Hyperinsulinemic normoglycemic clamp together with amino acids induces anabolism in patients undergoing cardiac surgery

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Abstract

Background: Cardiac surgery triggers an important stress response responsible for the development of hyperglycemia and protein catabolism that negatively impacts outcome. High-dose insulin therapy alone in the perioperative period can successfully maintain normoglycemia and attenuate protein breakdown, however patients remain in negative protein balance.

Objective: To determine whether perioperative amino acid supplementation improves whole-body protein balance in patients undergoing open-heart surgery and receiving hyperinsulinemic-normoglycemic clamp.

Methods: Twenty patients undergoing coronary artery bypass grafting surgery were divided into two groups. Both groups received intra and postoperative hyperinsulinemic-normoglycemic clamp. One of the groups additionally received an intravenous amino acid supplementation during and after the surgery. Whole-body protein and glucose metabolism was quantified before and after surgery with stable isotope tracers [6,6-$^{2}H_{2}$]glucose and L-[1-^{13}C]leucine. Various substrates, hormones and inflammatory markers in plasma and adipose tissue biopsies were compared between the two groups.

Results: The intervention of hyperinsulinemic-normoglycemic clamp together with amino acid supplementation successfully stimulated whole-body protein synthesis, resulting in a neutral whole-body protein balance (P<0.001). Endogenous glucose production was suppressed in both study arms. Amino acid supplementation was found to attenuate postoperative levels of plasma interleukin-6 (P=0.011). Interleukin-6 mRNA expression and translation were however increased at the adipose tissue level.

Conclusion: Hyperinsulinemic-normoglycemic clamp technique together with amino acid supplementation can induce anabolism after open-heart surgery, resulting in a neutral protein balance. Amino acid supplementation appears to modulate inflammatory marker expression, yet the underlying mechanism and significance remains unclear.

Résumé

Contexte : Le stress physiologique causé par la chirurgie cardiaque est responsable de dérangements métaboliques tels que l'hyperglycémie résultant en un impact nocif sur le rétablissement des patients. L'infusion d'une haute dose d'insuline durant la période périopératoire peut non seulement maintenir une glycémie normale mais elle peut aussi atténuer la dégradation protéique chez le patient. Malgré cet effet, le patient reste dans un état catabolique.

Objectif : Déterminer si la supplémentation d'acides aminés en plus du clamp hyperinsulinémique-normoglycémique peut améliorer la balance protéique chez le patient ayant recours à une chirurgie à cœur ouvert.

Méthodes : Vingt patients prévus pour une chirurgie de pontage aorto-coronariens ont été divisés en deux groupes. Chaque groupe a reçu le clamp hyperinsulinémique-normoglycémique durant et après la chirurgie. Un seul des deux groupes a reçu une supplémentation intraveineuse d'acides aminés durant et après la chirurgie. Pour quantifier et comparer les cinétiques du métabolisme des protéines et du glucose avant et après la chirurgie, des traceurs d'isotopes stables [6,6-$^{2}H_2$]glucose et L-[1-^{13}C]leucine ont été employés. Plusieurs autres substrats, hormones, ainsi que marqueurs d'inflammation dans le sérum et dans les tissus adipeux ont été mesurés et comparés parmi les groupes.

Résultats : L'intervention de clamp hyperinsulinémique-normoglycémique avec la supplémentation d'acides aminés peut stimuler la synthèse protéique, résultant en une balance protéique neutre même après une chirurgie à cœur ouvert (P<0.001). La production endogène de glucose était supprimée chez les deux groupes. La supplémentation d'acides aminés a réduit le niveau d'interleukin-6 en circulation (P=0.011). Par contre, l'expression et la traduction du mRNA de l'interleukin-6 étaient plus élevées dans les tissus adipeux.

Conclusion : Le clamp hyperinsulinémique-normoglycémique combiné avec une supplémentation intraveineuse d'acides aminés peut induire l'anabolisme même après une chirurgie à cœur ouvert, résultant en une balance protéique neutre. De plus, la supplémentation d'acides aminés semble moduler l'expression des marqueurs inflammatoires, mais le mécanisme sous-jacent reste à être élucidé.

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List of abbreviations

AA : amino acid

Akt : protein kinase B

ANOVA : analysis of variance

ATP : adenosine triphosphate

BCAA : branched chain amino acid

BMI : body mass index

CABG : coronary artery bypass grafting

CPB : cardiopulmonary bypass

CV : coefficient of variation

EAA : essential amino acid

EAR: estimated average requirement

EE : energy expenditure

ELISA : enzyme-linked immuno assay

GC/MS : gas chromatography/mass spectrometry

HNC : hyperinsulinemic normoglycemic clamp

ICU : intensive care unit

IL : interleukin

IRS : insulin receptor substrate

KIC : ketoisocaproic acid

LVEF : left ventricular ejection fraction

MAPK : mitogen activated protein kinase

MPE : molecules percent excess

mRNA : messenger ribonucleic acid

mTOR : mammalian target of rapamycin

NAD/NADH : nicotinamide adenine dinucleotide

NEAA : nonessential amino acid

PI3K : phosphoinositide 3 kinase

Ra : rate of appearance

RDA: recommended dietary allowance

RQ : respiratory quotient

SD : standard deviation

TNF : tissue necrosis factor

UPS : ubiquitin proteasome system

VCO_2 : carbon dioxide production

VO_2 : oxygen consumption

1. Introduction

Open-heart surgeries are known to trigger a physiological stress response associated with the release of multiple hormones that alter the metabolic function of various organ systems.[1] These reactions result in a shift of transport of glucose, amino acids, and free fatty acids, to favor efflux from the cells into the circulation.[2, 3] This catabolic state has been clearly shown to negatively impact clinical outcome. Hyperglycemia has been linked to increased mortality and morbidity in the perioperative setting as well as in many other disease states.[4] Excessive free fatty acids have been associated with worsened myocardial performance and increased risks of arrhythmias after a period of ischemia.[5] Accelerated rate of protein breakdown may slow the recovery process, particularly through a decreased function of the immune system and delayed wound repair.[6]

An intervention that could potentially normalize and reverse this catabolic process into an anabolic one involves the administration of perioperative administration of high-dose insulin. Recent evidence has demonstrated that this technique could safely and successfully 1) maintain normoglycemia,[7] 2) decrease circulating FFA levels,[8] but 3) failed to stimulate protein synthesis.[9] The primary aim of this study was to investigate the impact of high-dose insulin and exogenous amino acid supplementation on whole-body protein metabolism in patients undergoing cardiac surgery. We also explored the effect of such intervention on various perioperative hormonal and inflammatory marker profiles.

2. Literature review

2.1 Metabolic stress response

In 1932, Cuthbertson described for the first time the catabolic response in patients with lower limb injuries.[10] Injury (traumatic, surgical, burn) and sepsis result in altered glucose, protein, and lipid homeostasis, hence the importance attributed to this phenomenon in the medical community. This metabolic stress response is mediated by complex interactions between the nervous, endocrine and immune systems.[1] The increased circulating concentrations of stress hormones (glucagon, cortisol, catecholamines) as well as various cytokines (interleukin (IL)-1, IL-6, Prostaglandin E2, Tissue necrosis factor (TNF)) exert catabolic effects, either directly or indirectly, by impairing tissue insulin sensitivity and causing hyperglycemia and enhancing proteolysis and amino acid oxidation.[2, 11]

Growing evidence in the medical literature suggests that these metabolic alterations adversely impact on the course of illness.[12, 13] Hence, in the last decades, clinicians and researchers have put considerable effort in strategies aimed at minimizing the catabolic stress response. Such strategies include changing surgical technique, the use of multimodal anesthetic technique (neuraxial anesthesia), and more novel ones include the hyperinsulinemic normoglycemic clamp (HNC) technique.

2.2 Benefits of insulin in trauma and critical illness

In the acute phase of critical illness, hyperglycemia develops as a consequence of insulin resistance, enhanced glycogenolysis, and gluconeogenesis. Although no single unifying explanation has been proposed as to how perioperative insulin resistance develops, evidence points to a simultaneous reduction in glucose oxidation and non-oxidative glucose disposal.

Serine phosphorylation of insulin receptor substrate (IRS)-1 at serine-307 may be responsible for the defective intracellular insulin signaling, which in turn reduce the activation of glycogen synthase and the translocation of glucose transporters (GLUT-4).[14, 15]

High blood sugar levels is considered to lead to cellular glucose overload and toxicity in insulin independent tissues. For example, while microscopic analysis of hepatocytic mitochondria of ICU patients treated conventionally showed severe ultrastructural abnormalities, these were non-existent in ICU patients who had received high-dose insulin therapy.[16] This observation may explain how passive uptake of glucose by insulin independent cells leads to impaired cellular energy metabolism and organ dysfunction.[4] Intensive insulin therapy has also shown to be preventive for severe nosocomial infections in ICU patients.[17] This protective effect may be a result of improved polymorphonuclear neutrophil function from insulin therapy and normal glucose levels.[18] Insulin was shown to decrease C-reactive protein and mannose-binding lectin levels in one study[19], while it resulted in lower circulating levels of pro-inflammatory proteins in another.[20] Insulin's ability to enhance NO synthesis may have an antithrombotic effect that helps preserve organ blood flow and functional integrity.[21, 22] The use of intensive insulin therapy has gained attention in open-heart surgery as well, given recent evidence pointing toward a cardioprotective effect of the hormone.[23, 24]

2.3 Glucose-insulin-normoglycemia therapy

The hyperinsulinemic normoglycemic clamp (HNC) technique is a precise method of simultaneously infusing insulin and glucose while maintaining normoglycemia in a reliable and safe manner.[25] Blood glucose is maintained between 4 to 6mmol/L using a fixed infusion of insulin at 5 mU/kg/min 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.[25-27]

2.4 Protein metabolism

Tissue injury and sepsis accelerates protein breakdown and urinary nitrogen excretion, resulting in a net loss of whole body protein.[1, 28] Williamson et al. describe a 40% and 150% increase in urinary 3-methylhistidine excretion (as a direct marker of the breakdown of myofibrillar proteins in muscle) in patients undergoing elective surgeries and after accidental injuries, respectively.[28] Most amino acids released in the circulation as a result of the enhanced proteolysis are taken up by the liver to be used as the main substrate for gluconeogenesis and protein synthesis, particularly acute phase reactants.[2, 29] In muscle however, protein synthesis is depressed, even after minor surgeries such as cholecystectomies.[30] Protein catabolism has serious clinical implications; severely ill patients may lose up to 20% of body protein, which can lead to marked weight loss, muscle wasting, decreased respiratory muscle function, decreased gut function, impaired wound healing and impaired immune response in often already medically fragile patients.[29, 31] Of note, postoperative protein loss is greater in patients with type 2 diabetes by 50% when compared to their non-diabetic counterparts.[32] Given these negative consequences of protein breakdown during injury and sepsis, suppressing protein breakdown has been an important focus of research. For instance, intravenous or oral administration of glucose, growth hormone, and parenteral nutrition with amino acids have all demonstrated an attenuation of protein catabolism in surgical patients.[33-37]

2.5 Amino acids and their role in protein metabolism

Amino acids (AA) are organic compounds made from a carboxyl-carbon group, an amino-N group, and a functional group specific to each amino acid. There are a wide variety of amino acids, however only 20 of them are incorporated into mammalian protein and are commonly referred to as standard AA. These standard AA are further classified into essential AA (EAA) and non-essential AA (NEAA), depending on whether the human body can naturally synthesize them.

Amino acids are biologically very important compounds. They are the structural units (monomer) of longer polypeptide chains and proteins. Furthermore, AA are indispensable for the proper functioning of all organs and the immune system.[38] They help in wound healing processes and can also serve as a precursor of gluconeogenesis in the liver or as a fuel supply for the heart.[3, 39, 40] Significant loss of body protein can seriously impair various organ function, breathing, and can ultimately lead to death.

Since the 1970's, a subset of AA, the branched-chain amino acids (BCAA) leucine, isoleucine, and valine, have been described to have a regulatory role in muscle protein synthesis and to have an anti-catabolic effect.[41-43] Later studies however, have suggested that leucine appeared to be the main mediator of these effects, and not isoleucine or valine. Indeed, formulations mainly enriched with AA other than leucine were often ineffective.[44-46] One of the few potential mechanisms behind leucine's anabolic potential appears to involve the mTOR (mammalian target of rapamycin) pathway.[47] The mTOR is a large serine threonine protein kinase with sensitivity to inhibition by the antifungal agent rapamycin, hence its name. It is thought that leucine enhances an Akt-independent signaling cascade (as opposed to insulin which works via an Akt-dependent cascade) leading to the activation of the mTOR kinase complex and the

phosphorylation of p70s6k and 4E-BP1 proteins. This ultimately results in increased initiation of mRNA translation and enhanced protein synthesis.[48] Furthermore, numerous studies have suggested that leucine can exert these regulatory effects independently from insulin.[49] The details about how leucine activates mTOR remains however uncertain.

2.6 Insulin effect on protein metabolism

Insulin is the most potent anabolic hormone known. When insulin binds to the insulin receptor, it phosphorylates IRS proteins. The IRS proteins will then activate two signaling pathways. One of them, the phosphatidylinositol 3-kinase (PI3K)-AKT pathways, is responsible for the metabolic effects of insulin such as glycogen synthesis, glucose uptake, protein synthesis, and anti-proteolysis. The other pathway, the Ras-mitogen-activated protein kinase (MAPK) pathway, regulates gene expression and cellular growth/differentiation.[50]

As described previously, accelerated protein breakdown and muscle wasting is a metabolic response that occurs in stress conditions such as trauma, sepsis, and burn injuries. This response is believed to be mainly mediated by the ATP-dependent ubiquitin proteasome system (UPS).[51, 52]

Insulin deficiency or resistance is closely linked with increased rate of proteolysis. As observed by Lee et al., when PI3K activity was suppressed in muscle cells, the UPS was activated and muscle degradation occurred.[53] Conversely, when high-dose insulin was infused, the UPS was down regulated proteolysis was inhibited in rat muscles.[54]

Insulin's dose-dependent effect in reducing circulating AA concentrations was described 30 years ago.[55] More recently, a study demonstrated significant reductions in plasma AA levels when HNC was applied to patients undergoing cardiac surgery. Fifteen out of the 22 measured AA

were significantly lower in the treatment group when compared to standard care.[56] Hypoaminoacidemia observed in the presence of high-dose insulin is believed to be the result of an insulin-mediated decrease in whole-body protein breakdown. A follow up study, performed by the same authors, used a stable isotope technique to quantify protein kinetics in patients undergoing cardiac surgery with HNC. Interestingly, the authors found that the application of a HNC led to a reduction in whole-body protein breakdown as speculated, but insulin did not seem to stimulate protein synthesis in these patients. Hence, whole-body protein balance remained negative despite the presence of insulin therapy, and patients remained catabolic (see Table 2.1).[9] Given insulin's antiproteolytic and anabolic properties, numerous studies have attempted to study the effects of insulin and AA administration on protein synthesis. In most cases, the use of a HNC along with the infusion of a mixture of AA producing isoaminoacidemia or hyperaminocidemia in non-surgical healthy volunteers resulted in not only inhibition of protein breakdown but also in increased protein synthesis.[57-61] Maintaining circulating AA concentrations to normal levels has additive effects on the antiproteolytic property of insulin, hence further suppressing protein breakdown.[62] Although the effect of insulin and exogenous AA administration has been extensively studied in volunteers, data regarding these effects on surgical patients are still lacking.

Table 2.1. Whole-body leucine and glucose kinetics from a study by Hatzakorzian et al. The effect of HNC on protein and glucose kinetics was measured in patients undergoing open-heart surgery. The results show that HNC attenuates protein breakdown (Ra leucine) but fails to promote protein synthesis, when compared to standard surgery patients.

	Control group	Insulin group	P-value Time*	P-value Group†	P-value Interaction‡
Ra leucine ($\mu mol.kg^{-1}.h^{-1}$)					
Before surgery	95.0 ± 15.1	105.3 ± 9.8	0.001	0.759	<0.001
After surgery	97.5 ± 12.9	85.2 ± 9.2			
Leucine oxidation ($\mu mol.kg^{-1}.h^{-1}$)					
Before surgery	16.3 ± 3.2	16.6 ± 3.3	0.066	0.126	0.081
After surgery	16.6 ± 3.2	12.8 ± 4.1			
Protein synthesis ($\mu mol.kg^{-1}.h^{-1}$)					
Before surgery	78.6 ± 12.3	88.7 ± 8.7	0.002	0.927	<0.001
After surgery	80.9 ± 10.3	72.4 ± 8.4			
Leucine balance ($\mu mol.kg^{-1}.h^{-1}$)					
Before surgery	- 16.3 ± 3.2	- 16.6 ± 3.2	0.066	0.126	0.081
After surgery	- 16.3 ± 3.2	- 12.8 ± 3.2			
Endogenous Ra glucose ($\mu mol.kg^{-1}.min^{-1}$)					
Before surgery	9.3 ± 1.2	10.0 ± 1.6	0.005	<0.001	<0.001
After surgery	12.7 ± 1.2	3.7 ± 2.5			

Values are means ± SD, Ra, rate of appearance. *P* value was determined by repeated measure ANOVA. *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. (Hatzakorzian et al. 2014)

2.7 Amino acid supplementation and myocardial protection

Amino acids may be of little significance as a source of myocardial energy during cardiac surgery, yet some evidence points to their importance for the maintenance of intermediary metabolism of cardiomyocytes during and after ischemia.[63] Although taurine exhibits inotropic and antiarrhythmic properties via the modulation of Ca^{2+} availability in cardiomyocytes, and histidine have antioxidant properties that stimulate anaerobic energy formation[64], only the AAs involved with the malate-aspartate shuttle seem important in this respect. The malate-aspartate shuttle transports reducing equivalents across the mitochondrial membrane in order to promote glycolysis during periods of ischemia. Glutamate has also been involved in the clearance of lactate and ammonia. Along with aspartate, glutamate also serves as important intermediates in the Kreb's cycle, which may become depleted during periods of ischemia.[65-69] Glutamate and aspartate have been investigated further for their cardioprotective effect. Indeed, the infusion of both amino acids in an experimental model of coronary artery occlusion decreased the size of the infarct. Their addition into blood cardioplegia also appears to replenish the ATP pool of myocardiocytes in experimental models. Available data on the use of amino acid-enriched cardioplegia in humans may point toward improved hemodynamic recovery.[70] Postoperative glutamate infusion has also been associated with decreased lactate release, increased lactate uptake, and improved hemodynamic profiles, in patients undergoing cardiac surgery.[71]

2.8 Amino acids and inflammation

Amino acids play an integral role in the synthesis and optimal functioning of various cytokines and antibodies in the immune response. While their importance as a substrate has been

emphasized in the field of immunonutrition, very little is still known regarding the molecular mechanisms by which amino acids regulate the immune system and the inflammatory response. Each AA appear to play a specific role in immune responses. Just to name a few, alanine inhibits apoptosis and stimulates lymphocyte proliferation, arginine regulates cytokine production, and glutamate inhibits T-cell response and inflammation.[38] More specifically, there seems to be a paucity of data on how amino acid supplementation alters the cytokine production of an individual. A study involving athletes reports a lower production of TNF and IL-1 and IL-4 when supplemented with BCAA.[72, 73] A recent meta-analysis reports an improved T-cell proliferation response with L-arginine supplementation.[74] Improved thymic function, immune cells reactivity and wound healing has been described with arginine supplementation in rats.[75] The supplementation of glutamine and alanine in endotoxemic mice also attenuated the level of rise of TNF, IL-6, IL-1β, and IL-10.[76]

2.9 Rationale for perioperative nutrition

A positive protein balance in acutely ill patients can be induced with the parenteral infusion of hypercaloric amounts of energy together with amino acids.[77, 78] Although effective in conserving nitrogen, this strategy has several drawbacks.[79] High amounts of glucose can cause increased CO_2 production and respiratory distress,[80] and increased circulating norepinephrine concentrations.[81] The administration of large glucose doses will inevitably lead to hyperglycemia with its detrimental effects.[12, 82] Studies using isocaloric amounts of parenteral nutrition have demonstrated attenuated protein catabolism. However, these attempts mostly fail to achieve protein anabolism.[36, 78, 83] Although surgical pathways designed to fasten postoperative recovery are gaining in popularity, hypocaloric nutritional support remains a

mainstay strategy to feed surgical patients in North America. This strategy has the potential to provide nutritional support without exacerbating the stress-induced hyperglycemia and dyslipidemia, while also attenuating the negative nitrogen balance.[78, 84]

It should also be noted that preoperative fasting exacerbates the development of postoperative insulin resistance, as evidenced by a 26% reduction in patients who were administered a carbohydrate drink preoperatively.[14] Although the mechanism is unclear, the preoperative stimulation of insulin release appears to better preserve insulin action even after the surgery.

2.10 Nutritional requirements

In healthy humans, the estimated average requirement (EAR) and recommended dietary allowance (RDA) of protein is 0.65 and 0.83g/kg/d, respectively.[85] Under stress conditions however, this recommended amount of protein intake is not sufficient to meet the higher metabolic needs. Patients in catabolic state have protein requirements in the order of 1.5mg/kg/d.[86] Some evidence suggest protein intake as high as 2.5 to 3mg/kg/d to successfully achieve positive nitrogen balance in critically ill patients.[86, 87] In surgical patients, a protein intake of 1.7mg/kg/d was more efficient in achieving positive nitrogen balance than with 1mg/kg/d.[88]

It is well established that hyperalimentation with intravenous dextrose (approximately 36.5kcal/kg/d) has protein-sparing effect after major surgery.[89] However, hyperalimentation has its own limitations, as described previously. Based on our experience, the application of HNC on CABG patients results to an equivalent of 12-16g of glucose per hour, or 288-284g/d. If we consider that the REE of this patient population is between 1150 and 1600kcal/d, the HNC methodology provides an isocaloric amount of energy without causing hyperglycemia.

2.11 Amino acid tracer kinetics to calculate whole-body protein metabolism

The use of L-[1-^{13}C]leucine tracer is considered the reference method to obtain estimates of whole-body protein metabolism.[90] Since leucine is an essential amino acid, the transamination product of leucine, α-ketoisocaproate (KIC), has been suggested to reflect the intracellular enrichment of leucine throughout the body. When a primed continuous intravenous infusion is used, the venous α-KIC reaches a plateau after about 2h. The rate of appearance (Ra) of leucine can be calculated from the tracer dilution formula $Q = i(Ei/Ep - 1)$, where Q is the total leucine flux, i is the infusion rate of the tracer, Ei is the enrichment of the infused tracer and Ep is the enrichment of the plasma pool. During steady state, the total leucine flux Q is equal to Ra and can also be expressed as $Q = Ra = S+O = B+I$, where S is synthesis, O is oxidation, B is breakdown, and I is intake. The rate of leucine oxidation is determined by measuring breath $^{13}CO_2$ enrichment. Similarly to α-KIC, a plateau of $^{13}CO_2$ can be reached after 2h with priming of the bicarbonate pool. The total flux Q minus the intake of exogenous leucine will provide the rate of endogenous leucine release (breakdown, B). Similarly, the total flux Q minus the rate of oxidation (O) will give the rate of protein synthesis (S). Leucine oxidation is calculated by the equation: $\frac{I\,E_{CO2}\,V_{CO2}\,/\,K}{I\,E_{KIC}}$

where $I\,E_{CO2}$ is the breath CO_2 enrichment at isotope plateau, V_{CO2} is the CO_2 production rate measured by indirect calorimetry, $I\,E_{KIC}$ is the plasma KIC enrichment at isotope plateau, and K is the bicarbonate retention factor. This correction factor, traditionally set at 0.81, takes into account the incomplete recovery of labeled $^{13}CO_2$ from the bicarbonate pool.[91] More recently, Chevalier et al. have reported a correction factor of 0.67 in the postabsorptive state and 0.78 when HNC was applied. [57]

3. Hypothesis & objectives

3.1 Hypothesis

Infusion of AA at 20% of resting energy expenditure together with HNC will (1) achieve isoaminoacidemia during and after CABG surgery, (2) result in whole-body positive protein balance via a decrease in protein breakdown and an increase in protein synthesis in the immediate postoperative period, and (3) have anti-inflammatory effects.

3.2 Objectives

1. To investigate the anabolic effect of the HNC technique with a predetermined level of amino acid intake in patients undergoing CABG surgery. In order to assess the dynamic changes in protein and glucose metabolism (i.e. protein breakdown, AA oxidation, protein synthesis, glucose uptake and production) associated with such therapy, stable isotope tracers (L-[1-^{13}C]leucine, [6,6-$^{2}H_2$]glucose) will be applied. A positive protein balance (protein synthesis minus protein breakdown) will be used as an indicator of whole body anabolism.
2. To assess the metabolic and endocrine changes associated with the perioperative use of the HNC with AA intake as assessed by plasma concentrations of glucose, lactate, AA, insulin, cortisol, total protein, and albumin.
3. To study the effect of high-dose insulin with or without AA supplementation on plasma and adipose tissue inflammatory markers profiles.

4. Methods

4.1 Subject selection

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 <35g/L and body mass index <20kg/m^2), severe obesity (body mass index >35kg/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.

4.2 Metabolic interventions

Twenty patients scheduled for elective CABG surgery requiring cardiopulmonary bypass (CPB) were enrolled.

Insulin group (n=10): Patients received the HNC from the beginning of surgery until the end of the five-hour study period after surgery. No amino acids were given.

Insulin+AA group (n=10): Patients received the HNC and AA (Travasol 10%, Baxter, Deerfield IL. Table 4.1) during and after surgery in an amount equivalent to 20% of the patient's resting energy expenditure as measured before the operation.

	Travasol 10% AA profile (in grams of AA per 100g of total AA)
Isoleucine	6.00
Leucine	7.30
Valine	5.80
Lysine	5.80
Methionine	4.00
Cysteine	0.00
Phenylalanine	5.60
Tyrosine	0.40
Threonine	4.20
Tryptophan	1.80
Histidine	4.80
Arginine	11.50
Glycine	10.30
Alanine	20.70
Aspartate	0.00
Glutamate	0.00
Proline	6.80
Serine	5.00
Total EAA:Total AA	45:100

Table 4.1. Amino acid profile of Travasol 10%. All numbers are in grams of specific AA per 100g of total AA in Travasol 10% solution.

4.3 Perioperative and anesthetic care

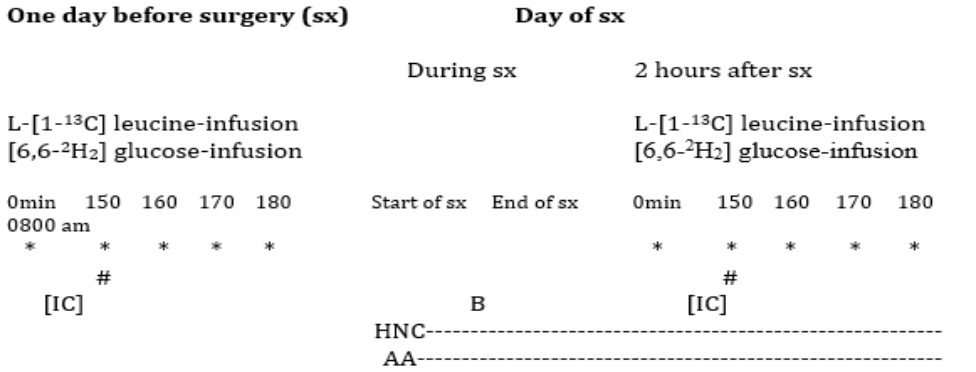
Patients were instructed to take all their cardiac medications up until the morning of operation. All patients received standard surgical and anesthetic care as established by the Departments of Anesthesia and Cardiac Surgery at the Royal Victoria Hospital. An arterial line and a central line were inserted prior to start of the operation. Heparin 400U/kg was administered intravenously with the aim to obtain an activated clotting time >500 seconds. 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, the aortic cross-clamp was removed and the patient separated from CPB. Protamine 1mg/100U of heparin was given, aortic and venous cannulas removed, hemostasis established and the pericardium and sternum closed.

4.4 Study protocol and measurements

Study protocol

All patients were fasted from midnight and arrived at the Cardiac surgery Unit on the day before surgery. The preoperative isotope measurements were performed on the day before surgery and the postoperative isotope measurements were commenced immediately after surgery.

Figure 4.1. Study protocol



* Isotopic enrichments of [1-^{13}C] ketoisocaproate and [6,6-$^{2}H_2$] glucose in plasma and expired $^{13}CO_2$
\# Hormones, metabolic substrates and plasma amino acids
[IC] Indirect calorimetry
B Adipose tissue biopsies
HNC Hyperinsulinemic-normoglycemic clamp
AA Amino acid infusion

Metabolic substrates, hormones, and inflammatory markers

Concentrations of glucose, lactate, AA, insulin, cortisol, total protein, albumin, CRP, IL-10, IL-1β, IL-6, IL-8, and TNF-α were determined at 150min of the pre- and postoperative study period.

Energy expenditure

Resting energy expenditure (EE) was determined one day before and two hours after surgery.

Hyperinsulinemic-normoglycemic clamp

Prior to the induction of anesthesia, a baseline blood glucose value was obtained. After obtaining baseline blood samples and hemodynamic measures, a 2U priming bolus of insulin (Humulin® R regular insulin, Eli Lilly and Company, Indianapolis, IN) was given followed by an insulin infusion of 5mU/kg/min. Additional boluses of insulin was given if the blood glucose remained >6.0mmol/L with incremental 2U of insulin for each 2mmol/L increase in blood glucose. Ten

minutes after commencing the insulin infusion and when the blood glucose was <6.0mmol/L, a variable continuous infusion of glucose (dextrose 20%) supplemented with potassium (40mEq/L) and phosphate (30mmol/L) was administered to preserve the blood glucose between 3.5 and 6.0mmol/L. The glucose infusion was started at 60mL/h. Insulin infusion was continued until the end of the study period approximately 5 hours after surgery. Arterial blood glucose was measured every 15min throughout the procedure using the Accu-chek® glucose monitor (Roche Diagnostics, Switzerland) and every 20-30min in the intensive care unit (ICU). We monitored glucose closely for three hours from the time the study was terminated.

Isotope studies

All isotopes were purchased from Cambridge Isotope Laboratories (Woburn, MA). Sterile solutions were prepared by Galenova pharmacy (St-Hyacnthe, QC) and tested to ensure sterility by the hospital department of microbiology (Royal Victoria Hospital, Montreal, QC) and absence from pyrogens by Limulus amoebocyte lysate test by Nucro-Technics Inc. (Scarborough, ON). On the day before surgery a cannula was inserted in a forearm vein for the infusion of isotopes and a second one into a dorsal vein in the contralateral hand to be used for blood sampling. On the day of surgery, all blood samples were collected from the arterial line, while the central venous line was used for the infusion of isotopes. Before beginning each stable isotope infusion, blood and expired air samples were collected to determine baseline enrichments of [1-^{13}C]α-ketoisocaproate ([1-^{13}C]α-KIC), [6,6-$^{2}H_2$]glucose, and expired $^{13}CO_2$.

Whole body leucine and glucose kinetics

Whole body kinetics was determined one day before and immediately after surgery. Priming of the bicarbonate pool was done by the oral administration of $NaH^{13}CO_3$ (0.1mg/kg). Glucose and leucine kinetics were determined by primed continuous infusions of L-[1-^{13}C]leucine and [6,6-$^{2}H_2$]glucose. Priming doses of L-[1-^{13}C]leucine (3.78μmol/kg, iv), and [6,6-$^{2}H_2$]glucose (18.68μmol/kg, iv) were administered followed by three-hour infusions of L-[1-^{13}C]leucine (0.063μmol/kg/min) and [6,6-$^{2}H_2$]glucose (0.22μmol/kg/min). Blood and expired breath samples were taken at 150, 160, 170 and 180min to determine metabolites enrichment.

Background [^{13}C] enrichment of plasma KIC and expired CO_2 validation studies

The dextrose solution infused in the clamp is derived of corn, which has a high [^{13}C] natural abundance. Infusion of large amounts of this solution will consequently increase [^{13}C] enrichment in expired CO_2. In order to correct for the unaccounted iatrogenic administration of ^{13}C, we conducted validation studies in five additional patients. Two (n=2) and three (n=3) additional patients undergoing CABG surgery, were studied under exactly the same conditions as in the insulin group protocol and the insulin+AA group protocol described above, respectively, with the exception that none of them received tracers. Frequent measurements of plasma glucose levels in these patients ensured that we achieved the same degree of normoglycemia as in the two study groups.

Biopsies

Biopsy specimens were obtained from mediastinal adipose tissue prior to chest closure at the end of surgery. The specimens were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

4.5 Analytical methods

Metabolic substrates, hormones and inflammatory markers

The combination of 0-phthalaldehyde and 9-fluorenylmethyl chloroformate allows the derivatization of primary and secondary AA, respectively. The concentrations of the following 20 plasma AA were analyzed by high-performance liquid chromatography with post-column fluorescence derivatization: isoleucine, leucine, valine, lysine, methionine, phenylalanine, threonine, tryptophan, alanine, arginine, asparagine, citrulline, glutamate, glutamine, glycine, histidine, ornithine, proline, serine, and tyrosine. Plasma samples were deproteinized by the addition of 300µl of methanol to 100µl of plasma followed by filtration with 0.2µm filters. All samples were analyzed in duplicate using an Agilent 1290 UHPLC (Agilent Technologies, Mississauga, Canada) on an Agilent Poroshell 120 EC-C18 4.6 x 150mm 2.7µm column. The AA were detected after precolumn derivatization with o-phthalaldehyde (OPA) and 9-fluorenyl-methyl chloroformate (FMOC). Separation was carried out at a flow rate of 1.5ml/min under gradient conditions. Mobile *phase A* was 10mM Na_2HPO_4, 10mM $Na_2B_4O_7$, pH 8.2, and mobile *phase B* was acetonitrile:methanol:water (45:45:10). Initial gradient was 2% *B* for 0.5min, then 2% to 57% *B* in 20min followed by 5min reequilibration before the next injection. Primary and secondary AA were measured by fluorescence with excitation and emission wavelengths of 230nm and 450nm, respectively. Data from standards and samples were analyzed using

MassHunter B.05 software. Circulating concentrations of insulin was measured by electrochemiluminescence immunoassay "ECLIA" (Roche Modular Analytics E170), glucose was measured using spectrophotometry (Advia 1800, Siemens), lactate was measured using colorimetric assay (Modular Analytics P Module Analyzer, Roche/Hitachi), cortisol was measured using immuno-chemiluminescence (Architect i2000, Abbott), and total protein and albumin were measured by spectrophotometry (Advia 188, Siemens). Human IL-1β, IL-6, IL-8, IL-10, and TNF-α cytokines were measured by suspension bead array immunoassay using a Luminex 200X-map instrument (Luminex Corp, Austin, TX, USA). The cytokine was measured using a Milliplex human cytokine kit following manufacturer's specifications (HCYTOMAG-60k, Millipore Corp, Bilerica, MA, USA). All samples were measured in duplicate and the kit had a sensitivity of 0.4pg/ml. Serial dilutions were made of a reconstituted human cytokine standard to produce a standard curve from 3.2 to 10,000pg/ml. The standards were mixed 1:1 with 25µl of serum matrix and added to the microtiter plate. The serum samples were mixed 1:1 with 25µl of assay buffer and transferred to the appropriate wells of the plate. After sonication, 25µl of diluted antibody coated beads were added to all standard, blank or sample wells. The plate was sealed and agitated on a plate shaker (Barnstead Int, Dubuque, IO, USA) for 16 hours at 4°C. Fluid was aspirated and then the plate was washed two times with 200µl of wash buffer. Following the wash, 25µl of detection antibody was added to all wells. The plate was again sealed and agitated at room temperature for 1 hour. Finally, 25µl of Streptavidin-Phycoerythrin was added and then incubated for an additional 30min with agitation. The fluid was then removed and the plate was washed two more times with wash buffer. The beads were resuspended in sheath fluid and agitated for 5min. The cytokines were analyzed on the Luminex instrument using MasterPlex CT 1.2 software (MiraiBio Inc, Alameda, CA, USA). Mean

fluorescence intensity was obtained from a minimum of 50 beads per sample. Concentrations were calculated from the standard curve generated by the MasterPlex QT 4.0 analysis software (MiraiBio Inc, Alameda, CA, USA).

Energy expenditure

Oxygen consumption (VO_2), carbon dioxide production (VCO_2) and EE were all measured and derived using the indirect calorimeter (Cosmed, Rome, Italy). The subjects were kept lying in a semi-recumbent position and breathing room air in the ventilated hood for 30min.

Whole body leucine and glucose kinetics

Plasma [1-^{13}C] KIC was derivatized into its pentafluorobenzyl ester and then analyzed by negative chemical ionization gas chromatography mass spectrometry (GC/MS) at m/z 129 and 130 to determine isotopic enrichment (Agilent 7890A/6975C, Agilent, Palo Alto, CA).[92] Isotopic enrichment of plasma [6,6-$^{2}H_2$]glucose was esterified to glucose penta-acetate. It was then analyzed using GC/MS electron-impact ionization by monitoring m/z 200 and 202. Isotopic enrichments were calculated as molecules percent excess (MPE). Expired $^{13}CO_2$ enrichment was analyzed by isotope ratio-mass spectrometry (IRMS Analytical Precision AP 2003, Manchester, UK).[93]

Detection of Adipose Tissue mRNA Levels

Adipose tissue biopsies were stored at -80°C until preparation of RNA for quantitative real-time PCR assays. Adipose tissue (50-100µg) was mechanically disrupted in RNA Later using a Brickman homogenizer. RNA was then purified using Quiagen RNA preparation kit as per the

manufacturer's protocol. cDNA was generated by reverse transcription from 2µg RNA (Superscript II, Invitrogen). Real-time PCR was performed on 2µl cDNA using Power Sybr Green Universal PCR master mix (ABI), as per the manufacturer's instructions. The following sequences were used for forward (F) and reverse (R) real-time PCR primers: IL-6 (F) 5'-AGGACTGCAAAATGAATGGG-3', (R) 5'-GGGTGCAGTTTGTTTCCACT-3'; TNF-α (F) 5'-CATTTATGGGACAAATGGGC-3', (R) 5'-CCGTCCTTTGAATTTCTCCA-3'; CD68 (F) 5'-GACACGCTGTCCTTTCCCTA-3', (R) 5'-GTCTGACACGCAGCAAAGTC-3'; GAPDH (F) 5'-CTTGTGTTGAATCCCGAACC-3', (R) 5'-AGCTCGAACCACTGTGACATC-3'. PCR reactions were carried out for 45 cycles (ABI 7500 Real Time PCR System). Results are expressed as fold induction in mRNA levels (± SEM) as calculated by the ΔΔCt method.[94]

Quantification of Adipose Tissue Protein Levels

Adipose tissue biopsies were stored at -80°C prior to protein extraction. Adipose tissues were mechanically disrupted using a Brickman mechanical homogenizer in homogenization buffer (20mM Tris pH 8.0, 0.5% Nonidet P-40, 1mM phenylmethanesulphonylfluoride, 50mM sodium fluoride, 1µg/ml aprotinin, 1µg/ml leupeptin, 100µM sodium orthovanadate). Homogenates were cleared by centrifugation at 16,000xG for 30min at 4°C. Supernatants were assayed for protein content by Bradford assay. Equal amounts of protein were assayed for IL-6, TNF-α, or CD68 by ELISA as per the manufacturers' recommendations (R&D Systems).

4.6 Statistics

Statistical analysis

Patients' characteristics and perioperative data were compared using the unpaired Student's *t*-test, or $\chi 2$ test where appropriate. Blood glucose levels and hemodynamic variables at different time points between the two groups were compared using repeated measures ANOVA. Whole-body leucine and glucose kinetics, gaseous exchange, glucose uptake and plasma concentrations of hormones and substrates between the two groups were analyzed using repeated measures ANOVA. The assumption of sphericity was tested with Mauchly's Sphericity test where appropriate. All data are presented as mean ± standard deviation (SD) unless otherwise specified. Statistical significance was set as $P<0.05$. All *P* values presented are 2-tailed. All statistical analyses were performed using JMP®11 (SAS institute inc.).

Calculation of sample size

Sample size calculation for the study was based on the hypothesis that augmenting the amino acid intake during the HNC would result in an increased postoperative leucine balance. A mean leucine balance difference of at least 10µmol/kg/h between the groups (insulin group : -5µmol/kg/h, insulin+AA group : +5µmol/kg/h) and a SD of 5 µmol/kg/h was assumed. To detect this difference with a type I error of 5% and a power of at least 90%, we determined that six patients in each group were required.

5. Results

5.1 Study population

A total of twenty-three patients were enrolled in the study. Ten patients were scheduled to receive the HNC protocol (insulin group) while ten were scheduled to receive the HNC and AA protocol (insulin+AA group). The perioperative and surgical characteristics of the two study groups are shown in Tables 5.1 and 5.2. Patient demographics including age, weight, height, and body mass index (BMI) were comparable between the two groups. There were three documented diabetics in the insulin group and five in the insulin+AA group (P=0.650). Preoperative left ventricular ejection fraction (LVEF) as assessed by preoperative angiography was similar between the two groups as well (P=0.175). There were no significant differences in the preoperative use of β-blockers, calcium channel blockers, or renin-angiotensin inhibitors. Surgical data such as length of surgery, graft number, blood loss and intraoperative transfusions were comparable between the two groups as well.

5.2 Inotropic support and hemodynamic variables

Hemodynamics of all study patients were recorded at four time points after the induction of anesthesia (Figure 5.1). Baseline hemodynamics were recorded after the induction of anesthesia and the floating of the pulmonary artery catheter. Another set of hemodynamics was collected at the end of surgery, once the sternum was wired and closed. Two other sets of hemodynamics were sampled at 2h and 5h after transfer to the ICU. Overall, the two groups had similar hemodynamics throughout the study period. Systolic pulmonary artery pressures, diastolic pulmonary artery pressures, central venous pressures, cardiac output, and cardiac index demonstrated a significant difference in time, in both groups (P<0.01).

When needed, patients were started on inotropic and vasopressor therapy (dobutamine 2.5µg/kg/min or norepinephrine 1-10µg/min) intraoperatively to maintain the systolic blood pressure above 100 mmHg and/or a CI above 2.0 L/min/m^2 (Table 5.2). There were no significant difference in dobutamine or norepinephrine usage between the two groups (*P*=0.474 and *P*=1.0, respectively).

5.3 Blood glucose and high-dose insulin therapy

Both groups had similar blood glucose values at baseline and throughout the study period (Figure 5.8). The application of the HNC in all study patients led to a comparable blood glucose levels between the 2 groups, at all time points and without a noticeable trend. Blood glucose was successfully maintained between 4.0 and 6.0mmol/L with the HNC in all patients. Furthermore while the total administered dose of insulin was similar between the two groups (insulin group: 219 ± 44U *vs* insulin+AA group: 225 ± 37U ; *P*=0.745), the insulin group required a higher infusion rate of 20% dextrose solution through the study period (insulin group: 95.7 ± 31.4ml/h vs insulin+AA group: 66.7 ± 12.7ml/h, *P*=0.01), and consequently had a higher glucose uptake compared to the insulin+AA patients (Figure 5.9).

5.4 Background [^{13}C] enrichment of plasma KIC and expired CO_2 validation studies

Two (n=2) and three (n=3) patients were additionally studied under exactly the same conditions as in the insulin and insulin+AA group, respectively, with the exception that none received tracers. The data and results of these validation studies are found in Table 5.4, Figures 5.5 and 5.6. The difference in protocol did not affect the background Delta^{13}C among the controls (insulin+AA protocol: -17.726 Delta^{13}C vs insulin protocol: -18.119 Delta^{13}C, *P*=0.36).

According to the plateau achieved, the average correction in Delta^{13}C needed for the insulin group is of 1.996, and that for the insulin+AA group is of 1.914. All subsequent results were determined after the application of these correction factors. Furthermore, the relationship between dextrose infusion rate and the background [^{13}C] enrichment (as mole percent excess, MPE) was determined to be linear, $y = 0.0914x + 0.0005$ (Figure 5.7, r^2=0.724).

5.5 Leucine and glucose kinetics

Steady states were successfully achieved for L-[1-^{13}C]leucine, [6,6-$^{2}H_2$]glucose and expired $^{13}CO_2$ in all patients in both groups (Coefficient of variation, CV <5%) (Figure 5.2-4). Leucine and glucose kinetics during steady state are summarized in Table 5.5. Both groups had similar baseline leucine and glucose kinetics. Not only did the insulin group have a lower leucine Ra (protein breakdown) after the surgery, it also had a lower level of protein synthesis and hence remained in a negative leucine balance at all time. Conversely, the insulin+AA group was able to maintain a sustained level of protein synthesis postoperatively. This group was able to virtually attain a neutral leucine balance despite the major surgery (P<0.001). Rate of leucine oxidation remained relatively constant between the groups and throughout the perioperative period. Leucine oxidation and leucine balance values differ depending on whether correction for background [^{13}C] enrichment is applied or not (Table 5.6).

Glucose Ra had a similar increase after surgery in both groups, although endogenous glucose production was suppressed in both groups (P<0.001).

5.6 Plasma AA concentrations

Preoperative and postoperative plasma AA concentrations are summarized in Table 5.9. Baseline plasma EAA and NEAA concentrations were all similar between the two groups except for citrulline. The insulin group had a significant decrease in all nine (histidine, threonine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine) EAA and ten (asparagine, serine, glutamine, glycine, citrulline, arginine, alanine, tyrosine, ornithine, proline) of the NEAA from baseline to ICU. The insulin+AA group, however, showed a significant decrease only in five (threonine, tryptophan, isoleucine, leucine, lysine) of the EAA and six (asparagine, serine, glutamine, tyrosine, ornithine, proline) of the NEAA. While the average % changes of EAA and NEAA in the insulin group were of -51% and -49 respectively, these were attenuated to -18% and -24% in the insulin+AA group.

5.7 Gaseous exchange

Whole body VO_2, VCO_2 and the respiratory quotient (RQ) are outlined in Table 5.3. Values for VO_2 were similar between the two groups at baseline. Both groups exhibited increased VO_2 postoperatively, but this increase was greater in the insulin+AA group (P=0.019). Baseline VCO_2 was higher in the insulin+AA group. This discrepancy was magnified postoperatively (P=0.010). The RQ consistently increased in both groups postoperatively, suggesting a higher carbohydrate metabolism. There were no significant differences in RQ between the two groups.

5.8 Hormones and substrates

Concentrations of hormones, substrates, and inflammatory markers in plasma are summarized in Tables 5.7 and 5.8. Preoperative plasma lactate, insulin, and cortisol concentrations were similar

in the two groups. Each of them were increased in both groups after surgery compared to preoperative values. On the contrary, plasma total protein and albumin levels consistently decreased in both groups postoperatively, while being similar preoperatively.

All inflammatory markers with the exception of TNF-α had similar baseline values. Postoperatively, there was a significant increase in CRP, IL-10, IL-6, IL-8, and TNF-α for both groups. IL-1β was the only marker that showed significant decrease from baseline values equally in both groups. Overall, a statistically significant interaction was only noted for the IL-6 levels between the two groups. The elevation in IL-6 level appears attenuated in the insulin+AA group compared to the insulin group.

5.9 Adipose tissue inflammatory markers

Amino acid supplementation (insulin+AA group) was associated with significantly higher adipose tissue IL-6, in accordance with the intracellular transcriptional controls showing higher IL-6 mRNA expression. This was however not demonstrated for TNF-α levels, which showed a higher TNF-α mRNA expression but a similar TNF-α level compared to the insulin group (see Figure 5.10). Systemic inflammation is associated with recruitment of tissue macrophages, which may be a source of inflammatory cytokines. However, the increase in TNF-α and IL-6 mRNA in the insulin+AA group was accompanied by a decrease in the macrophage marker CD68.

Table 5.1. Patient characteristics

	Insulin group	Insulin + AA group	*P* value
n	10	10	
Age (y)	66 ± 7	60 ± 8	0.094
Gender (M/F)	10/0	9/1	0.305
Weight (kg)	78.4 ± 14.7	86.1 ± 16.4	0.331
Height (m)	1.74 ± 0.09	1.74 ± 0.10	0.953
BMI (kg/m^2)	25.7 ± 3.7	28.2 ± 3	0.153
DM	3/10	5/10	0.650
Diabetic therapy			
Oral agents	3	4	
Insulin	0	1	
HbA1c (%)	6.1 ± 0.6	6.9 ± 1.9	0.282
LVEF (%)	52 ± 13	43 ± 13	0.175
Beta blockers	5/10	8/10	0.350
Renin-angiotensin inhibitors	2/10	5/10	0.350

Values are means ± SD or no. of patients, M, male; F, female; BMI, body mass index; DM, diabetes mellitus; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction. *P* values were determined by unpaired *t*-test and χ^2 test where appropriate.

Table 5.2. Perioperative and surgical data

	Insulin group	Insulin + AA group	*P* value
Anesthesia time (min)	359 ± 21	327 ± 42	0.059
Surgical time (min)	272 ± 26	246 ± 26	0.066
CPB time (min)	137 ± 22	127 ± 16	0.278
Aortic x-clamp time (min)	117 ± 21	105 ± 16	0.182
Grafts (n)	5.1 ± 0.9	5.3 ± 0.9	0.567
EBL (ml)	575 ± 127	439 ± 262	0.184
PRBC transfusions (units)	2.1 ± 1.6	2.4 ± 1.7	0.990
Inotropic and vasopressor therapy			
Dobutamine 2.5 µg/kg/min	0	2	0.474
Norepinephrine 1-10 µg/min	8	8	1.0

Values are means ± SD or no. of patients, CPB, cardiopulmonary bypass; EBL, estimated blood loss; PRBC, packed red blood cells; *P* values were determined by unpaired *t*-test and χ^2 test where appropriate.

Table 5.3. Gaseous exchange

	Insulin group	Insulin + AA group	*P* value Time*	*P* value Group †	*P* value Interaction ‡
VO_2 (ml/min)					
Before surgery	238 ± 41	228 ± 53	0.032	0.114	0.019
After surgery	230 ± 42	311 ± 83			
VCO_2(ml/min)					
Before surgery	175 ± 32	188 ± 37	<0.001	0.008	0.010
After surgery	203 ± 28	292 ± 77			
RQ					
Before surgery	0.74 ± 0.10	0.84 ± 0.10	<0.001	0.134	0.357
After surgery	0.90 ± 0.15	0.94 ± 0.06			

Values are means ± SD. VO_2 indicates whole body oxygen consumption; VCO_2, whole body carbon dioxide production; RQ, respiratory quotient. *P* value was determined by repeated measures ANOVA. * 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 5.4. Comparison of study patient characteristics to controls

	Insulin group	Insulin + AA group	Insulin control group	Insulin + AA control group
n	10	10	2	3
Age (y)	66 ± 7	60 ± 8	67 ± 3	57 ± 15
Gender (M/F)	10/0	9/1	2/0	2/1
Weight (kg)	78.4 ± 14.7	86.1 ± 16.4	84.5 ± 9.8	94.8 ± 36
Height (m)	1.74 ± 0.09	1.74 ± 0.10	1.76 ± 0.0	1.70 ± 0.14
BMI (kg/m^2)	25.7 ± 3.7	28.2 ± 3	27.3 ± 3.2	32.9 ± 9.2
DM	3/10	5/10	0/2	2/3
Diabetic therapy				
Oral agents	3	4	0/2	2/3
Insulin	0	1	0	0
HbA1c (%)	6.1 ± 0.6	6.9 ± 1.9	6.3 ± 0.6	9.0 ± 2.6
LVEF (%)	52 ± 13	43 ± 13	50 ± 21	40 ± 10
Beta blockers	5/10	8/10	2/2	3/3
Renin-angiotensin inhibitors	2/10	5/10	0/2	1/3

Values are means ± SD or no. of patients, M, male; F, female; BMI, body mass index; DM, diabetes mellitus; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction. *P* values were determined by unpaired *t*-test and χ^2 test where appropriate.

Table 5.5. Whole-body leucine and glucose kinetics using average plateaus of validation studies as correction

	Insulin group	Insulin + AA group	*P* value Time*	*P* value Group†	*P* value Interaction‡
Ra leucine (μmol/kg/h)					
Before surgery	102.9 ± 10.4	103.4 ± 12.6	0.005	0.163	0.084
After surgery	84.6 ± 9.2	104.1 ± 16.8			
Leucine oxidation (μmol/kg/h)					
Before surgery	16.7 ± 4.0	19.9 ± 7.5	0.153	0.067	0.638
After surgery	13.6 ± 4.5	18.3 ± 5.3			
Protein breakdown (μmol/kg/h)					
Before surgery	102.9 ± 10.4	103.4 ± 12.6	<0.001	0.491	0.431
After surgery	84.6 ± 9.2	83.3 ± 13.7			
Protein synthesis (μmol/kg/h)					
Before surgery	86.8 ± 8.5	83.5 ± 9.4	0.006	0.310	0.001
After surgery	71.0 ± 9.3	85.9 ± 15.0			
Leucine balance (μmol/kg/h)					
Before surgery	- 16.0 ± 3.5	- 19.9 ± 7.5	<0.001	<0.001	<0.001
After surgery	- 13.6 ± 4.5	2.1 ± 5.4			
Ra glucose (μmol/kg/min)					
Before surgery	10.0 ± 1.6	10.8 ± 2.5	<0.001	0.199	0.165
After surgery	24.6 ± 7.2	21.0 ± 5.4			
Endogenous Ra glucose (μmol/kg/min)					
Before surgery	10.0 ± 1.7	11.1 ± 2.8	<0.001	0.193	0.230
After surgery	0.0 ± 3.8	1.6 ± 1.6			

Values are means ± SD, Ra, rate of appearance. *P* value was determined by repeated measures ANOVA. *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 5.6. Effect of applying correction from validation studies

	Insulin group	Insulin+AA group
MPE $^{13}CO_2$ Plateau (%)		
No correction	$8.47 \times 10^{-3} \pm 1.4 \times 10^{-3}$	$8.34 \times 10^{-3} \pm 1.6 \times 10^{-3}$
Correction (avg)	$6.56 \times 10^{-3} \pm 1.4 \times 10^{-3}$	$6.17 \times 10^{-3} \pm 1.6 \times 10^{-3}$
Correction (D20)	$6.22 \times 10^{-3} \pm 1.6 \times 10^{-3}$	$6.70 \times 10^{-3} \pm 1.8 \times 10^{-3}$
Leucine oxidation (μmol/kg/h)		
No correction	17.4 ± 5.0	25.1 ± 6.7
Correction (avg)	13.6 ± 4.5	18.3 ± 5.3
Correction (D20)	12.5 ± 3.3	19.9 ± 5.9
Leucine balance (μmol/kg/h)		
No correction	-17.4 ± 5.0	-4.6 ± 6.1
Correction (avg)	-13.6 ± 4.5	2.1 ± 5.4
Correction (D20)	-12.5 ± 3.3	0.6 ± 6.2

Values are means ± SD, MPE, mole percent excess; avg, correction value derived from average plateau of validation studies; D20, individualized correction value derived from equation $y = 0.0914x + 0.0005$.

Table 5.7. Plasma concentrations of hormones and substrates

	Insulin group	Insulin + AA group	*P* value Time*	*P* value Group†	*P* value Interaction‡
Lactate(mmol/L)					
Before surgery	0.7 ± 0.3	1.2 ± 0.7	<0.001	0.030	0.708
After surgery	1.3 ± 0.3	1.6 ± 0.4			
Insulin (pmol/L)					
Before surgery	44.3 ± 18.5	44.7 ± 21.8	<0.001	0.970	0.540
After surgery	>2100	>2100			
Cortisol (nmol/L)					
Before surgery	125 ± 64	227 ± 111	<0.001	0.060	0.702
After surgery	632 ± 233	729 ± 224			
Total protein (mmol/L)					
Before surgery	64.1 ± 3.9	65.8 ± 5.7	<0.001	0.319	0.342
After surgery	41.7 ± 6.5	45.0 ± 4.7			
Albumin(mmol/L)					
Before surgery	39.0 ± 2.3	40.7 ± 5.0	<0.001	0.118	0.594
After surgery	26.6 ± 3.8	28.8 ± 3.1			

Values are means ± SD. *P* value was determined by repeated measures ANOVA. *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 5.8. Plasma concentrations of inflammatory markers

	Insulin group	Insulin + AA group	*P* value Time *	*P* value Group†	*P* value Interaction‡
C-Reactive Protein (mg/L)					
Before surgery	3.2 ± 5.2	4.7 ± 5.7	<0.001	0.906	0.273
After surgery	6.0 ± 4.7	5.8 ± 4.4			
IL-10 (pg/mL)					
Before surgery	7.8 ± 8.1	4.0 ± 3.2	<0.001	0.129	0.186
After surgery	111.2 ± 104.8	57.2 ± 47.1			
IL-1β (pg/mL)					
Before surgery	2.1 ± 4.9	2.4 ± 5.1	0.023	0.281	0.290
After surgery	0.4 ± 0.7	0.5 ± 0.7			
IL-6 (pg/mL)					
Before surgery	2.1 ± 3.1	3.4 ± 1.9	<0.001	0.011	0.011
After surgery	246.6 ± 111.2	124.5 ± 79.3			
IL-8 (pg/mL)					
Before surgery	10.6 ± 3.9	9.7 ± 3.5	<0.001	0.179	0.207
After surgery	86.3 ± 65.8	53.5 ± 39.2			
TNF-α (pg/mL)					
Before surgery	15.0 ± 5.9	9.5 ± 4.2	0.001	0.007	0.153
After surgery	24.9 ± 10.6	13.8 ± 7.0			

Values are means ± SD. *P* value was determined by repeated measures ANOVA. *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 5.9. Plasma AA concentrations

	Insulin			Insulin+AA			*P* values		
	BL	ICU	%Change	BL	ICU	%Change	Time*	Group†	Interaction‡
				Plasma EAA concentrations, μmol/L					
Histidine	70 ± 11	54 ± 10	-24	73 ± 15	83 ± 19	+14	0.477	0.003	0.007
Threonine	124 ± 32	49 ± 15	-60	127 ± 36	76 ± 19	-40	<0.001	0.121	0.129
Valine	237 ± 50	154 ± 28	-35	276 ± 42	236 ± 50	-14	<0.001	<0.001	0.176
Methionine	22 ± 11	3 ± 5	-88	19 ± 6	17 ± 7	-9	<0.001	0.059	<0.001
Tryptophan	52 ± 11	29 ± 9	-44	49 ± 10	38 ± 7	-22	<0.001	0.371	0.025
Phenylalanine	80 ± 24	66 ± 15	-18	70 ± 13	77 ± 21	+10	0.366	0.919	0.022
Isoleucine	74 ± 19	7 ± 7	-91	81 ± 19	56 ± 20	-31	<0.001	<0.001	0.003
Leucine	145 ± 30	68 ± 15	-53	161 ± 28	95 ± 26	-41	<0.001	0.011	0.561
Lysine	183 ± 37	94 ± 20	-49	183 ± 30	131 ± 23	-28	<0.001	0.080	0.020
				Plasma NEAA concentrations, μmol/L					
Glutamate	71 ± 29	75 ± 29	+6	69 ± 26	73 ± 28	+6	0.043	0.088	0.728
Asparagine	38 ± 14	12 ± 8	-67	44 ± 9	9 ± 3	-80	<0.001	0.745	0.013
Serine	102 ± 18	37 ± 8	-64	113 ± 37	67 ± 18	-41	<0.001	0.035	0.085
Glutamine	736 ± 122	382 ± 107	-48	618 ± 81	398 ± 79	-36	<0.001	0.173	0.007
Glycine	187 ± 45	119 ± 32	-36	215 ± 71	248 ± 62	+16	0.098	0.003	<0.001
Citrulline	45 ± 16	18 ± 7	-61	26 ± 11	18 ± 7	-30	<0.001	0.030	0.002
Arginine	67 ± 9	30 ± 8	-55	62 ± 21	74 ± 18	+19	0.022	0.001	<0.001
Alanine	310 ± 50	169 ± 22	-45	359 ± 87	362 ± 97	+1	0.007	<0.001	0.005
Tyrosine	63 ± 11	38 ± 10	-40	70 ± 20	23 ± 8	-66	<0.001	0.403	0.012
Ornithine	75 ± 24	27 ± 10	-65	88 ± 26	55 ± 11	-38	<0.001	0.008	0.145
Proline	197 ± 45	79 ± 28	-60	252 ± 74	158 ± 40	-37	0.026	<0.001	0.389

Values are means ± SD. EAA, essential amino acids; NEAA, nonessential amino acids; BL, baseline; ICU, intensive care unit; % Decrease are calculated from the mean values (% of ICU values decrease from BL). *P* value was determined by repeated measures ANOVA. *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.

Figure 5.1. Hemodynamic variables

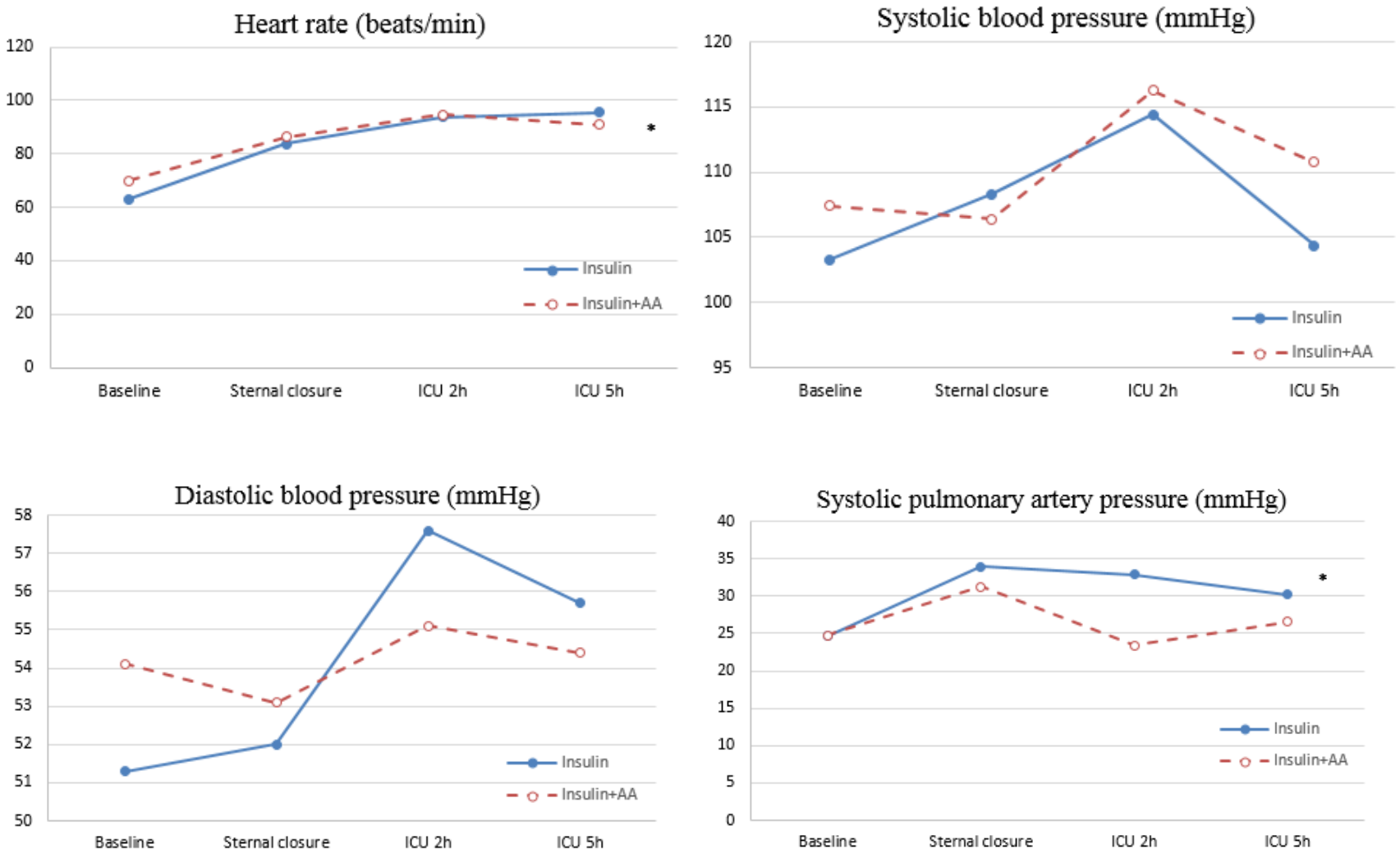
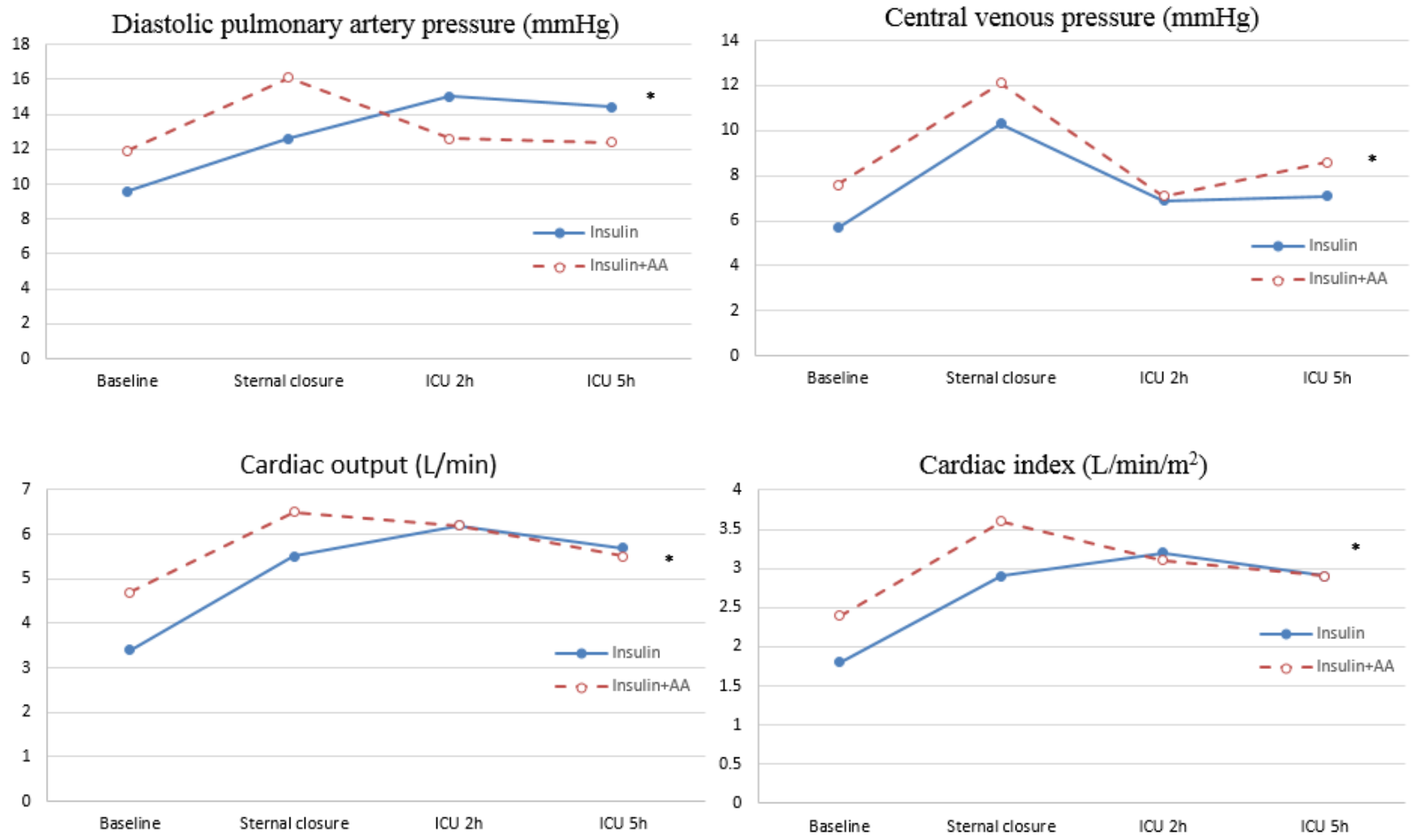


Figure 5.1. Hemodynamic variables (continued)



Values are means. ICU, intensive care unit. *P* value was determined by repeat measure ANOVA. *Probability that values change with time.

Figure 5.2. *A*: Isotopic enrichment of $^{13}CO_2$ before surgery in the insulin group. *B*: Isotopic enrichment of $^{13}CO_2$ before surgery in the insulin+AA group. *C*: Isotopic enrichment of $^{13}CO_2$ after surgery in the insulin group. *D*: Isotopic enrichment of $^{13}CO_2$ after surgery in the insulin+AA group. All values are mean ± SD.

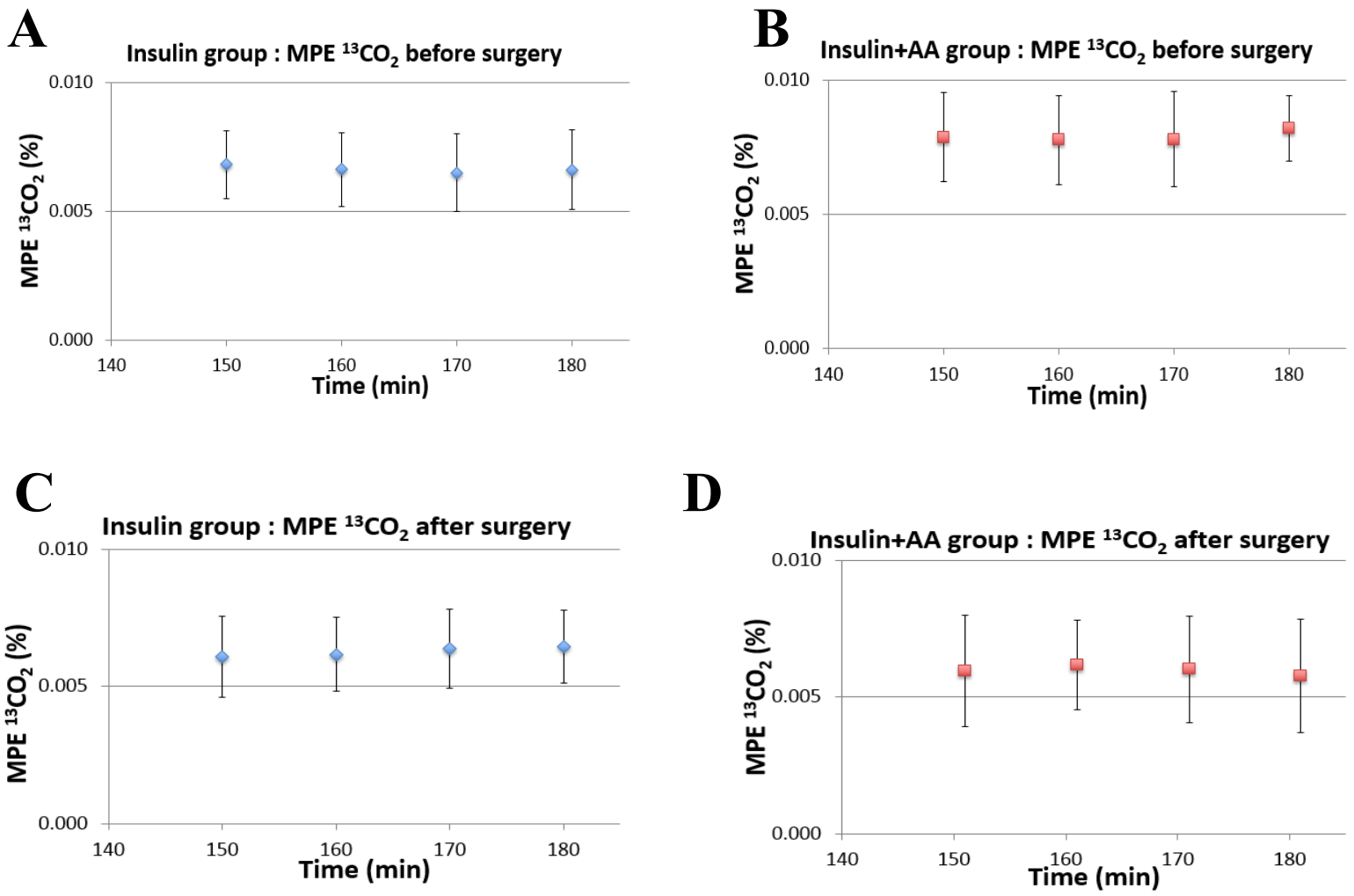


Figure 5.3. *A*: Isotopic enrichment of [1-^{13}C]α-KIC before surgery in the insulin group. *B*: Isotopic enrichment of [1-^{13}C]α-KIC before surgery in the insulin+AA group. *C*: Isotopic enrichment of [1-^{13}C]α-KIC after surgery in the insulin group. *D*: Isotopic enrichment of [1-^{13}C]α-KIC after surgery in the insulin+AA group. MPE, mole percent excess. All values are mean ± SD.

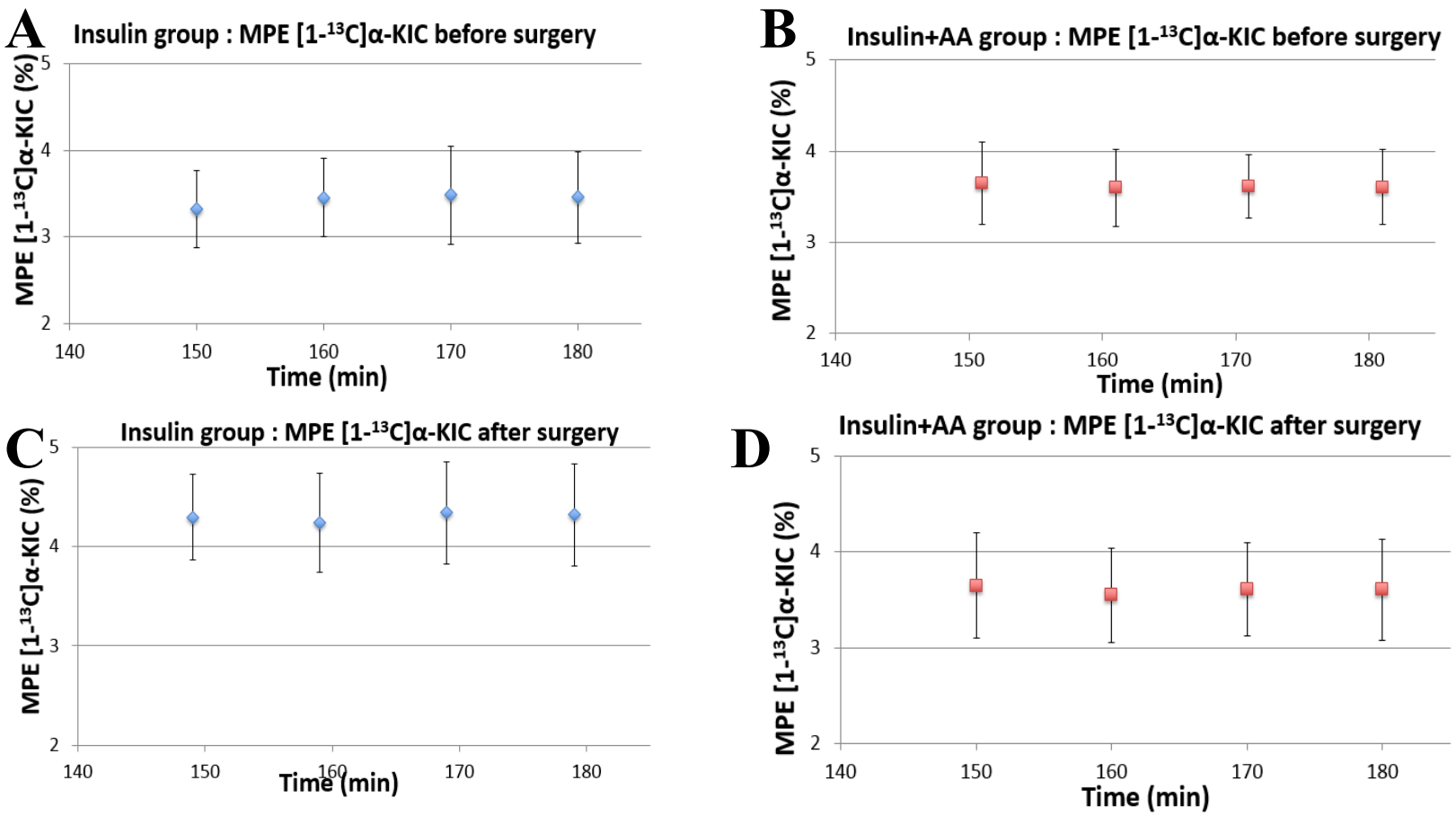


Figure 5.4. *A*: Isotopic enrichment of [6,6-$^2H_2$]glucose before surgery in the insulin group. *B*: Isotopic enrichment of [6,6-$^2H_2$]glucose before surgery in the insulin+AA group. *C*: Isotopic enrichment of [6,6-$^2H_2$]glucose after surgery in the insulin group. *D*: Isotopic enrichment of [6,6-$^2H_2$]glucose after surgery in the insulin+AA group. MPE, mole percent excess. All values are mean ± SD.

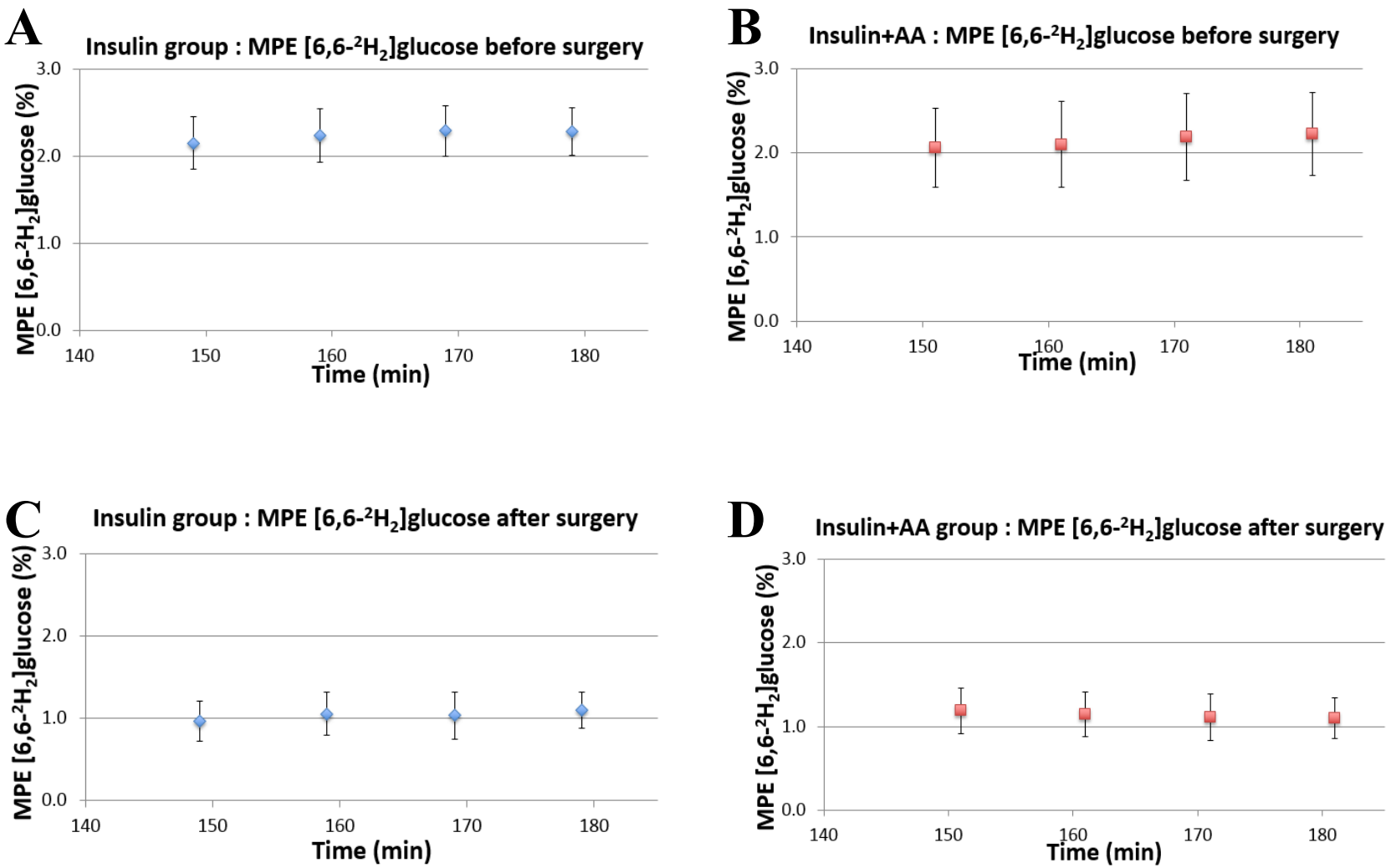


Figure 5.5. $^{13}CO_2$ isotopic enrichment with and without tracer infusion for insulin protocol. The insulin group (infused with tracer isotopes, n=10) served as a comparison to the control group (no infusion of tracer isotopes, n=2). During the plateau phase (at 150-180 min), the dextrose infusion accounted for 39% of the total ^{13}C enrichment.

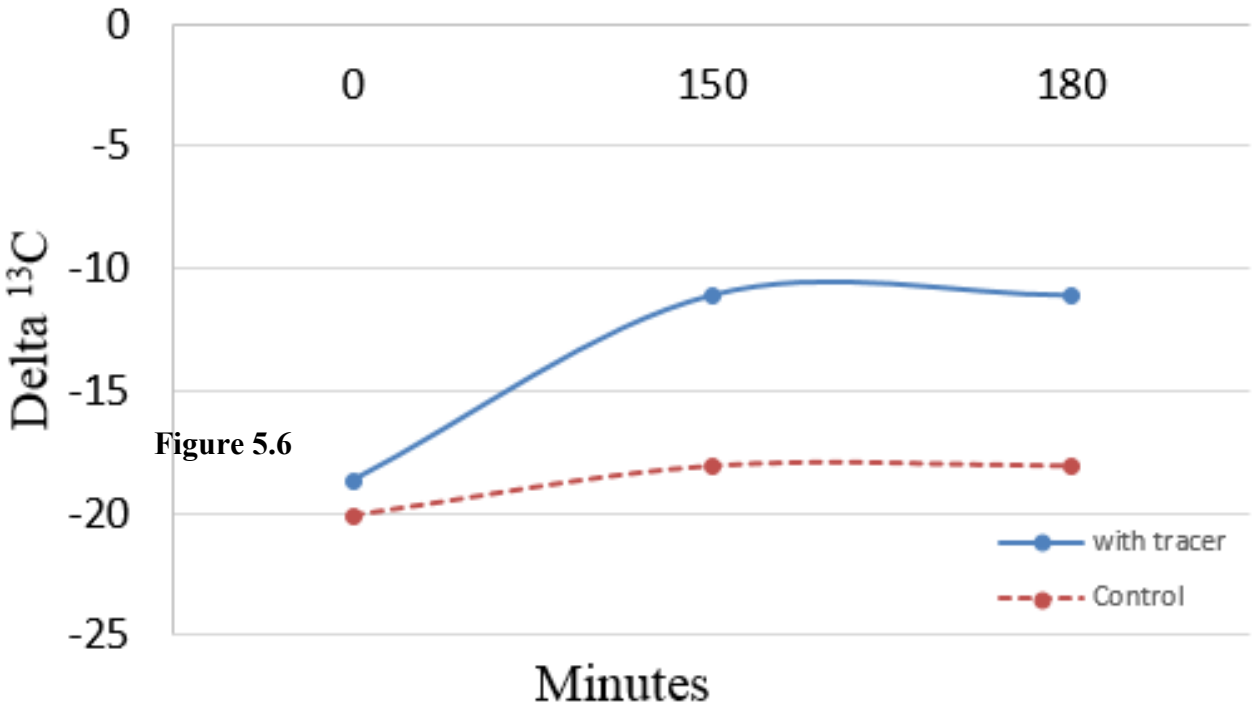


Figure 5.6

Figure 5.6. $^{13}CO_2$ isotopic enrichment with and without tracer infusion for insulin+AA protocol. The insulin+AA group (infused with tracer isotopes, n=10) served as a comparison to the control group (no infusion of tracer isotopes, n=3). During the plateau phase (at 150-180 min), the dextrose infusion alone accounted for 34% of the total ^{13}C enrichment.

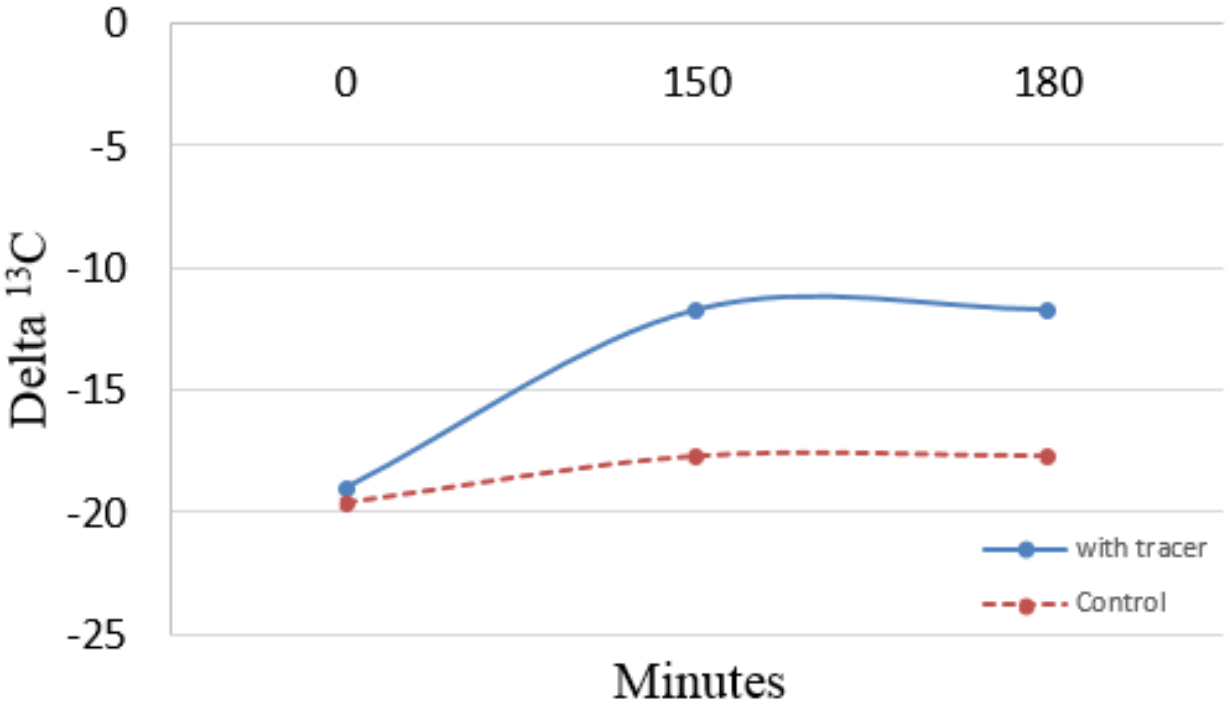


Figure 5.7. Relationship between MPE $^{13}CO_2$ and dextrose 20% infusion rate in controls. A linear relationship (expressed as $y = 0.0914x + 0.0005$, $r^2=0.724$) can help predict and individualize the MPE $^{13}CO_2$ from the rate of the dextrose infusion required for each subject.

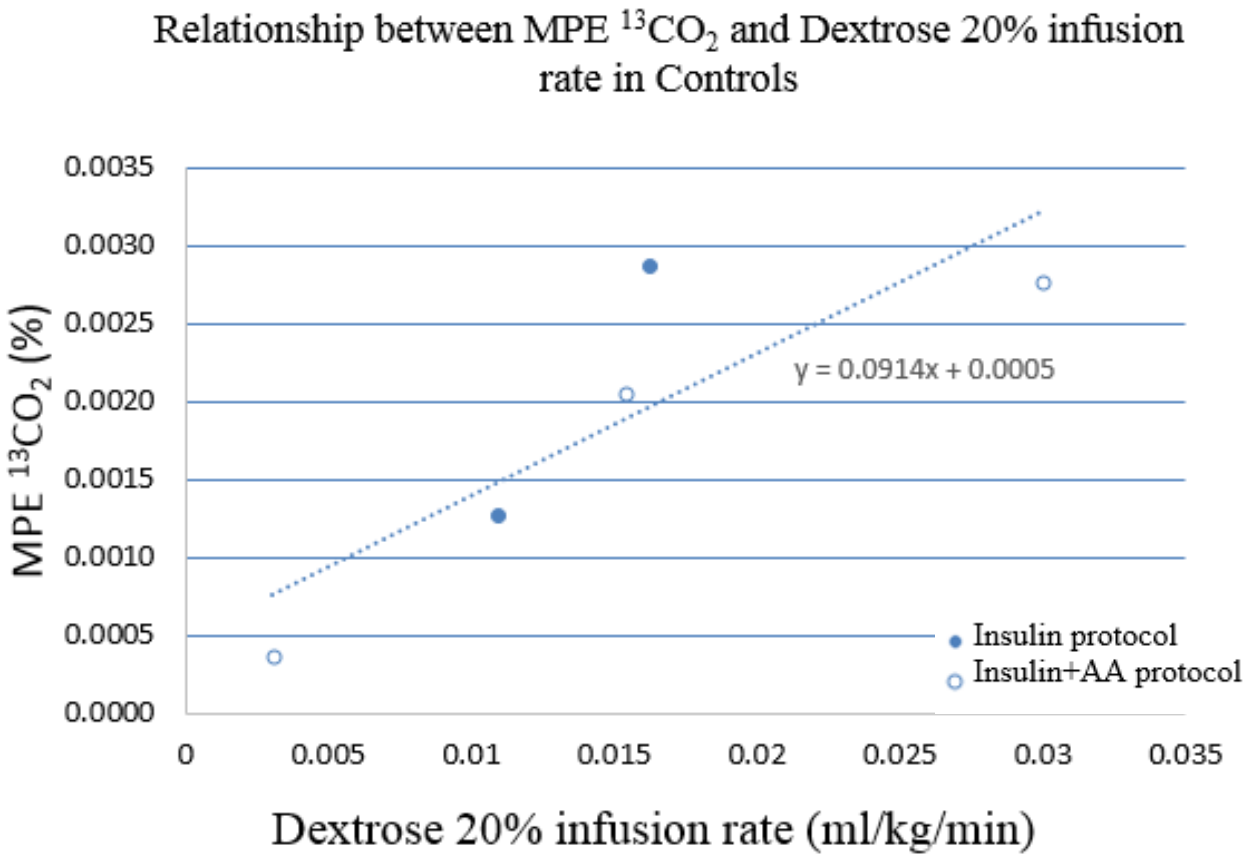


Figure 5.8. Perioperative blood glucose concentrations. Values are means ± SD, CPB, cardiopulmonary bypass; ICU, intensive care unit; *P* value was determined by repeated measures ANOVA. No statistical significance in group, time, or interaction.

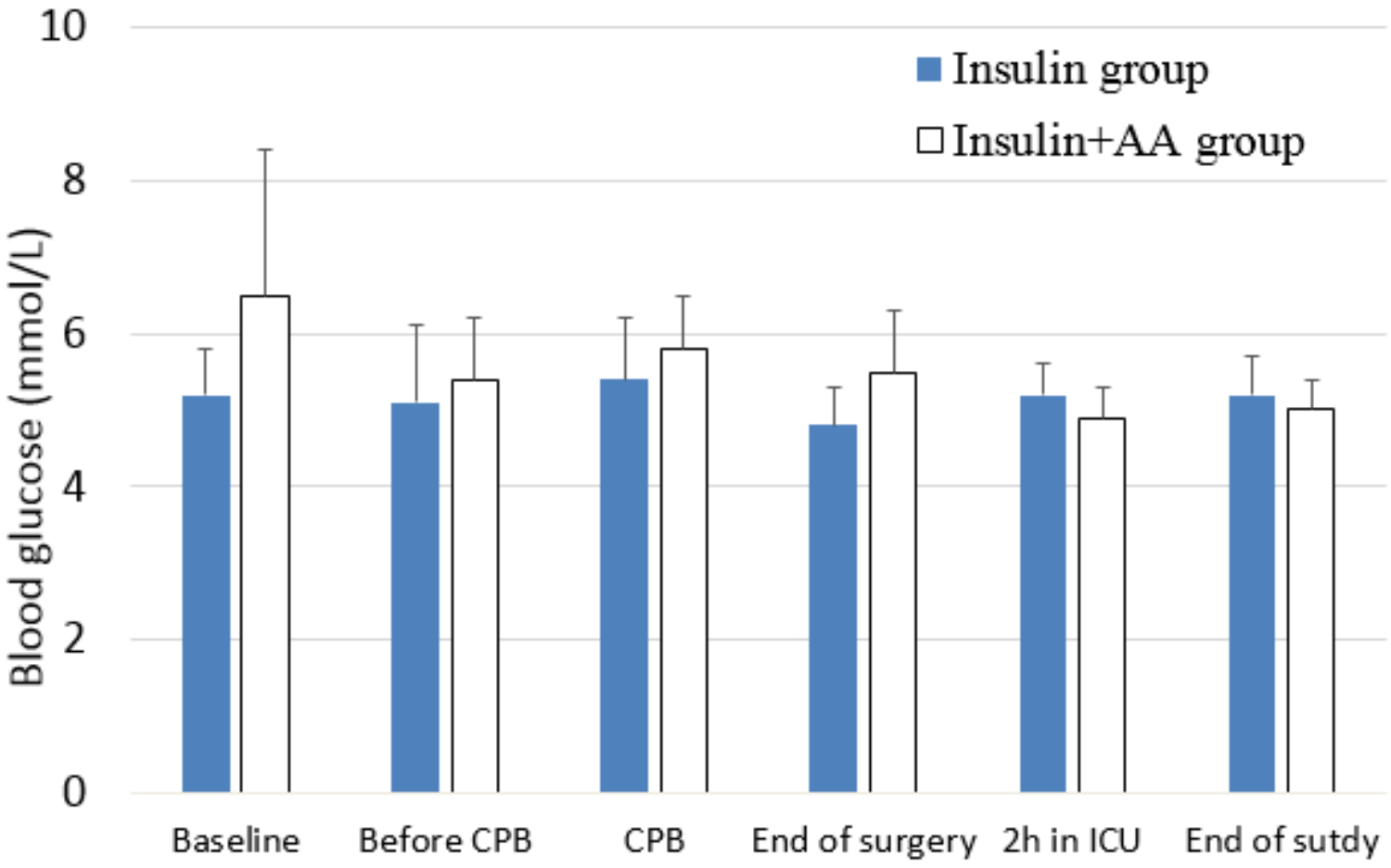


Figure 5.9. Perioperative glucose uptake. Values are in means ± SD, CPB, cardiopulmonary bypass; ICU, intensive care unit. *P* value was determined by repeated measures ANOVA. **P* < 0.05 that the values are different between groups only (not significant in time or interaction).

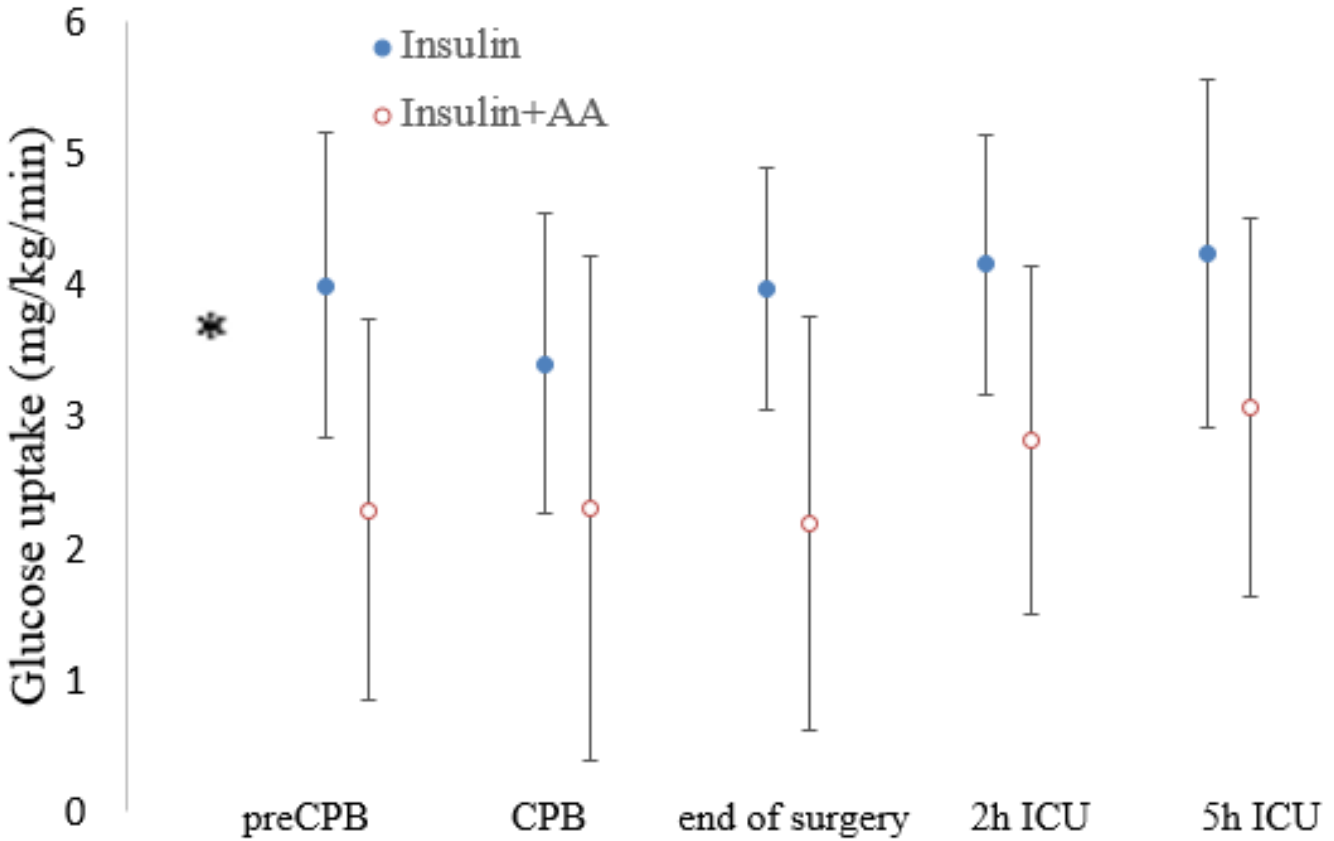
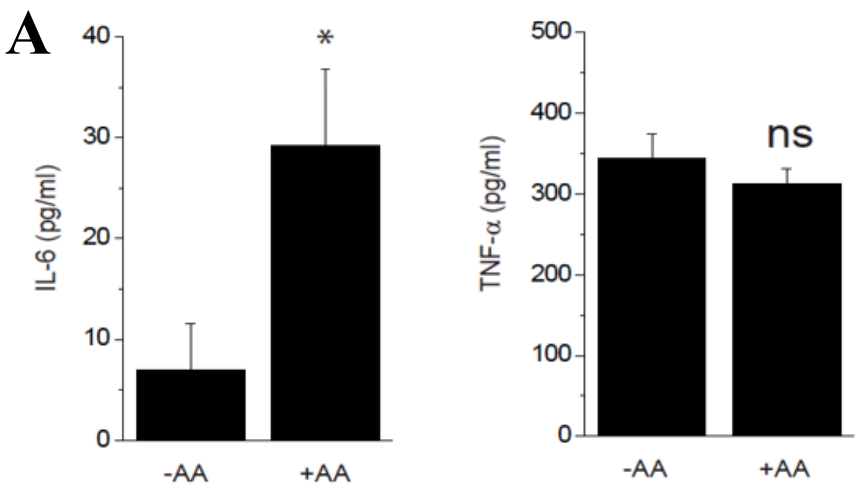
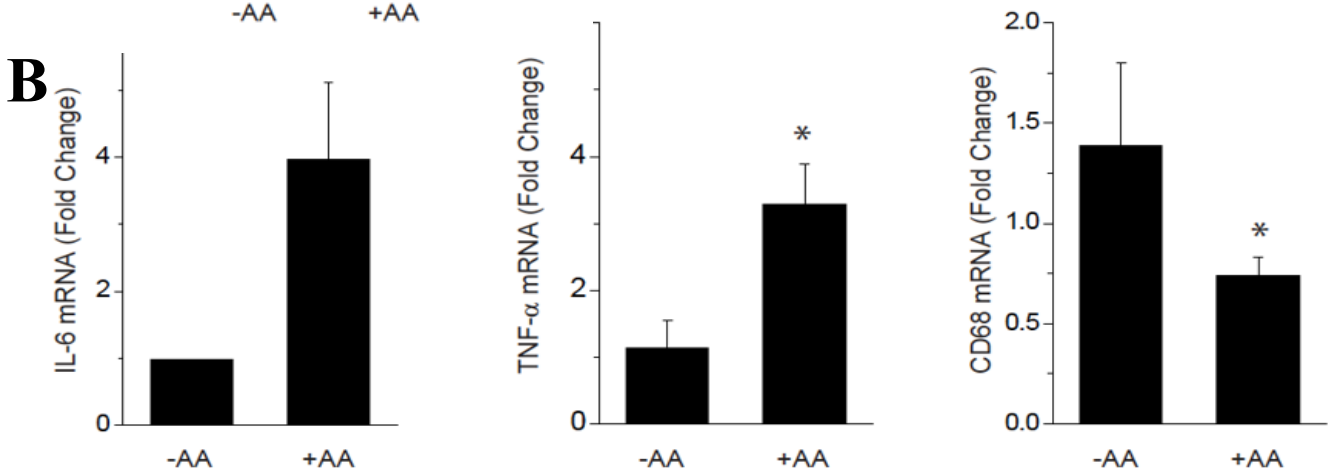


Figure 5.10. Inflammatory profiles of adipose tissue



A: Effect of AA on adipose tissue IL-6 and TNF-α protein levels in the insulin (-AA) or insulin+AA (+AA) group. Results are the means of IL-6 or TNF-α protein levels ± SD in insulin group (n=7) or insulin+AA group (n=13). Samples from each patient were measured in triplicate. * P <0.05 +AA *vs.* –AA; ns, not significant.



B: Effect of AA on adipose tissue IL-6 and TNF-α mRNA levels in the insulin (-AA) or insulin+AA (+AA) group. Results are the means of IL-6, TNF-α, or CD68 fold change in mRNA levels ± SD in insulin group (n=7) or insulin+AA group (n=13). Samples from each patient were measured in triplicate. * P <0.05 +AA vs. –AA.

6. Discussion

The results of this study demonstrates 1) high-dose insulin therapy combined with the infusion of amino acid equivalent to 20% of the subject's resting EE was insufficient to maintain isoaminoacidemia in cardiac surgery, 2) perioperative HNC with AA supplementation suppresses protein breakdown while stimulating synthesis, resulting virtually in a neutral protein balance, 3) HNC with AA supplementation exhibits anti-inflammatory properties in plasma, while exhibiting pro-inflammatory properties in the adipose tissue.

The infusion of Travasol 10% at an amount equivalent to 20% of the patient's resting EE resulted in a rate averaging 0.006ml/kg/min (0.6mg/kg/min) in our patients. This rate is similar to rates previously used to maintain stable postabsorptive plasma AA concentrations in humans.[57] Although we were able to attenuate the decrease in circulating EAAs and NEAAs compared to the insulin group, a higher AA infusion rate appears to be necessary to maintain baseline concentrations of most of the measured amino acids (See Table 5.9).

Since the dextrose solution we used is derived from corn (with a higher natural [^{13}C] occurrence), its [^{13}C] enrichment can potentially contaminate the endogenous ^{13}C pool derived from oxidation of metabolites from a mixed diet. This would lead to an overestimation of leucine oxidation, and inversely, an underestimation of protein synthesis. Hence, performing background [^{13}C] enrichment study without tracers to determine the MPE of $^{13}CO_2$ due to the dextrose 20% infusion is of critical importance (Figure 5.5 and 5.6). The consequence of not accounting for this source of contamination results in a significantly different set of results (Table 5.6). Furthermore, assuming that the source of ^{13}C contamination is mainly due to the dextrose 20% solution (and not from the AA solution), we hypothesized that the degree of ^{13}C contamination in each subject directly correlates to the rate of dextrose 20% infusion needed to maintain the clamp

(Figure 5.7). Hence we propose a more accurate and individualized method to predict ^{13}C enrichment caused by the dextrose infusion, a relationship defined by:

$$MPE\ ^{13}CO_2\ (\%) = 0.0914*[\text{dextrose 20\% infusion rate (ml/kg/min)}]+0.0005$$

The effects of such an individualized correction is presented for comparison in Table 5.6.

One of the other determinants of leucine oxidation calculation is the [^{13}C] bicarbonate recovery factor. This recovery factor has been estimated in previous studies. While Hoerr et al. obtained a recovery factor of 0.70 and 0.82 for postabsorptive and fed states respectively,[95] Chevalier et al. obtained 0.67 and 0.78 for postabsorptive and clamp states respectively.[57] In our study, Chevalier et al.'s recovery factors were used, since they likely represent closest the conditions in which patients were studied. The precise bicarbonate recovery factor for a patient undergoing CABG surgery, with HNC, and with or without AA remains to be determined.

The effect of high-dose insulin supplemented with AA infusion has been well described in healthy volunteers. Hyperinsulinemia has been shown to consistently lead to hypoaminoacidemia, particularly the BCAAs, via the suppression of protein breakdown. Chevalier et al. have reported that the infusion of 40mU/m^2·min insulin in healthy volunteers, while maintaining postabsorptive concentrations of plasma AA, resulted in unchanged protein oxidation, 18% decrease in protein breakdown and a 142% increase in protein balance.[57] Until now, the impact of such therapy in cardiac surgery patients had not been elucidated. Our current study results are in concordance with these previous findings, with a comparable 19% decrease in protein breakdown and a 110% increase in protein balance even after a major surgery. Another study, performed almost two decades ago by Castellino et al., had also documented the anabolic response to insulin clamp and AA infusions to maintain isoaminoacidemia in healthy volunteers.[96] The authors describe leucine kinetics in four different study arms. Among these,

two have particular relevance to our current study; the 1) "insulin clamp only" group (*vs* the insulin group in our study), and the 2) "insulin clamp with isoaminoacidemia" group (*vs* the insulin+AA group in our study). The similarities between Castellino et al.'s results and ours are striking; Castellino et al. report decreased leucine rate of appearance, leucine oxidation, and protein synthesis in the context of "insulin clamp only," and we conclude the same. As for "insulin clamp and isoaminoacidemia," the leucine rate of appearance and protein synthesis remain unchanged, and protein breakdown decreased, all which are again in concordance with the results from our study. The only discrepancy is the unchanged leucine oxidation in our insulin+AA group. Although leucine oxidation is typically increased in the context of iso-/hyperaminoacidemia with and without insulin clamp, we fail to observe this trend in our results.[60, 96, 97] It should be also noted that Castellino et al. were able to better achieve an isoaminoacidemic state, with a Travasol 10% infusion rate set at 0.011ml/kg/min (*vs* an average of 0.006ml/kg/min in our study).

Some studies have investigated the potential benefits of AA on the myocardium. Amino acids may play an important protective role in the ischemic myocardium, through the replenishment of the Krebs cycle intermediates and enhancing myocardial anaerobic metabolism. More specifically, glutamate and aspartate have been described to reduce myocardial infarct size,[98] increase ischemic tolerance in patients known to have coronary artery disease,[99] and increase lactate clearance[100] in animals and medical patients. Our study was not designed to analyze the effect of AA infusion on the hemodynamics and/or cardiac function of cardiac surgery patients, or even outcome. Hence, our data cannot support nor reject the possibility of a cardioprotective effect of AA infusion.

While glycogenolysis and gluconeogenesis typically increases following major surgery due to the activation of the stress response, the administration of HNC has consistently shown to suppress endogenous glucose production even after cardiac surgery and CPB.[9] Both our groups had similar baseline and postoperative endogenous glucose production, with a significant reduction after the surgery (Table 5.5). An interesting finding is the lower glucose uptake in the insulin+AA group, which suggests higher insulin resistance (Figure 5.9). This difference in insulin resistance was present prior to the onset of CPB, and the trend persisted throughout the study period. This may be caused by a difference in the baseline insulin resistance between the two groups that we failed to recognize. However, given that both groups had a similar number of diabetics, similar preoperative HbA1c levels, similar HOMA-IR scores (insulin group: 1.5 ± 0.7 vs insulin+AA group: 1.5 ± 0.7, P=1.0) and nonsignificant difference in baseline blood glucose (insulin group: 5.2 ± 0.6mmol/L vs insulin+AA group: 6.5 ± 1.9mmol/L, P>0.05), the possibility of the AA infusion inducing insulin resistance must be contemplated. Indeed, the development of insulin resistance has been associated with higher circulating AA concentrations in medical patients.[101] The molecular mechanism by which AA impairs insulin action is not fully elucidated. Current understanding implicates the overactivation of the mTor/S6 kinase pathways and inactivation of the PI-3 kinase, which leads to impaired insulin-mediated suppression of glucose production and glucose disposal in skeletal muscles.[102-104] Furthermore, a recent study by Robinson et al. found that healthy volunteers undergoing AA infusion together with insulin clamping had lower exogenous glucose requirements to maintain normoglycemia.[105] It is very well known that insulin resistance and hyperglycemia increases morbidity and mortality in medical and surgical patients.[17] In fact, some evidence suggest that the degree of insulin resistance, rather than the diagnosis of diabetes per se, is associated with worsened cardiac

function and higher rates of complications after surgery.[106, 107] Hence, if AA supplementation is indeed responsible for worsening perioperative insulin resistance in patients, concerns regarding its use naturally arises. Although none of our subjects who received AA infusion had any postoperative complication to our knowledge, the potential benefits and complications of AA infusions need further investigations.

Insulin has previously been shown to have anti-inflammatory properties, as demonstrated by a decrease in CRP, TNF-α, IL-6, IL-1β, while also increasing the anti-inflammatory cytokine IL-10.[19, 20, 108-110] Investigations looking into the anti-inflammatory potential of AA supplementation suggest promising results as well. Recent evidence suggests that the supplementation of some specific AA may play a role in attenuating cytokine release and inflammatory response in periods of stress. Some amino acids of interest include L-glutamate, L-alanine, and L-arginine. They all have been implicated in the modulation of immune function, attenuation of oxidative stress and inflammation.[74-76]

The measurement and analysis of inflammatory markers in our study was a secondary outcome. While most inflammatory markers exhibited a similar increase in plasma levels between the two groups (except IL-1β, which exhibited similar decrease between the two groups), only IL-6 showed a significant decrease in postoperative plasma levels that appeared to be related to the AA supplementation. Interleukin-6 and TNF-α are well known to be proinflammatory, and thus, this finding may be associated to an anti-inflammatory effect of AA intake. Hall and colleagues report an inverse correlation between postoperative IL-6 levels and functional recovery in elderly patients undergoing hip arthroplasty.[111] Hence, further investigations regarding the possible anti-inflammatory property of AA in the clinical setting is warranted. Contrary to the plasma cytokines, mRNA expression of IL-6 and TNF-α in adipose tissue were both increased in the

insulin+AA group compared to the insulin group (Figure 5.10B). These findings were further corroborated by a higher concentration of IL-6, but not TNF-α, in adipose tissue (Figure 5.10A). Interestingly, expression of CD68 mRNA, a macrophage marker, was decreased in adipose tissue from the insulin+AA patients. This finding would suggest that the increased mRNA expression of IL-6 and TNF-α are independent of tissue macrophage recruitment associated with systemic inflammation. It is worth to note that higher levels of adipose tissue IL-6 and TNF expression has been associated with insulin resistance and obesity in healthy vounteers.[112] Consequently, we can hypothesize that the higher mRNA expression of adipose tissue cytokines may be directly associated with the increased insulin resistance in the insulin+AA group. Possibly, AA effects are different among various tissue types, leading to anti-inflammatory responses in some, while causing pro-inflammatory responses in others. The relevance and interpretation of these findings are complex and beyond the scope of our study. Further investigations dedicated to differences in AA-induced inflammatory response are necessary.

7. Study limitations

There are certain limitations to this study. The inherent variability associated with the studying of human subjects in the perioperative setting potentially introduces confounders which may not be fully accounted for. Moreover, because of the very labor-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. Although the performance of validation studies for background $^{13}CO_2$ enrichment allows for some correction, the possibility that the control groups may not be fully comparable to the treatment groups should be noted. Additional control subjects are needed to refine the correction and its relationship to the dextrose 20% infusion rate. The study design does not explore specific organ protein turnover, nor is it powered to analyze the effect of the intervention on hormones and inflammatory markers. Finally, the analysis limited to adipose tissue may not reflect molecular changes in other organ systems, or systemically. At times, small amounts of biopsies can complicate the subsequent analysis. Moreover, adipose tissue *per se* may not play a significant role in inflammation and glucose and protein kinetics, compared to skeletal muscles and/or liver, for example. Correlation with other tissue sites would be interesting.

8. Conclusion

In summary, this study further demonstrates that stable isotope tracer technique can be reliably used in patients undergoing open-heart surgery. Furthermore, despite its labor-intensive nature, the use of high-dose insulin therapy with or without intravenous AA supplementation can be performed safely, while significantly impacting glucose and protein metabolism perioperatively. This study demonstrates that anabolism and neutral protein balance can be successfully induced, even in the context of open-heart surgery, with the combination of HNC and AA. By maintaining the availability of plasma circulating AA, HNC can suppress protein breakdown, while stimulating protein synthesis. Lastly, AA supplementation also alters the inflammatory pattern associated with a major surgery. Further investigations should be aimed at maximizing anabolic potential with higher doses of AA, investigating the role of AA on insulin resistance in the perioperative setting, and organ-specific inflammatory responses to AA supplementation.

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