its membrane receptors decreases whole body protein wasting primarily by regulating ubiquitination of proteins (Wing, 2005). In fact, Larbaud and colleagues have demonstrated that 3 hours of high-dose insulin infusion downregulates Ub-proteasome dependent proteolysis in rat muscles (Larbaud et al, 2001).

Insulin has been shown to stimulate muscle protein synthesis and wound healing in burn patients, particularly if hypoaminoacidemia was avoided by the simultaneous provision of AA (Gore et al, 2004; Adegoke et al, 2009). Chevalier and colleagues showed that hyperinsulinemia caused an increase in whole body protein synthesis when administered with exogenous AA by intravenous (IV) infusion with a view to maintaining postabsorptive plasma AA levels (Chevalier et al, 2004).

Although the metabolic effects of insulin in non-surgical patients and healthy volunteers have been studied extensively, only scarce data are available on its influence on the catabolic changes induced by major surgical tissue trauma. Results of a study conducted by Hatzakorzian and colleagues demonstrated a significant reduction in plasma AA levels in patients treated with the hyperinsulinemic-normoglycemic clamp during cardiac surgery (Hatzakorzian et al, 2011). Twenty patients scheduled for elective CABG surgery were included in the study. Patients were randomly allocated to receive either standard metabolic care (target glycemia 6.0-10.0 mmo/L, control group, n=10) or high-dose insulin therapy (insulin group, n=10). Insulin was administered at 5 $mU \cdot Kg^{-1} \cdot min^{-1}$

together with 20% dextrose infused at a variable rate adjusted to maintain glycemia between 4.0 and 6.0 mmol/L. Plasma concentrations of all AAs decreased in the insulin group, with 15 out of 22 AAs, including BCAAs (valine, leucine, isoleucine), lysine, methionine, phenylalanine, threonine, tryptophan, alanine, asparagine, citrulline, glycine, ornithine, serine and tyrosine being significantly lower at the end of the operation when compared to the control group (Table 2.1 and 2.2).

The measurement of static plasma AA concentrations does not allow any conclusion to be made as to the underlying mechanism causing the observed decrease in AA levels in the presence of high-dose insulin. Stable isotope tracer technique measures whole body protein flux and can potentially be used to elucidate the mechanism behind the reduction in plasma AA levels in subjects receiving the hyperinsulinemic-normoglycemic clamp during cardiac surgery (Wagenmakers, 1999).

Table 2.1 Plasma EAA concentrations in patients undergoing CABG surgery receiving standard insulin treatment (control) and high-dose insulin (insulin)

	Control group		**Insulin group**		
EAA (μmol/L)	Baseline	Sternal closure	Baseline	Sternal closure	P for differences between groups at sternal closure
Isoleucine	56 ± 11	44 ± 14 ‡	63 ± 5	14 ± 6 *	<0.001
Leucine	110 ± 18	99 ± 16	123 ± 12	38 ± 14 *	<0.001
Valine	207 ± 37	186 ± 25 ‡	233 ± 15	125 ± 18 *	<0.001
Lysine	188 ± 20	176 ± 23	198 ± 25	128 ± 25 *	<0.001
Methionine	13 ± 2	15 ± 5	15 ± 4	6.8 ± 2 *	<0.001
Phenylalanine	49 ± 6	43 ± 8 †	53 ± 4	25 ± 6 *	<0.001
Threonine	102 ± 9	83 ± 15 †	115 ± 28	58 ± 15 *	<0.001
Tryptophan	39 ± 8	22 ± 6 *	46 ± 9	15 ± 8 *	0.043

EAA, essential amino acid.
CABG, coronary artery bypass surgery.
Values are means ± SD.
There are no significant differences in the two groups at baseline.
* $p<0.001$, † $p<0.01$, ‡ $p<0.05$ for differences between baseline and sternal closure in each group.
(Hatzakorzian et al, 2011)

Table 2.2 Plasma NEAA concentrations in patients undergoing CABG surgery receiving standard insulin treatment (control) and high-dose insulin (insulin)

	Control group		**Insulin group**		
NEAA (μmol/L)	Baseline	Sternal closure	Baseline	Sternal Closure	P for differences between groups at sternal closure
Alanine	287 ± 33	320 ± 63	308 ± 51	267 ± 29 ‡	0.027
Arginine	99 ± 10	102 ± 36	110 ± 12	75 ± 19 *	0.052
Asparagine	44 ± 7	38 ± 11 ‡	45 ± 8	27 ± 10 *	0.025
Aspartic Acid	20 ± 7	14 ± 5 ‡	21 ± 8	11 ± 3 ‡	0.122
Citrulline	34 ± 8	25 ± 6 *	31 ± 6	15 ± 2 *	< 0.001
Cysteine	1.7 ± 0.4	3.5 ± 1.8 *	1.4 ± 0.6	4.2 ± 3.6 *	0.570
Glutamic acid	215 ± 58	154 ± 57 *	217 ± 50	135 ± 49 †	0.436
Glutamine	315 ± 67	307 ± 75	307 ± 83	245 ± 77 †	0.084
Glycine	215 ± 57	177 ± 37 †	184 ± 45	128 ± 27 *	0.003
Histidine	69 ± 13	61 ± 12 ‡	76 ± 19	53 ± 10 *	0.199
Ornithine	79 ± 11	61 ± 16 †	73 ± 19	44 ± 11 *	0.015
Proline	139 ± 30	142 ± 32	184 ± 56	114 ± 41 *	0.104
Serine	100 ± 14	68 ± 8 *	89 ± 13	42 ± 9 *	< 0.001
Tyrosine	51 ± 6	44 ± 10 †	61 ±17	30 ± 11 *	0.008

NEAA, non-essential amino acid.
CABG, coronary artery bypass surgery.
Values are means ± SD.
There are no significant differences in the two groups at baseline.
* $p<0.001$, † $p<0.01$, ‡ $p<0.05$ for differences between baseline and sternal closure in each group.
(Hatzakorzian et al, 2011)

2.5 Assessment of whole body protein and glucose turnover

Nitrogen balance studies have been the traditional method used to analyze whole body protein loss. This method cannot distinguish between changes in the rates of protein synthesis and breakdown. Stable isotope tracer technique has become the more accepted method to calculate whole body glucose and protein metabolism (Wagenmakers, 1999). Substrates labeled by stable isotopes (i.e. [6,6-$^{2}H_2$]glucose and L-[1-^{13}C]leucine) are given as a continuous infusion until steady state conditions are achieved. Steady state is usually attained within 3 hours. Subsequently measurements of plasma isotopic enrichments are used to calculate rates of breakdown and synthesis. [6,6-$^{2}H_2$]glucose tracer is used to assess whole body glucose turnover (endogenous glucose production and glucose clearance) in vivo. L-[1-^{13}C]leucine is the amino acid most commonly used as tracer to measure whole body protein kinetics (protein synthesis, breakdown and oxidation) *in vivo*. When leucine is irreversibly committed to oxidation, its carboxyl carbon is removed, and after equilibrating with the bicarbonate pool, is exhaled as CO_2. Therefore oxidation can be measured from rate of appearance of $^{13}CO_2$ in breath during constant infusion of L-[1-^{13}C]leucine with correction for retention of the label in the bicarbonate pool (Matthews et al,1980). Plasma [1-^{13}C]α-ketoisocaproic acid (α-KIC) is used to calculate the flux and oxidation of leucine. α-KIC is formed intracellularly from reversible transamination of leucine with some release into the systemic circulation. Therefore plasma α-KIC reflects the intracellular precursor pool enrichment more accurately than plasma leucine itself (Matthews et al, 1982; Wagenmakers, 1999). Whole body protein turnover,

synthesis and breakdown can be extrapolated from leucine kinetics based on the (8%) leucine content of body protein.

3. Hypothesis and Objectives

3.1 Hypothesis

CABG surgery provokes a catabolic response which is characterized by hyperglycemia and increased systemic proteolysis. Insulin is a hormone traditionally used to counteract hyperglycemia, but also has significant effects on protein turnover. Plasma AAs in particular the BCAAs leucine, isoleucine and valine, have been shown to decrease after high-dose insulin treatment in open heart surgery. The purpose of this pilot project was to establish a protocol and to investigate the feasibility of using stable isotope tracers (L-[1-^{13}C]leucine and [6,6-$^{2}H_2$]glucose) to assess the effect of the hyperinsulinemic-normoglycemic clamp, in the absence of exogenous AAs, on protein and glucose metabolism in patients undergoing CABG surgery. We hypothesize that the reduction of plasma AA levels as seen in patients undergoing CABG surgery and receiving the hyperinsulinemic-normoglycemic clamp while maintaining normoglycemia is a consequence of an inhibition of whole body proteolysis as assessed by L-[1-^{13}C]leucine tracer kinetics.

3.2 Objectives

I. To establish a protocol and to assess the feasibility of undertaking a stable isotope tracer study in an acutely sick surgical population, i.e postoperative CABG surgery, receiving hyperinsulinemic-normoglycemic clamp in the intensive care unit (ICU).

II. To study the effect of the hyperinsulinemic-normoglycemic clamp on whole body protein and glucose metabolism, including systemic protein breakdown, protein synthesis, AA oxidation and glucose rate of appearance in patients undergoing CABG.

III. To assess the changes in the metabolic-endocrine milieu associated with the perioperative use of the hyperinsulinemic-normoglycemic clamp as assessed by plasma concentrations of glucose, insulin, cortisol, lactate, total protein and albumin.

4. Methods

4.1 Study population

The study was approved by the Ethics Committee of the Royal Victoria Hospital. Patients scheduled for elective CABG surgery were eligible for enrollment in the study after written informed consent was obtained. Subjects were recruited in the preoperative clinic or on the ward the day prior to surgery. Exclusion criteria included patients with severe malnutrition (weight loss >20% in the preceding three months, albumin level <35 g/L and body mass index <20 kg/m^2), severe obesity (body mass index >35 kg/m^2), chronic liver disease (cirrhosis, documented chronic viral hepatitis and abnormal liver function tests), left ventricular ejection fraction <20%, active cancer, dialysis, and <18 years of age.

Consenting patients were randomly allocated to receive the hyperinsulinemic-normoglycemic clamp or standard metabolic care. Patients were blinded to treatment. The personnel collecting and performing the data analysis were blinded to the patient's treatment. Randomization was performed by a computer program (Plan procedure, SAS software).

4.2 Perioperative and anesthetic care

Patients received all their cardiac medications up until the morning of their operation. Patients were administered standard surgical and anesthetic care as established by the Departments of Anesthesia and Cardiac Surgery at the Royal

Victoria Hospital. All patients were operated by the same surgeon. At arrival to the operating room, IV and arterial lines were inserted prior to the start of the anesthesia. Patients received IV midazolam 1 mg/kg, sufentanil 1 µg/kg and rocuronium 1 µg/kg at induction. Sevolflurane with a continuous IV infusion of sufentanil 0.5 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ was used to maintain anesthesia during the operation until its completion. A central line with an indwelling pulmonary catheter was inserted after induction prior to surgical incision to measure cardiac output. Heparin 400 U/kg was administered intravenously with the aim to obtain an activated clotting time >500 seconds. Tranexamic acid, 2 g IV bolus followed by a continuous infusion of 5 $mg \cdot kg^{-1} \cdot hr^{-1}$ until chest closure, was used as the antifibrinolytic agent. The ascending aorta and the right atrium were cannulated and the patient was placed on CPB. Subsequently aortic cross-clamp was applied and cardioplegia administered. Once all the coronary anastomoses were sutured and the aortic cross-clamp removed, the patient was separated from CPB. Dobutamine 2.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ IV was started if the cardiac index (CI) remained $<2.2\ L \cdot min^{-1} \cdot m^{-2}$ despite adequate fluid resuscitation. Norepinephrine 1-10 µg/min IV was used if the systolic blood pressure (SBP) remained consistently <100 mmHg. Protamine 1mg/100U of heparin was given, aortic and venous canulas removed, homeostasis established and the pericardium and sternum closed. The patient was transferred to the ICU intubated and ventilated on continuous IV infusion of low dose propofol 10-25 $\mu g \cdot kg^{-1} \cdot min^{-1}$ to maintain adequate sedation. Once the patient was deemed to be hemodynamically stable, the sedation was slowly decreased and the patient was extubated 6 to 10 hours post-surgery.

4.3 Study protocol

4.3.1 Control group

Prior to the induction of anesthesia, a baseline blood glucose value was obtained. Arterial blood glucose measurements were performed every 30 minutes while in the operating room. If the blood glucose was ≥10.0 mmol/L an insulin (Humulin® R regular insulin, Eli Lilly and Company, Indianapolis, IN) bolus of 2U followed by an infusion of 2U/h was started. The insulin infusion was then adjusted according to the following sliding scale to a maximum of 20 U/h.

If blood glucose:	Action
<4.1 mmol/L	stop insulin infusion and administer 25 mL dextrose 50%
4.1- 6.0 mmol/L	stop insulin infusion
6.1 – 10.0 mmol/L	maintain current infusion rate
>10.0 mmol/L	increase infusion by 2 U/h

4.3.2 Hyperinsulinemic-normoglycemic clamp group (insulin group)

Prior to the induction of anesthesia, a baseline blood glucose value was obtained. After procuring baseline blood samples and hemodynamic measures, a 2U priming bolus of insulin was given followed by an insulin infusion of 5 $mU \cdot Kg^{-1} \cdot min^{-1}$. Additional boluses of insulin were given if the blood glucose remained >6.0 mmol/L with incremental 2U of insulin for each 2 mmol/L increase in blood glucose. Ten minutes after commencing the insulin infusion and when the blood

glucose was ≤6.0 mmol/L, a variable continuous infusion of glucose (dextrose 20 %) supplemented with potassium (40 mEq/L) and phosphate (30 mmol/L) was administered to maintain the blood glucose between 4.0 and 6.0 mmol/L. The glucose infusion was started at 60 mL/h. Insulin infusions were continued until the end of the study period approximately six hours after surgery. Arterial blood glucose was measured every 15 minutes throughout the procedure using the Accu-chek® glucose monitor (Roche Diagnostics, Switzerland) and every 20 to 30 minutes in the ICU. We monitored blood glucose closely for three hours after the end of the study.

4.4 Measurements and stable isotope tracer studies

Stable isotope tracers, [6,6-$^{2}H_2$]glucose and L-[1-^{13}C]-leucine, were bulk tested to be pyrogen-free by the manufacturer (Cambridge Isotope Laboratories, Cambridge, MA). Each tracer solution was made by pharmacy under laminar flow, injected into sterile single use vials, and then tested for sterility and pyrogenicity using the USP rabbit test. Each lot was given a 12-month expiry date, and vials were stored in the dark at 4^{0}C.

The preoperative whole body leucine and glucose kinetics were studied from 9:00h to 12:00h the day before the operation. Postoperative studies were conducted 2 hours after arrival to the ICU when the patient was deemed hemodynamically stable and there was no evidence of major bleeding. Patients were fasting > 8 hours for both of the measurements. A graphical illustration of the study protocol is presented in Figure 4.1.

A cannula was placed in a forearm vein to administer the stable isotope infusions through an in-line 0.22 mm filter. A second superficial vein in the contralateral arm was used for blood drawing. Plasma kinetics of glucose and leucine were determined by a primed constant infusion of tracer quantities of [6,6-$^{2}H_2$]glucose and L-[1-^{13}C]leucine**.** Before beginning each experiment, blood and expired air samples were obtained to establish baseline enrichments of [1-^{13}C]α-KIC and expired $^{13}CO_2$. Priming doses of $NaH^{13}CO_3$ (1 μmol/kg, po), L-[1-^{13}C]leucine (4 μmol/kg, iv) and [6,6-$^{2}H_2$]glucose (22 μmol/kg, iv) were administered followed immediately by continuous infusions of [6,6-$^{2}H_2$]glucose (0.22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and L-[1-^{13}C]leucine (0.06 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). The isotope infusion was uninterrupted throughout the entire study period. At 150, 160, 170 and 180 min four expired breath samples and blood samples were taken to determine isotopic enrichments in plasma of [1-^{13}C]α-KIC, [6,6-$^{2}H_2$]glucose and expired $^{13}CO_2$. Blood samples were also collected at the end of the preoperative and postoperative studies to measure plasma concentrations of metabolic substrates and hormones; insulin, cortisol, glucose, lactate, total protein and albumin. Breath samples were collected through a mouthpiece in a 3-l bag and transferred immediately to 10-ml vacutainers until analysis. During the postoperative period while the patients were on controlled ventilation, expired gases were collected by a one-way valve connected to the ventilator. Blood samples were immediately transferred to a heparinized tube, centrifuged at 4 °C (3,000 g, 15 min) and the resulting plasma was stored at -70°C until analysis.

Oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory quotient (RQ) were measured by indirect calorimetry (Datex Instrumentation Deltatrac, Helsinki, Finland) 50 min prior to end of each study measurement. The average over a period of 20 min were taken for each of the values, with a coefficient of variation <10%.

4.5 Analytic methods

Plasma [1-^{13}C]α-KIC enrichment was analyzed by electron-impact selected-ion monitoring gas chromatography mass spectrometry (GC/MS), after it was derivatized to its pentafluorobenzyl ester (Hewlett-Packard 5988A GC/MS, Palo Alto, CA) (Mamer et al, 1988; Hackey et al, 1991). The [6,6-$^{2}H_2$]glucose enrichment was quantified by GC/MS using electron-impact ionization, after plasma glucose was derivatized to its pentaacetate compound (Kalhan, 1996). Isotopic enrichments were calculated as molecules percent excess (MPE). Expired $^{13}CO_2$ enrichment for the calculation of leucine oxidation was analyzed by isotope ratio mass spectrometry (IRMS Analytical Precision AP2, 003, Manchester, UK) (Schricker et al, 2000a).

Plasma glucose was measured by a glucose-oxide method using a Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA). Plasma lactate was measured by an assay founded on lactate oxidase using the Synchron CX system (Beckman Instruments, Fullerton, CA). Albumin and total protein concentrations were measured with an automated Bromocresol Green method. Plasma cortisol and

insulin were analyzed by sensitive and specific double-antibody radioimmunoassay (Amersham International, Amersham, Bucks, UK).

4.6 Calculations

Whole body glucose and leucine kinetics were calculated by conventional isotope dilution technique using a two-pool random model during steady state conditions. At isotopic steady state the rate of appearance (Ra) of unlabeled substrate in plasma is derived from the plasma isotope enrichment, expressed as MPE, according to the following equation: $Ra = I.(MPE_{inf}/MPE_{pl} - 1)$, where I is the infusion rate of the tracer, MPE_{inf} is the enrichment of the tracer in the infusate and MPE_{pl} is the tracer enrichment in plasma. The final MPE values represent the mean of all the MPE measurements during each isotopic plateau. Isotopic steady state conditions were regarded as valid when the coefficient of variation (CV) of the MPE values at isotopic plateau was < 5%.

At isotopic steady state leucine flux (Q) is quantified by the following formula; Q = S+O = B+I, where S is the rate of synthesis of protein from leucine, O is the rate of oxidation, B is protein breakdown and I is the dietary intake. Furthermore Q is equal to Ra (Ra = B + I) and the rate of disappearance (Rd; Rd = S + O). When tracer studies are done in fasting states, leucine flux equals B. The rate of protein synthesis is calculated by subtracting leucine oxidation from leucine flux (S = Q – O). Protein balance is calculated as protein synthesis minus leucine Ra with positive values indicating anabolism and negative values catabolism. Plasma [1-^{13}C]α-KIC is used to calculate the flux and oxidation of leucine. α-KIC is formed

intracellularly from leucine and is released into the systemic circulation. It reflects the intracellular precursor pool enrichment more accurately than plasma leucine itself (Matthews et al, 1982; Wagenmakers, 1999). In the calculation of leucine oxidation, a correction factor of 0.76 was used in the fasted state to account for the fraction of $^{13}CO_2$ released from leucine but retained within slow turnover rate pools of the body (Matthews et al, 1980).

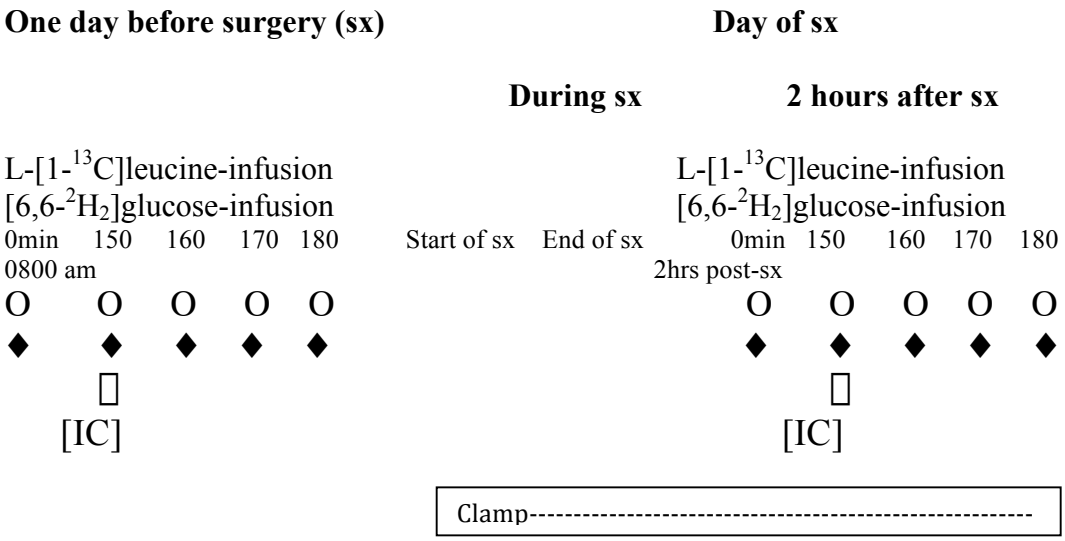
Ra glucose is identical to the rate of endogenous glucose production in the fasted state. During the hyperinsulinemic-normoglycemic clamp protocol where glucose (dextrose 20%) is being infused, the exogenous glucose infusion rate at steady state was subtracted from the total Ra glucose.

4.7 Statistical analysis

Patients' characteristics and perioperative data were compared using the unpaired Student's t-test, Wilcoxon-Mann-Whitney test and Fisher's exact test where appropriate. Blood glucose levels and hemodynamic variables at different time points between the two groups were compared using linear mixed models. Unpaired Student's t-test was used to compare mean blood glucose value between the two groups. Whole body leucine and glucose kinetics, gaseous exchange and plasma concentrations of hormones and substrates between the two groups were analyzed by using ANOVA for repeated measurements (before and after surgery). All data are presented as mean ± SD unless otherwise specified. Statistical significance was set as $P < 0.05$. All p-values presented are 2-tailed. Patients who opted out of the study were analyzed in their original treatment group as an

intention to treat analysis. All statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Chicago, IL, USA).

Figure 4.1 Study protocol



O Isotopic enrichments of [1-^{13}C]ketoisocaproate in plasma and expired ^{13}CO2
♦ [6,6-$^{2}H_2$]glucose enrichment in plasma
 Hormones, metabolic substrates
[IC] Indirect calorimetry

5. Results

5.1 Study population

Fifteen patients scheduled for elective CABG were enrolled in the pilot study; seven in the control group and eight in the insulin group receiving the hyperinsulinemic-normoglycemic clamp protocol. One patient in the control group refused to further participate in the study after completing the preoperative measurements. This patient's results were included in the analysis.

The preoperative characteristics and the perioperative and surgical data are outlined in Tables 5.1 and 5.2. There were no differences between the two groups regarding gender, age, weight, height and BMI. There were three patients with a diagnosis of diabetes mellitus in the insulin group compared to one in the control group, although this was not statistically significant (P= 0.569). Glycosylated hemoglobin (A1c), a marker of glycemic control over three to four months prior to its measurement, was similar in both groups. Left ventricular ejection fraction, a measure of myocardial function, was comparable in both groups. The prevalence of preoperative cardiac medications including β-blockers, calcium channel blockers and renin-angiotensin inhibitors, were similar in both groups.

There was no significant difference between the two groups regarding the number of grafts and the duration of anesthetic, surgical, CPB and aortic cross-clamp time. Estimated blood loss and packed red blood cell transfusions during the study period were comparable in both groups.

5.2 Inotropic support and hemodynamic variables

Hemodynamic variables were recorded at four different time points during the study. The baseline values are taken after induction of anesthesia and placement of the pulmonary artery catheter. These variables are presented in Table 5.3. There were no major statistically significant differences between the control and insulin groups. Baseline pulmonary artery pressures were slightly higher in the control group ($P<0.05$). There was also a trend towards a higher CI in the insulin group at sternal closure, 2 hrs and 5 hrs in the ICU, however it did not reach statistical difference.

The number of patients requiring inotropic (dobutamine 2.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and vasopressor (norepinephrine 1-10 μg/min) therapy during the study period are presented in Table 5.2. Three patients in the control group required dobutamine infusion after dissociation from CPB and in the ICU compared to none in the insulin group ($P= 0.769$). Most of the patients in both groups required norepinephrine infusion to maintain SBP over 100 mmHg ($P= 0.466$).

5.3 Blood glucose and high-dose insulin therapy

Perioperative blood glucose and insulin therapy data are summarized in Table 5.4 and 5.5. Blood glucose levels at baseline, the start of anesthesia were similar in both groups ($P= 0.602$). Blood glucose concentration progressively rose in the control group during and after surgery. However none in the patients in the control group had a blood glucose value >10 mmol/L. Consequently none of

these patients required insulin administration.

In the insulin group blood glucose levels in all eight patients were maintained between 4.0-6.0 mmol/L with the hyperinsulinemic-normoglycemic clamp. No episode of hypoglycemia (blood glucose <3.5 mmol/L) was reported during or after surgery. The mean dose of insulin provided to the insulin group was 222 ± 53 U. During the study period the mean rate of 20% dextrose infusion was 84 ± 19 cc/hr and the mean glucose dose administered was 3.6 ± 0.7 $mg \cdot kg^{-1} \cdot min^{-1}$. There was a significant difference in the mean blood glucose values between the groups ($P<0.001$). This was also true for the blood glucose values taken at all four key points during the study period: CPB, end of surgery, 2h in the ICU, and end of study ($P<0.001$).

5.4 Leucine and glucose kinetics

Isotopic plateaus of L-[1-^{13}C]leucine, [6,6-$^{2}H_2$]glucose and expired $^{13}CO_2$ were attained in all patients in both groups (CV< 5%). The isotopic plateaus need to be achieved to use the steady state equation where the rate of appearance of unlabeled substrate in plasma can be calculated from the plasma isotope enrichment (MPE). The data are summarized in Figures 5.1 to 5.3. Whole body leucine and glucose kinetics are presented in Table 5.6. There were no statistically significant differences in baseline preoperative leucine and glucose kinetics between the control and insulin groups. Postoperative leucine rate of appearance and protein synthesis decreased in patients receiving high-dose insulin compared to preoperative baseline ($P<0.01$). In contrast, postoperative leucine rate of

appearance and protein synthesis in the control group remained the same. There was no change in the postoperative leucine oxidation and leucine balance in both groups when compared to their baseline values.

Postoperative glucose rate of appearance increased in both groups compared to preoperative baseline, but this increase was much more pronounced in the high-dose insulin group where 20% dextrose was infused to maintain normoglycemia ($P<0.001$). 20% dextrose infusion in the insulin group suppressed postoperative endogenous glucose rate of appearance compared to baseline ($P= 0.011$) and to the control group ($P<0.001$).

5.5 Gaseous exchange

Whole body O_2 consumption, CO_2 production and the RQ are outlined in Table 5.7. RQ is the ratio of the volume of CO_2 produced to the volume of O_2 consumed per unit of time by the body under steady state conditions. The ratio varies for different metabolic fuels: for carbohydrates it is 1.0, for lipids 0.7 and for proteins 0.82. Postoperative whole body O_2 consumption was similar to the preoperative baseline in both groups. Patients who received high-dose insulin and 20% dextrose infusion had their whole body CO_2 production stimulated resulting in an increase in the RQ ($P<0.017$). The mean core body temperature in both groups, measured by the pulmonary artery catheter, was >36.0 ^{o}C at the start of the isotopic study in the ICU and >37.0 ^{o}C at the end of the study. There was no statistical difference in core body temperature between the control and insulin groups at start of the isotopic study in ICU and at the end of the study.

5.6 Hormones and substrates

Concentrations of hormones and substrates in plasma are summarized in table 5.8. Lactate concentration was higher in both groups after surgery compared to preoperative values. There was no statistical difference in lactate levels between the two groups measured before and after surgery. Preoperative plasma insulin and cortisol concentrations were similar in the two groups. In the insulin group postoperative plasma insulin levels were significantly elevated compared with patients in the control group ($P<0.001$). Plasma cortisol levels increased postoperatively without showing any significant difference between the two groups. Plasma total protein and albumin levels were similar in the two groups before surgery and decreased in the postoperative period.

Figures

Figure 5.1

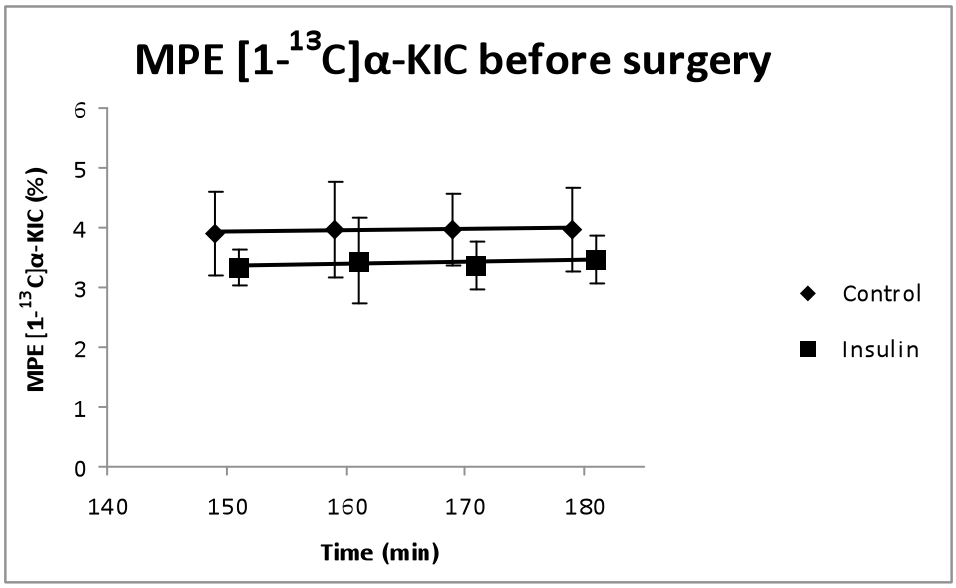


Figure 5.1A Isotopic enrichments (MPE= molecules percent excess) of [1-^{13}C]α-KIC before surgery.

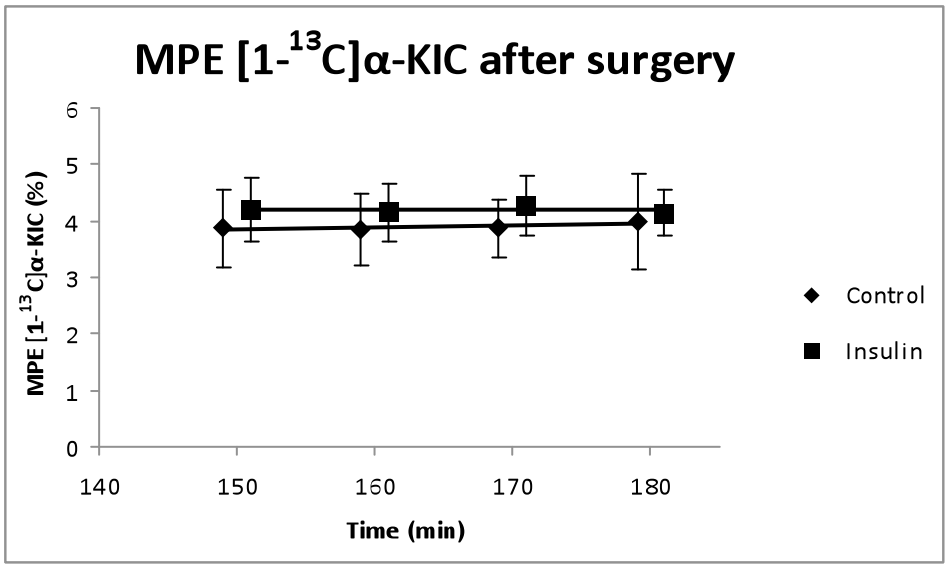


Figure 5.1B Isotopic enrichments (MPE= molecules percent excess) of [1-^{13}C]α-KIC after surgery.

Figure 5.2

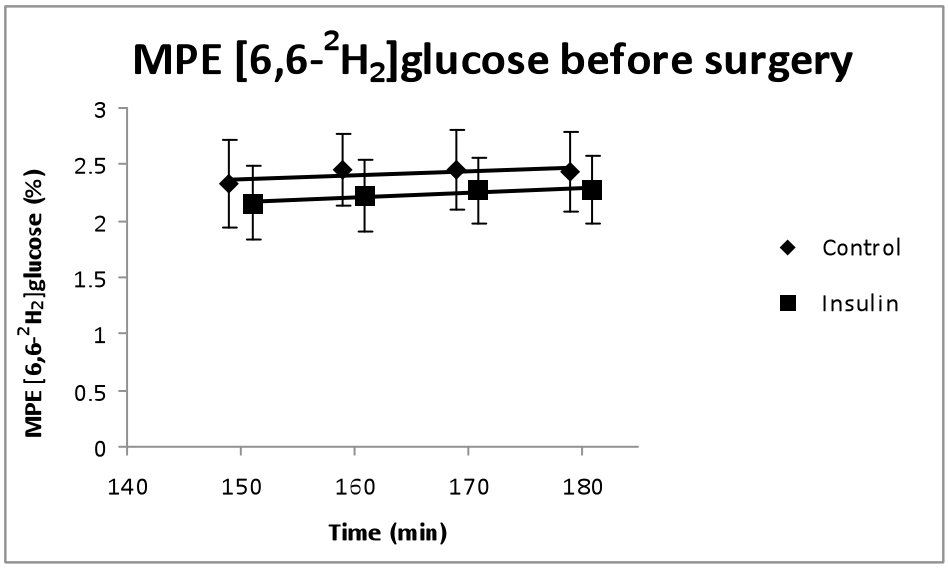


Figure 5.2A Isotopic enrichments (MPE= molecules percent excess) of [6,6-$^2H_2$]glucose before surgery.

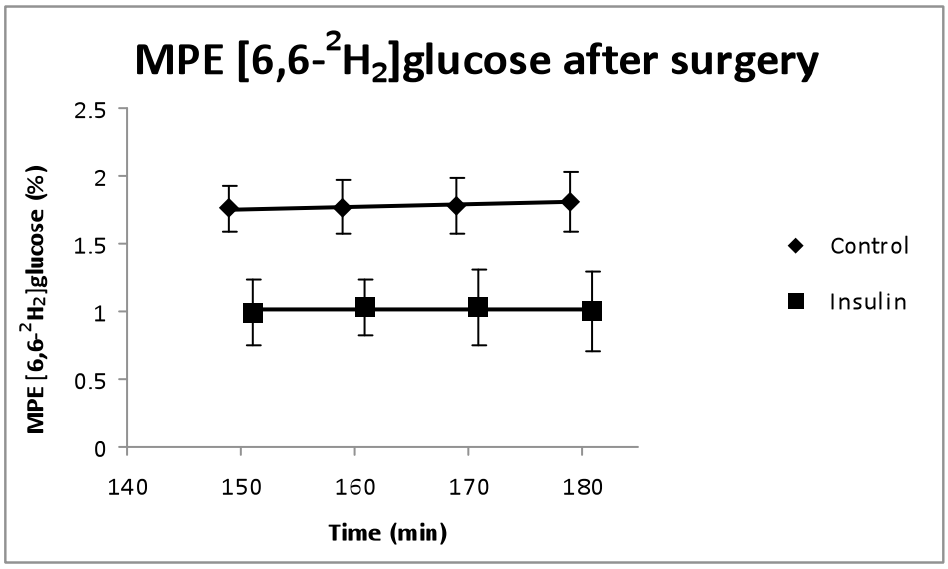


Figure 5.2B Isotopic enrichments (MPE= molecules percent excess) of [6,6-$^2H_2$]glucose after surgery.

Figure 5.3

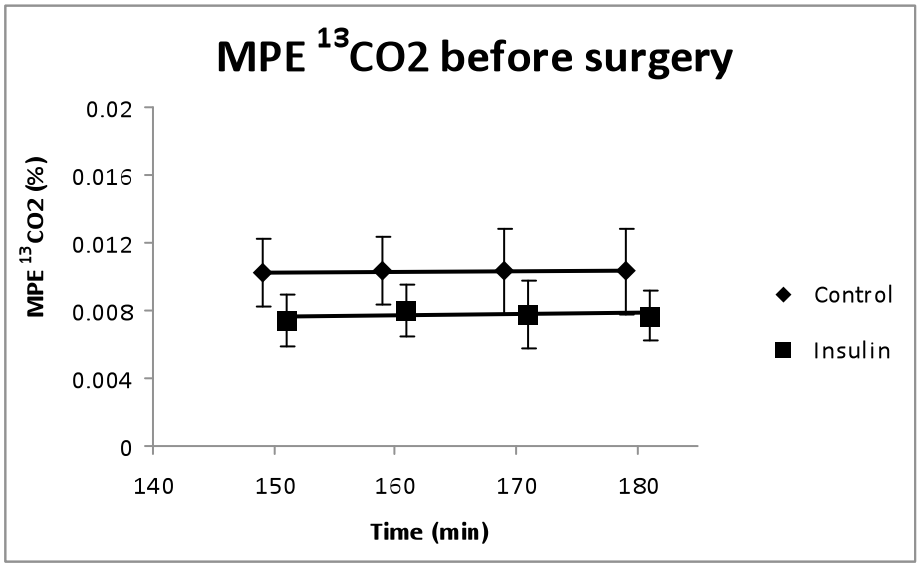


Figure 5.3A Isotopic enrichments (MPE= molecules percent excess) of $^{13}CO_2$ before surgery.

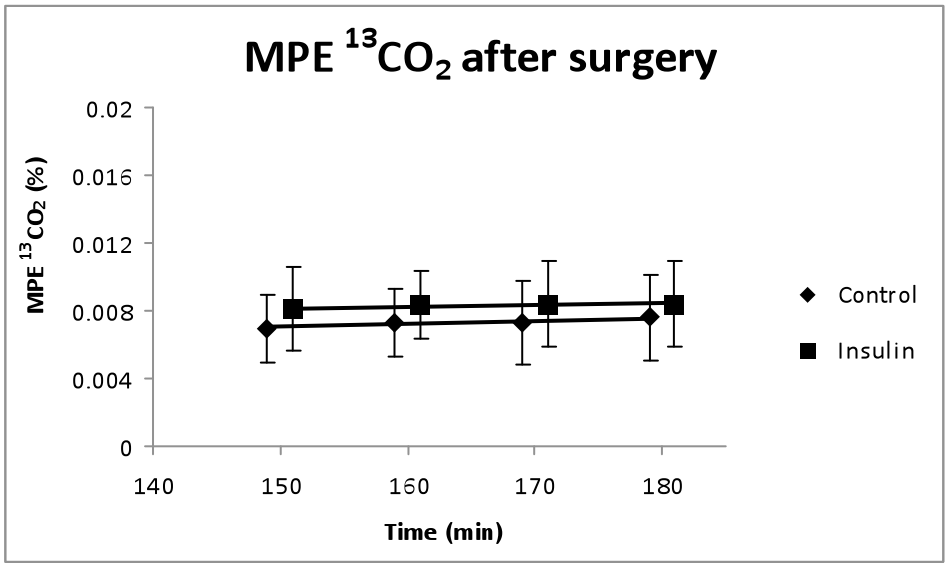


Figure 5.3B Isotopic enrichments (MPE= molecules percent excess) of $^{13}CO_2$ after surgery.

Tables

Table 5.1 Patient characteristics

	Control group	Insulin group	P value
n	7	8	
Age (y)	58 ± 10	65 ± 7	n.s. (0.187)
Gender (M/F)	4/3	7/1	n.s. (0.282)
Weight (kg)	80.6 ± 14.3	80.0 ± 16.2	n.s. (0.942)
Height (m)	1.7 ± 0.1	1.7 ± 0.1	n.s. (0.181)
BMI (kg/m^2)	28.9 ± 4.1	26.1 ± 4.3	n.s. (0.199)
DM	1	3	n.s. (0.569)
Diabetic therapy			
Oral agents	1	3	
Insulin	0	0	
HbA1c (%)	6.2 0.7	6.1 ± 0.2	n.s.(0.752)
LVEF (%)	44 ± 15	50 ± 13	n.s.(0.451)
ß-blockers	6	6	n.s. (1)
Calcium channel blockers	2	1	n.s. (0.569)
Renin-angiotensin inhibitors	5	3	n.s. (0.315)

Values are means ± SD or number of patients.
P values were determined by unpaired t-test and Fisher's exact test where appropriate.
M indicates male; F, female; BMI, body mass index; DM, diabetes mellitus; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction.

Table 5.2 Perioperative and surgical data

	Control group	Insulin group	P value
Anesthesia time (min)	336 ± 46	372 ± 53	n.s. (0.187)
Surgical time (min)	257 ± 40	282 ± 38	n.s. (0.239)
CPB time (min)	121 ± 29	140 ± 22	n.s.(0.168)
Aortic x-clamp time (min)	103 ± 31	119 ± 21	n.s. (0.256)
Grafts (n)	4.7 ± 1.7	5.3 ± 0.9	n.s. (0.947)
EBL (cc)	485 ± 160	580 ± 140	n.s. (0.246)
PRBC transfusions (units)	2.9 ± 1.1	2.3 ± 1.6	n.s. (0.425)
Inotropic and vasopressor therapy			
Dobutamine 2.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$	3	0	n.s. (0.769)
Norepinephrine 1-10 µg/min	7	6	n.s. (0.466)

Values are means ± SD or number of patients.
P values were determined by unpaired t-test, Wilcoxon-Mann-Whitney test and Fisher's exact test where appropriate.
CPB indicates cardiopulmonary bypass; EBL, estimated blood loss; PRBC, packed red blood cell.

Table 5.3 Hemodynamic variables

	Baseline	Sternal closure	ICU 2h	ICU 5h end of study
HR (beats/min)				
Control	66 ± 8	85 ± 11	93 ± 18	93 ± 15
Insulin	64 ± 11	83 ± 9	93 ± 10	94 ± 11
sBP (mmHg)				
Control	109 ± 10	114 ± 6	115 ± 12	110 ± 12
Insulin	104 ± 8	106 ± 6	115 ± 12	105 ± 7
dBP (mmHg)				
Control	56 ± 7	55 ± 5	59 ± 6	58 ± 7
Insulin	52 ± 4	51 ± 4	57 ± 6	57 ± 8
CVP (mmHg)				
Control	9 ± 3	13 ± 3	9 ±3	9 ± 2
Insulin	7 ± 2	11 ± 3	8 ± 4	10 ± 6
sPAP (mmHg)				
Control	30 ± 6*	37 ± 8	32 ± 7	35 ± 6
Insulin	25 ± 3*	34 ± 8	34 ± 7	32 ± 7
dPAP (mmHg)				
Control	13 ± 3*	17 ± 3*	14 ± 4	16 ± 4
Insulin	10 ± 3*	13 ± 3*	16 ± 4	15 ± 5
CI (L/min/m^2)				
Control	1.8 ± 0.2	2.7 ± 0.4	3.0 ± 0.6	2.8 ± 0.5
Insulin	1.8 ± 0.3	2.9 ±0.5	3.3 ± 0.5	3.0 ± 0.5

Values are means ± SD.
P values were determined by linear mixed models. * $P < 0.05$ for differences between groups at baseline, sternal closure, ICU 2h and end of study.
ICU indicates intensive care unit; HR, heart rate (beats per minute); sBP, systolic blood pressure; dBP, diastolic blood pressure; CVP, central venous pressure; sPAP, systolic pulmonary artery pressure; dPAP, diastolic pulmonary artery pressure; CI, cardiac index.

Table 5.4 Perioperative mean plasma glucose value and Insulin/Dextroxe 20% therapy (start of anesthesia to end of study-5h in the ICU)

	Control group (n=7)	Insulin group (n=8)	P value
Administration of insulin (n)	0	8	-
Insulin dose (U)	0	222 ± 53	-
Dextrose 20% infusion (cc/hr)	0	84 ± 19	-
Glucose ($mg \cdot kg^{-1} \cdot min^{-1}$)	0	3.6 ± 0.7	-
Mean plasma glucose (mmol/L)	7.5 ± 0.7	5.0 ± 0.7	<0.001
Number of measures	78	246	-

Values are means ± SD or number of patients.
P value was determined by unpaired student's t-test. P value indicates the difference between the two groups.

Table 5.5 Blood glucose values at baseline, end of surgery and end of study (5h in the ICU)

	Control group	Insulin group	P value (difference between groups at each time point)
Baseline	6.5 ± 0.9	6.1 ± 1.5	n.s (0.602)
Before CPB	6.2 ± 1.2	5.2 ± 1.1	n.s. (0.129)
CPB	6.9 ± 1.0	5.3 ± 0.8	<0.001
End of surgery	7.1 ± 1.2	4.8 ± 0.5	<0.001
2h in the ICU	7.4 ± 0.5	5.2 ± 0.5	<0.001
End of study	7.9 ± 0.9	5.2 ± 0.5	<0.001

Values are means ± SD.
P value was determined by linear mixed models. P values indicate the difference between the two groups at each time point.
CPB indicates cardiopulmonary bypass; ICU, intensive care unit.

Table 5.6 Whole body leucine and glucose kinetics

	Control group	Insulin group	P-value Time*	P-value Group†	P-value Interaction‡
Ra leucine ($\mu mol.kg^{-1}.h^{-1}$)					
Before surgery	93.5 ± 17.2	105.3 ± 8.8	0.004	n.s. (0.967)	0.003
After surgery	95.8 ± 14.8	85.5 ± 9.9			
Leucine oxidation ($\mu mol.kg^{-1}.h^{-1}$)					
Before surgery	14.3 ± 3.4	14.2 ± 2.7	n.s. (0.455)	n.s. (0.614)	n.s. (0.344)
After surgery	14.5 ± 2.8	16.6 ± 5.3			
Protein synthesis ($\mu mol.kg^{-1}.h^{-1}$)					
Before surgery	79.2 ± 14.3	91.1 ± 7.8	<0.001	n.s. (0.854)	<0.001
After surgery	81.4 ± 12.5	69.9 ± 8.5			
Leucine balance ($\mu mol.kg^{-1}.h^{-1}$)					
Before surgery	- 14.3 ± 3.4	- 14.2 ± 2.7	n.s. (0.455)	n.s. (0.614)	n.s. (0.344)
After surgery	- 14.5 ± 2.8	- 16.6 ± 5.3			
Ra glucose ($\mu mol.kg^{-1}.min^{-1}$)					
Before surgery	9.3 ± 1.2	10.0 ± 1.8	<0.001	0.001	<0.001
After surgery	12.7 ± 1.4	22.2 ± 4.8			
Endogenous Ra glucose ($\mu mol.kg^{-1}.min^{-1}$)					
Before surgery	9.3 ± 1.2	10.0 ± 1.8	0.0106	<0.001	<0.001
After surgery	12.7 ± 1.4	3.7 ± 2.5			

Values are means ± SD. Ra indicates rate of appearance.

* Probability that values change after surgery.

† Probability that values are different between the two groups.

‡ Probability that postoperative changes are different between the two groups.

Table 5.7 Gaseous exchange

	Control group	Insulin group	P value Time*	P value Group†	P value Interaction‡
VO_2 (ml/min)					
Before surgery	230 ± 44	234 ± 44	0.935	0.803	0.906
After surgery	232 ± 45	233 ± 43			
VCO_2(ml/min)					
Before surgery	170 ± 38	170 ± 30	0.043	0.045	0.380
After surgery	170 ± 39	199 ± 28			
RQ					
Before surgery	0.74 ± 0.08	0.73 ± 0.02	0.017	0.023	0.005
After surgery	0.73 ± 0.05	0.86 ± 0.09			

Values are means ± SD.

* Probability that values change after surgery.

† Probability that values are different between the two groups.

‡ Probability that postoperative changes are different between the two groups.

VO_2 indicates whole body oxygen consumption; VCO_2, whole body carbon dioxide production; RQ, respiratory quotient.

Table 5.8 Plasma concentrations of hormones and substrates

	Control group	Insulin group	P value Time*	P value Group†	P value Interaction‡
Lactate(mmol/L)					
Before surgery	0.9 ± 0.2	0.7 ± 0.3	<0.001	0.147	0.774
After surgery	1.6 ± 0.7	1.3 ± 0.3			
Insulin (pmol/L)					
Before surgery	56 ± 25	48 ± 17	<0.001	<0.001	<0.001
After surgery	56 ± 39	3614 ± 560			
Cortisol(nmol/L)					
Before surgery	177 ± 80	137 ± 59	<0.001	0.061	0.083
After surgery	882 ± 311	667 ± 222			
Total protein (mmol/L)					
Before surgery	62.3 ± 4.2	65.4 ± 3.2	<0.001	0.995	0.023
After surgery	45.3 ± 3.1	41.8 ± 7.4			
Albumin(mmol/L)					
Before surgery	38.6 ± 3.0	39.4 ± 2.4	<0.001	0.206	0.051
After surgery	30.1 ± 1.8	26.6 ± 4.1			

Values are means ± SD.
* Probability that values change after surgery.
† Probability that values are different between the two groups.
‡ Probability that postoperative changes are different between the two groups.

6. Discussion

6.1 The effect of high-dose insulin on whole body protein metabolism in cardiac surgery

Metabolic intervention has garnered much interest over the last four decades as a potent therapy for myocardial dysfunction in the setting of myocardial tissue malperfusion secondary to coronary artery occlusion and during cardiac surgery and CPB (Svedjeholm et al, 1995).

The most effective metabolic support for the ischemic myocardium has been glucose-insulin-potassium (GIK) therapy, which has been demonstrated to have a favorable effect on outcome after cardiac surgery. GIK therapy maintains normoglycemia, promotes glucose uptake by the myocardium, lowers circulating FFA levels, stimulates the activity of the pyruvate dehydrogenase enzyme and enhances Krebs cycle metabolism (Svedjeholm et al, 1991b, Knuuti et al, 1995, Kobayashi et al, 1983).

The hyperinsulinemic-normoglycemic clamp is the gold standard in maintaining normoglycemia while benefiting from all the favorable effects of high-dose insulin including antifibrinolytic, anti-inflammatory and cardioprotective properties (Sato et al, 2010; Albacker et al, 2008; Carvalho et al, 2011).

In the cardiac surgical population the focus of current research has been on the effect of high-dose insulin on glucose and lipid metabolism (Sato et al, 2010; Zuurbier et al, 2008). Insulin also has a powerful effect on protein metabolism which to date has not been examined in great detail in the context of cardiac

surgery. A recently published study demonstrated that insulin administered as part of a hyperinsulinemic-normoglycemic clamp protocol causes significant hypoaminoacidemia in patients undergoing open heart surgery (Hatzakorzian et al, 2011). Plasma AA levels do not give insight into the dynamics of AA metabolism and do not distinguish between changes in the rates of protein synthesis and degradation. Stable isotope tracer technique (L-[1-^{13}C]leucine) can assess whole body protein metabolism, i.e. protein breakdown, synthesis and AA oxidation. Because leucine is an essential AA, dependent on dietary intake, its rate of plasma appearance in the post-absorptive state is determined by body protein breakdown.

In this context the main findings of this pilot study are summarized as follows: (1) Isotopic plateaus of L-[1-^{13}C]leucine, ([6,6-$^{2}H_2$]glucose and expired $^{13}CO_2$ were attained in all patients in both groups (CV< 5%), (2) Protein breakdown and synthesis both decreased in patients who received high-dose insulin, while AA oxidation remained stable (3) Postoperative protein breakdown, synthesis and oxidation were similar to the preoperative measurements in patients who did not receive insulin therapy (4) Protein balance was negative in both groups and was not affected by either surgery or insulin treatment and (5) Endogenous glucose production was almost completely suppressed by the administration of high-dose insulin and 20% dextrose solution.

6.1.1 Use of stable isotope tracers after cardiac surgery

Stable isotope tracer technique has been used to assess whole body protein metabolism in healthy volunteers and in patients undergoing non-cardiac surgical procedures (Fukagawa et al, 1985; Schricker et al, 2005;2007). This pilot study demonstrates that the effect of the hyperinsulinemic-normoglycemic clamp can be accurately assessed by L-[1-^{13}C]leucine and [6,6-$^{2}H_2$]glucose kinetics in cardiac surgical patients. The principal elements necessary for calculating leucine and glucose turnover are the plasma [1-^{13}C]α-KIC, [6,6-$^{2}H_2$]glucose and the expired $^{13}CO_2$ plateau enrichments. The complex immediate postoperative cardiac surgical setting did not affect the accuracy of these measurements. All the values obtained are comparable with previous studies conducted in healthy volunteers and other surgical patients (Fukagawa et al, 1985; Schricker et al, 2005;2007)..

6.1.2 High-dose insulin and protein breakdown

This pilot study demonstrates the acute physiologic effect of high-dose insulin on whole body protein breakdown assessed by the leucine rate of appearance in cardiac surgical patients. High-dose insulin decreases the leucine rate of appearance due to a reduction in protein breakdown. At isotopic steady state leucine rate of appearance equals protein breakdown and dietary intake. During fasting conditions leucine rate of appearance reflects protein breakdown.

Insulin has been shown to suppress most plasma AA levels in nonsurgical healthy volunteers. BCAAs are most sensitive to the effects of insulin. In the CABG

population, hyperinsulinemic-normoglycemic clamp at 5 $mU \cdot kg^{-1} \cdot min^{-1}$ (21 U/h in a 70-kg subject; equivalent to 120 $mU \cdot m^{-2} \cdot min^{-1}$ in a 1.7 m^2 patient) produced hypoaminoacidemia and a 70% reduction in isoleucine and leucine (Hatzakorzian et al, 2011).

The data from the present study demonstrated an 18% reduction in leucine rate of appearance in the presence of a high-dose insulin infusion of 5 $mU \cdot kg^{-1} \cdot min^{-1}$ compared to its preoperative baseline. Both the preoperative and postoperative measurements were done in overnight fasting conditions. This is consistent with previous observations made in healthy human subjects (Fukagawa et al, 1985; Tessari et al, 1986; Castellino et al, 1987). Fukagawa and colleagues studied the effect of insulin on overnight-fasted healthy subjects and showed a 25% decrease in leucine rate of appearance at an insulin infusion rate of 30 $mU \cdot m^{-2} \cdot min^{-1}$ and a 30% decrease with their highest insulin dosage of 400 $mU \cdot m^{-2} \cdot min^{-1}$ (Fukagawa et al, 1985). Plasma insulin concentrations were approximately 520 pmol/L (75 μU/ml) at an insulin dosage of 30 $mU \cdot m^{-2} \cdot min^{-1}$ and 16,600 pmol/L (2,400 μU/Ml) at an insulin rate of 400 $mU \cdot m^{-2} \cdot min^{-1}$. In the present study mean plasma insulin concentrations were 3,600 pmol/L (520 μU/ml). Tessari and colleagues also looked at the effect of insulin at different doses on protein breakdown on healthy overnight-fasted subjects. They showed a 30% decrease at a maximal insulin dosage of 500 $mU \cdot m^{-2} \cdot min^{-1}$ (Tessari et al, 1986).

Patients receiving high-dose insulin had a glucose infusion administered at 3.6 ± 0.7 $mg \cdot kg^{-1} \cdot min^{-1}$. Glucose in surgical patients has protein-sparing properties. Exogenous glucose administration inhibits gluconeogenesis and thus lowers the

need for protein breakdown to supply glucoplastic AAs. This observation has not been shown in surgical patients (Schricker et al, 2000a,b). Protein breakdown does not decrease with the administration of high-dose glucose infusion. The decrease in protein breakdown demonstrated in this study seems to be secondary to the direct effect of insulin in the absence of exogenous AAs or clamping of AA concentrations.

In a catabolic condition such as surgical stress, protein breakdown is expected to increase. Patients who did not receive insulin therapy showed no reduction in the leucine rate of appearance because during cardiac surgery high doses of IV narcotics and benzodiazepines are generally used to induce and maintain anesthesia. This type of anesthesia has been shown to blunt the surgical stress response (Desborough et al, 2000). All of these patients are deeply sedated in the first 4 to 6 hours in the ICU with IV propofol infusion, a powerful sedative, and intermittent boluses of narcotics.

In the insulin group preoperative leucine rate of appearance was 10% higher than in the control group, although this was not statistically significant. Variability in kinetics can be attributed to the factors that can affect whole body protein breakdown such as age, weight and diabetic status. The subjects' mean age was slightly higher in the insulin group. Studies have shown that advanced age is associated with lower whole body protein turnover (Short et al, 2000), therefore it is unclear why preoperative rate of proteolysis in the insulin group was found to be higher in our study. Mean weight was identical in both groups. There were 3 diabetic subjects in the insulin group compared to one in the control group, all

diagnosed with type 2 diabetes. Nair and colleagues demonstrated that type 1 diabetic patients with poor levels of plasma insulin had a 35 % higher rate of protein breakdown (Nair et al, 1983). Type 2 diabetic patients suffer from insulin resistance and may also have lower plasma insulin levels due to pancreatic beta-cell insufficiency. Therefore they may also be at risk of having higher rates of proteolysis.

6.1.3 High-dose insulin and protein synthesis/oxidation

The primary effect of insulin on protein metabolism, in the absence of exogenous AAs, is an inhibition of endogenous proteolysis as demonstrated in the present study. Insulin also has effects on protein synthesis and oxidation.

Studies conducted in healthy subjects where high-dose insulin was administered demonstrated that leucine oxidation either increased or did not change (Fukagawa et al, 1985; Tessari et al, 1986; Castellino et al, 1987). In the present study, leucine oxidation was similar before and after surgery in both groups. However, there was a trend towards higher rates of leucine oxidation in the postoperative period in patients who were on high-dose insulin and 20% dextrose infusion. This elevation can be explained by an increase in CO_2 production secondary to glucose administration. The inability to demonstrate a clear increase in leucine oxidation may be due to the fact that all patients were heavily sedated in the ICU where the isotopic measurements were done.

Protein synthesis declined by almost 25% in patients who received exogenous high-dose insulin. Insulin decreases proteolysis and thus lowers plasma AA levels. The lack of circulating AAs is probably one of the main reasons why protein synthesis is inhibited in the presence of insulin. However, insulin has been shown to stimulate muscle protein synthesis and wound healing in burn patients, particularly if hypoaminoacidemia was avoided by the simultaneous provision of AA (Gore et al, 2004; Adegoke et al, 2009; Chevalier et al, 2004). In an elegant study using leucine methodology, Chevalier and colleagues showed that hyperinsulinemia indeed caused an increase in whole body protein synthesis when administered with exogenous AA with a view to maintaining postabsorptive plasma AA levels. To achieve this, exogenous AA had to be infused at rates greater than the rate of protein degradation (Chevalier et al, 2004).

Muscle protein synthesis has been shown to decrease after CABG and major abdominal procedures, whereas synthesis rates in the liver appear to increase. Caso and colleagues demonstrated that the rate of hepatic protein synthesis, assessed by synthesis of fibrinogen, increased after elective CABG surgery while muscle protein synthesis declined (Caso et al, 2008). This elevation in liver protein synthesis has also been observed in patients after traumatic injury (Mansoor et al, 1997; Thompson et al, 1989). In the present study, whole body protein synthesis rates in patients on the standard insulin protocol did not change after surgery. This neutral result is most likely due to a decrease in muscle protein synthesis and a concomitant increase in protein synthesis in the liver and heart.

The effect of surgical stress on protein synthesis appears to be organ and tissue specific (Boirie et al, 2001).

All subjects were maintained normothermic during the study period. Hypothermia has been demonstrated to play an important role in protein metabolism in the surgical population. Maintaining normothermia prevents muscle protein loss in patients undergoing general surgery (Carli et al, 1986).

6.2 Hyperinsulinemic-normoglycemic clamp and glucose metabolism

Glucose homeostasis in cardiac surgical patients has gained considerable attention because hyperglycemia has been associated with increased rates of infection and poor overall outcome after surgery (Estrada et al, 2003; Gandhi et al, 2005). Hyperinsulinemic-normoglycemic clamp with insulin infusion doses of 5 $mU \cdot Kg^{-1} \cdot min^{-1}$ has been shown to be used safely during cardiac surgery but has never been studied in the postoperative period at these high doses (Sato et al, 2010). In the present study, the hyperinsulinemic-normoglycemic clamp was started after induction of anesthesia and was continued for 6 to 8 hours in the ICU. This pilot study demonstrated that the hyperinsulinemic-normoglycemic technique can safely be used in the ICU without causing any episodes of hypoglycemia while successfully maintaining normoglycemia. Insulin at high doses has vasodilatory and inotropic effects and this has been shown in cardiac surgical patients (Sato et al, 2011; Koskenkari et al, 2005). In this study hemodynamic parameters were

similar between the both groups. Myocardial function assessed by pulmonary artery catheter demonstrated a trend towards a higher cardiac index and lower dobutamine usage in the insulin group.

In this study the preoperative glucose production rate in the fasted state was comparable to previous studies done in healthy volunteers and surgical patients scheduled to undergo major abdominal interventions (Schricker et al, 2000a). As expected, endogenous glucose production increased after surgery in patients who were on the standard insulin protocol. Whole body glucose production increases during and after major surgery secondary to the hypermetabolic stress response. Glucose production is dependent on two biochemical pathways: glycogenolysis and gluconeogenesis. In the initial phase of surgery, glucose production is dependent on hepatic glycogenolysis. But as the surgical stress continues and glycogen stores in the liver get consumed, hepatic gluconeogenesis becomes the main contributor to whole body glucose production. The stable isotope tracer technique used to assess glucose production does not differentiate which of the two pathways, glycogenolysis or gluconeogenesis, contributes to the production of whole body glucose. It has been demonstrated that after 42 hours of fasting, gluconeogenesis accounts for 90% of the whole body glucose production in healthy subjects (Chandramouli et al, 1997). Therefore we can presume that the rate of gluconeogenesis is almost equivalent to the rate of total glucose production in patients who did not receive high-dose insulin therapy. Hepatic glycogen stores were likely almost completely depleted in the study patients as they just had major

surgery and were fasting for at least 26 hours at the time of the isotopic measurements.

Administration of high-dose insulin at 5 $mU \cdot kg^{-1} \cdot min^{-1}$ and glucose at a mean infusion rate of 3.6 ± 0.7 $mg \cdot kg^{-1} \cdot min^{-1}$ almost completely suppressed glucose production in all patients. High-dose insulin therapy needs to be administered with supplementation of glucose to avoid severe hypoglycemia. In healthy volunteers and surgical patients, administration of a glucose infusion of 4 $mg \cdot kg^{-1} \cdot min^{-1}$ without any insulin almost completely suppressed endogenous glucose production (Schricker et al, 2000a). Exogenous glucose at high doses seems to inhibit hepatic gluconeogenesis secondary to the direct effect of glucose and/or to a rise in endogenous insulin concentrations. The inhibition of endogenous glucose production in the presence of hyperinsulinemic-normoglycemic clamp can be explained by the direct effect of high-dose insulin and glucose administration on hepatic gluconeogenesis.

6.3 High-dose insulin and circulating levels of hormones and substrates

Hyperinsulinemic-normoglycemic clamp is a metabolic intervention that has important effects on various hormones and substrates. In the present study, the plasma insulin concentrations were measured to be 3,600 pmol/L (520 μU/ml) in patients receiving high-dose insulin therapy at a rate of 5 $mU \cdot Kg^{-1} \cdot min^{-1}$ (21 U/h in a 70-kg subject; equivalent to 120 $mU \cdot m^{-2} \cdot min^{-1}$ in a 1.7 m^2 patient). These

supraphysiologic levels are consistent with studies using hyperinsulinemic-normoglycemic clamps. In one study, plasma insulin concentrations were approximately 520 pmol/L (75 μU/ml) at an insulin dosage of 30 $mU \cdot m^{-2} \cdot min^{-1}$ and 16,600 pmol/L (2400 μU/Ml) at 400 $mU \cdot m^{-2} \cdot min^{-1}$ (Fukagawa et al, 1985). Cortisol is one of the key modulators of the stress response to surgery. Cortisol concentrations increase during and after surgery. This response depends on the severity of the surgical tissue trauma and can be modified by anesthesia (Desborough et al, 2000). Cortisol levels increased as expected after CABG surgery in all patients enrolled in the study regardless of whether high-dose insulin was used (van Wezel et al, 2006).

Plasma total protein and albumin concentrations were found to be lower after surgery in both groups. The decrease in albumin levels is most likely due to transcapillary escape of albumin and not a decline in the rate of albumin synthesis (Fleck et al, 1985). In a recent study, albumin synthesis was shown to increase 8 hours after cardiac surgery (Caso et al, 2008). The effect of high-dose insulin on albumin production was outside the scope of this study however.

Plasma lactate levels slightly increased at the end of the study in the ICU in all fifteen patients. Elevation in plasma lactate during and after CABG surgery is secondary to whole body ischemia due to CPB and/or an increase in glycolytic activity. Insulin stimulates skeletal muscle glycolysis and may also enhance myocardial and hepatic lactate uptake (Ferrannini et al, 1993). The hyperinsulinemic-normoglycemic clamp was not associated with substantially

altered plasma lactate levels. This pilot study was not powered to look at biochemical markers such as lactate however.

6.4 Study limitations

There are certain limitations to this study. There were four diabetic patients in the study (three in the insulin group). It is possible that these patients had an altered protein metabolism and this had an impact on overall results. Furthermore, the study design does not explore specific organ protein turnover and the effect of simultaneous provision of AA on whole body protein synthesis, breakdown and oxidation. Because of the very labour-intensive nature of the study protocol, only a limited number of patients could be recruited. The relatively small study size likely limited our ability to detect small differences between groups.

7. Conclusion

In summary, this study demonstrated that stable isotope tracer technique can be used to accurately measure whole body protein and glucose metabolism in patients after cardiac surgery receiving high-dose insulin therapy. The hyperinsulinemic-normoglycemic clamp is a metabolic intervention that when used in the perioperative setting of cardiac surgery, can have a significant effects on glucose and protein metabolism. This study showed that achieving supraphysiological levels of exogenous insulin while maintaining euglycemia after CABG surgery decreases whole body protein breakdown and synthesis, without a change in AA oxidation. Nonetheless, leucine balance remained negative and not different than in control subjects.

Exogenous high-dose insulin suppresses whole body protein breakdown. We cannot discount the possibility that the decrease in whole body protein synthesis was due to lack of AA precursors. We postulate that protein synthesis may be enhanced in specific organs such as the liver and the heart, especially in catabolic conditions, and this may in fact be beneficial for the organ's function and/or healing. For any prolonged benefit to arise from the hyperinsulinemic-normoglycemic clamp, exogenous AA would have to be infused concomitantly and AA levels would have to be clamped to determine the specific impact of high-dose insulin on AA kinetics. Future studies are needed to examine specific organ protein kinetics in the context of exogenous hyperinsulinemia and whether it can be modified by the administration of exogenous AA.

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