Parenteral Amino Acids Improve Leucine Balance Without Aggravating Hyperglycemia In Patients With Type 2 Diabetes Undergoing Colorectal Cancer Surgery.

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ABSTRACT

Insulin resistance of type 2 diabetes mellitus may accentuate the catabolic response that is seen after colorectal cancer surgery. The objective of the present study was to investigate the effect of a prolonged parenteral nutrition support regimen based on a moderate dose of amino acids in patients with and without type 2 diabetes undergoing colorectal cancer surgery. Whole body glucose and protein kinetics and synthesis rates of hepatic secretory proteins were assessed by stable isotope tracers D-[6, 6-$^{2}H_{2}$] glucose, L-[1-^{13}C] leucine and L-[ring-$^{2}H_{5}$] phenylalanine respectively.

In the fasted state, patients with type 2 diabetes were more catabolic and moderately hyperglycemic than controls. Parenteral amino acid administration improved balance in both groups with normoglycemia in controls. However, patients with type 2 diabetes had lower protein balance and higher blood glucose levels than controls. Overall, parenteral amino acids supported the acute phase response without attaining net neutral balance in either group. Thus the regimen was successful in maintaining glycemic control with partial success in improving protein balance.

RÉSUMÉ

La résistance à l'insuline du diabète de type 2 peut accentuer la réponse catabolique qui suit une chirurgie pour un cancer colorectal. L'objectif de ce projet était d'évaluer l'effet d'une nutrition parentérale prolongée composée d'une dose modérée d'acides aminés chez les patients avec ou sans le diabète de type 2 subissent une chirurgie pour un cancer colorectal. Les cinétiques du métabolisme protéique et du glucose et des taux de synthèse des protéines ont été évaluées à l'aide des isotopes stable D-[6, 6-$^{2}H_{2}$] glucose, L-[1-^{13}C] leucine and L-[ring-$^{2}H_{5}$] phénylalanine respectivement.

À l'état de jeûne, les patients atteints du diabète de type 2 étaient dans un état plus catabolique avec les taux de glycémie modérément élevée par rapport aux témoins. L'administration parentérale d'acides aminés à amélioré l'équilibre de la protéine chez les deux groupes. Cependant, l'équilibre de la protéine était plus bas et la glycémie était plus haut chez les patients atteints du diabète de type 2 par rapport aux témoins. De façon générale, les acides aminés parentéraux ont favorisée la synthèse des protéines de phase aiguë sans mener l'équilibre des protéines vers le neutre dans les deux groupes. Ce régime a réussi à maintenir la glycémie avec une réussite partielle de but de balance protéique.

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AA	Amino acids
APE	Atoms percent excess
APR	Acute phase response
ASR	Absolute synthesis rate
BCAA	Branched chain amino acids
CRCsx	Colorectal cancer surgery
CV	Coefficient of variance
EAA	Essential amino acids
EDA	Epidural analgesia
Endo Glu Ra	Endogenous glucose rate of appearance
Endo Leu Ra	Endogenous leucine rate of appearance
FSR	Fractional synthesis rate
Glu Cl	Glucose clearance
i.v.	Intravenous
IR	Insulin resistance
Leu Bal	Leucine balance

Leu Ox	Leucine oxidation
Leu Ra	Leucine rate of appearance
NEAA	Non-essential amino acids
Phe/tyr	Phenylalanine to Tyrosine ratio
PNS	Parenteral nutrition support
PO	Per oral
POD	Postoperative day
REE	Resting energy expenditure
RMR	Resting metabolic rate
RQ	Respiratory quotient
T2DM	Type 2 diabetes mellitus
TAA	Total amino acids
TP	Total proteins
VLDL Apo B100	VLDL Apo lipoprotein B100
VCO_2	Carbon dioxide production
VO_2	Oxygen consumption
α-KIC	α- ketoisocaproate

CONTRIBUTION OF AUTHORS

The study protocol was developed by Dr. Schricker and Dr. Wykes. Patients were recruited by Dr. Schricker. In collaboration with Dr. Schricker and Dr. Lattermann, I performed pre and post-operative stable isotope infusion studies. I prepared samples for analysis and performed GC/MS analysis with help from Mr. Evan Nitschmann. I also collected data and did the calculations and statistical analysis. I was responsible for writing the paper and creating the tables of results. Dr. Wykes, Dr. Lattermann and Dr. Schricker provided guidance and assisted with the manuscript.

1. INTRODUCTION

Surgical injury leads to a typical metabolic milieu that is characterized by insulin resistance (IR) and stress induced catabolism. This catabolism is considered compensatory in nature conferring survival advantage. Unimpeded surgical stress leads to hyperglycemia and depleted state, often associated with complications that increase morbidity and mortality (Desborough 2000). Patients with type 2 diabetes mellitus (T2DM) may have a mild form of an injury response that is similar to that seen in infection, cancer or trauma (Richardson and Tayek 2002). Thus patients with T2DM, undergoing colorectal cancer surgery (CRCsx), may experience a greater catabolic response. Glucose based parenteral nutrition support (PNS) employed to offset the depletion may aggravate hyperglycemia especially in T2DM. This study examines the effects of amino acid (AA) based PNS regimen in patients with and without T2DM with respect to glucose and protein kinetics.

2. LITERATURE REVIEW

2.1. SURGICAL STRESS RESPONSE

Colorectal surgery serves as a major injury that leads to activation of a cascade, which has neuronal, hormonal and immune-cytological components. The neuronal arm involves stimulation of afferent sensory and sympathetic fibers by surgical tissue trauma leading to activation of efferent hypothalamo-pituitary pathways. This leads to alteration of the hormonal milieu with dominance of counter-regulatory hormones like glucagon, cortisol, catecholamine, growth hormone, and reduced levels of insulin, T3 and T4 (Desborough 2000). Local release of inflammatory mediators called cytokines from activated immune cells at the site of surgical injury (IL-1, IL-6, TNF-α) initiate and propagate this response (Hill 2000). This combination of changes is called the surgical stress response and it has profound effects on substrate metabolism characterized by accelerated catabolism diverting substrates centrally for energy supply, tissue repair or homeostasis.

2.1.1. INSULIN RESISTANCE

Insulin resistance is a state of reduced biological effect for any given concentration of insulin (Wallace and Matthews 2002) and often accompanies the postoperative state. Numerous mechanisms have been postulated for its occurrence, mainly lipid accumulation, unfolded protein response pathway as well as immune mediated pathways (Samuel and Shulman 2012). The degree of IR has been linked to the extent of surgical tissue trauma (Thorell, Nygren et al. 1999) and is a significant contributor to

hyperglycemia, a common perioperative occurrence. Intensive glycemic control is not backed by clear evidence supporting its implementation and is accompanied by significant risk of hypoglycemia (Griesdale, de Souza et al. 2009). Thus proactive measures aimed at preserving insulin sensitivity such as use of perioperative epidural analgesia (EDA) (Uchida, Asoh et al. 1988), carbohydrate loading (Ljungqvist, Nygren et al. 2002) or minimally invasive surgery (Thorell, Nygren et al. 1996) are preferred. In addition to impact on glucose metabolism, IR may also affect protein metabolism (Pereira, Marliss et al. 2008).

2.1.2. GLUCOSE METABOLISM IN SURGERY

The counter-regulatory hormones increased by the stress response act by increasing glucogenic substrate supply to and its uptake by the liver leading to increased hepatic gluconeogenesis. The mobilization of glycogen stores and facilitation of glucose release by the liver, ultimately increase blood glucose levels (Bagry, Raghavendran et al. 2008). The peripheral IR as well as the relative lack of insulin secretion (Akhtar, Barash et al. 2010) hampers glucose disposal by insulin sensitive tissues such as the muscle and adipose tissue. The result is hyperglycemia with starvation of insulin sensitive tissues. The non-insulin sensitive tissues such as brain are exposed to the cytotoxic effects of hyperglycemia mediated by the free radicals generated through the glycolytic pathway (Brownlee 2005).

2.1.3. ADVERSE EFFECTS RELATED TO HYPERGLYCEMIA

Hyperglycemia was originally thought to be an essential part of the stress response maintaining energy supply to the brain (Desborough 2000). However, in the era of modern medicine, hyperglycemia has been consistently linked to a series of adverse outcomes. These may include reduced immunity leading to infections (Karunakar and Staples 2010); increased incidence of neurologic, pulmonary and renal complications as well as mortality (Gandhi, Nuttall et al. 2005). In effect hyperglycemia leads to increased length of hospital and ICU stay (Frisch, Chandra et al. 2010). Hence avoidance of hyperglycemia is of vital importance.

2.1.4. PROTEIN METABOLISM IN SURGERY

Changes in protein metabolism begin in the intraoperative period with suppression of catabolism and synthesis of proteins (Lattermann, Carli et al. 2002). In the absence or limitation of exogenous nutrient supply, the postoperative period is characterized by increased protein flux and negative nitrogen balance i.e. net loss of body proteins. This results from accelerated protein breakdown mainly in the skeletal muscle and to some extent in the visceral muscles (Douglas and Shaw 1989). This proteolysis increases supply of essential amino acid (EAA) precursors for processes such as hepatic gluconeogenesis, oxidative catabolism, synthesis of acute phase proteins, or protein synthesis related to tissue repair or immune response (Desborough 2000). These processes are mediated by the hormones and cytokines associated with the stress response e.g. cortisol activation of the ubiquitin proteolytic pathway (Price, England et al. 1994) leading to increased

catabolism of proteins (Brillon, Zheng et al. 1995), glucagon signaling of amino acid oxidation (Tessari, Inchiostro et al. 1996) or IL-6 mediated increase in synthesis of hepatic acute phase proteins. The acute phase response (APR) is the symptom sign complex that is seen during the early stages following an illness or injury. This is accompanied by a typical profile of plasma proteins that fulfill important functions in tissue repair and recovery. Plasma protein levels also serve as prognostic indicators (Al-Joudi 2005; Groblewska, Mroczko et al. 2008; Aoki, Iwamoto et al. 2009; Wang, Hsieh et al. 2009; Aki, Suyani et al. 2012). There is an increase in levels of certain proteins called the positive acute phase reactants e.g. Fibrinogen, C-reactive protein. Levels of other proteins show a drop e.g. albumin, pre-albumin, retinol binding protein: leading to their classification as negative acute phase reactants (Fleck 1989). Recent evidence suggests that hepatic rate of albumin synthesis is actually increased in stress (Rittler, Jacobs et al. 2007) and factors such as accelerated catabolism, redistribution or loss to tissue spaces maybe responsible for the drop in levels (Fleck 1989). In the absence of exogenous substrates, increased synthesis of plasma proteins, particularly the negative acute phase reactant albumin, may compromise muscle protein synthesis or increase breakdown, ultimately compromising the anabolic response (Reeds, Fjeld et al. 1994; Mackenzie, Warren et al. 2003). In the setting of CRCsx the postoperative rates of albumin synthesis were found to be significantly higher along with greater leucine balance in patients who were fed preoperatively compared to those who were fasted (Schricker, Meterissian et al. 2008). Thus APR is amenable to modification by nutritional support and this may ultimately affect the whole body protein balance.

2.1.5. EFFECTS OF PROTEIN CATABOLISM

Proteins are important structural and functional components of the human body. Catabolic reaction to surgical stress is a cause of significant perioperative morbidity (Kehlet 1984). Since skeletal muscle is the largest reservoir of proteins, proteolysis leads to loss of skeletal muscle mass (Tisdale 2001) and hence reduced muscle strength (Watters, Clancey et al. 1993). Perioperative loss of lean body mass, or deterioration of nutritional status is associated with increased incidence of respiratory complications, reduced immunity and infection, delayed wound healing and prolonged recovery (McClave, Snider et al. 1999).

2.2. METHODS OF MODULATING THE RESPONSE TO SURGICAL STRESS

Numerous interventions are undertaken perioperatively to minimize the magnitude of stress response to surgical injury or its effects. Some of them are discussed below.

2.2.1. USE OF EPIDURAL ANALGESIA

Preoperative initiation and postoperative continuation of EDA results in significant reduction of postoperative IR through the attenuated neuroendocrine responses to surgical tissue trauma (Uchida, Asoh et al. 1988). It, however, does not modify the inflammatory cascade which involves release of cytokines and acute phase proteins. The beneficial effects of EDA occur through the modification of the surgical stress response. This is seen by the lack of metabolic benefits in patients not subjected to surgical injury (Schricker, Klubien et al. 2000).The surgical stress response is least modifiable in the

immediate perioperative period with epidural analgesia showing beneficial effects only on glucose metabolism (Lattermann, Carli et al. 2002; Lattermann, Carli et al. 2003). In the postoperative period, however, the maintenance of insulin sensitivity favorably modifies both glucose and protein metabolism. Thus glucose disposal is improved (Uchida, Asoh et al. 1988) in turn improving glycemic control. Improvement in glucose utilization decreases the use of proteins as fuel and leads to protein sparing (Schricker, Wykes et al. 2000). Carli et al. have also demonstrated a decrease in protein breakdown (Carli, Webster et al. 1991). These favorable effects of epidural only occur in the fed state (Schricker, Wykes et al. 2000).

2.2.2. AVOIDANCE OF PREOPERATIVE FASTING

Patients scheduled for CRCsx may be in a net catabolic state as a result of cancer cachexia (Tijerina 2004). Perioperatively, oral intake maybe limited for numerous reasons. These may include, but are not limited to, cancer associated anorexia (Tijerina 2004), preoperative bowel preparation, mandatory overnight fast for preventing aspiration of gastric contents under anesthesia, postoperative paralytic ileus and nausea and vomiting. This fasting is responsible for significant perioperative weight loss (Kinney, Long et al. 1968).

Oral intake maybe halted in advance of CRCsx for bowel preparation and for avoidance of aspiration of residual gastric contents. On the other hand, accruing evidence links preoperative fasting with insulin resistance and worsening of surgical outcome (Ljungqvist, Nygren et al. 2002; Ljungqvist, Soop et al. 2007). Preoperative

feeding, by maintaining insulin sensitivity, improves postoperative protein metabolism (Thorell, Nygren et al. 1999; Yuill, Richardson et al. 2005). Schricker et al. have shown that net anabolism cannot be achieved with preoperative fasting despite higher protein synthesis, while maintenance of a preoperative fed state leads to a positive protein balance by a reduced protein breakdown and amino acid oxidation (Schricker, Meterissian et al. 2008). Thus avoidance of fasting appears to improve the metabolic scenario in the state of surgical stress.

2.3. NUTRITIONAL SUPPORT

Nutritional support provides substrates to support tissue repair thus limiting the use of body tissues as protein source. Depending on the energy provided, nutrition may be classified as hypo-, iso- or hyper caloric and maybe administered via the enteral or parenteral routes.

2.3.1. TOTAL PARENTERAL NUTRITION (TPN)

TPN, either iso- or hyper-caloric, is generally indicated in severe malnutrition and intolerance to or ineffectiveness of enteral nutrition after 7-10 days of administration (Braga, Ljungqvist et al. 2009). Administration requires central venous access. Institution is gradual with readjustments over time and necessitates intensive monitoring for significant biochemical derangements such as hyperglycemia and electrolyte imbalance which can adversely affect outcomes (Svanfeldt, Thorell et al. 2006; Karunakar and Staples 2010). Hyperalimentation-associated increases in oxygen consumption (VO_2)(Muller, Muller et al. 1995) and carbon dioxide production (VCO_2) (Liposky and

Nelson 1994) maybe dangerous in patients with compromised cardio-respiratory reserves. Other common complications include infections (Sena, Utter et al. 2008), steatosis and cholestasis (Raman and Allard 2007). Benefits of TPN are restricted to patients with severe malnourishment. Risk of adverse effects outweighs benefits in patients with lesser degrees of malnutrition or tolerance of enteral nutrition (1991; Sandstrom, Drott et al. 1993; Brennan, Pisters et al. 1994; Sena, Utter et al. 2008).

2.3.2. HYPOCALORIC PERIPHERAL PARENTERAL NUTRITION SUPPORT (PNS)

Hypo-caloric PNS on the other hand can be administered through peripheral intravenous access. It avoids the metabolic stress imposed on the body by hyper- or iso-caloric PNS (Muller, Muller et al. 1995), is associated with significantly reduced incidence of infectious complications (Jiang, Sun et al. 2011) and hence it is the preferred mode of parenteral nutritional support in perioperative patients with mild to moderate degrees of malnutrition (Lu, Chuang et al. 2010). Patients presenting for CRCsx have greater prevalence of mild to moderate degrees of malnutrition (Garth, Newsome et al. 2010). Also with the emergence of the benefits of Enhanced Recovery After Surgery (ERAS) protocol (Gustafsson, Scott et al. 2012), the period of perioperative obligate nil per oral status has contracted significantly. Nonetheless, even a short interruption of nutritional intake can have significant effect on the nutritional status (Kinney, Long et al. 1968). Therefore, hypo-caloric PNS may avoid deterioration of nutritional status by minimizing interruptions in nutritional intake in uncomplicated patient undergoing CRCsx.

2.3.3. ADJUSTMENT OF NUTRITION TO INDIVIDUAL PATIENT'S NEEDS

Energy expenditure determines energy requirement and should be the basis of prescribing PNS in order to improve its efficiency. Numerous equations provide an estimate of resting energy expenditure (REE) from calculations based on known parameters (Frankenfield, Roth-Yousey et al. 2005). However, predictive equations are inherently inaccurate in estimating REE in individuals (Frankenfield, Rowe et al. 2003; Boullata, Williams et al. 2007). Moreover, the daily REE in critically ill patients can vary significantly (McClave and Snider 1992). Since both over- and under- nutrition can be harmful (Bartlett, Dechert et al. 1982), use of indirect calorimetry, which calculates the resting metabolic rate (RMR) through measurement of VO_2 and VCO_2, is the method of choice (McClave and Snider 1992; Frankenfield and Ashcraft 2011).

2.4. EFFECT OF DIABETES MELLITUS TYPE 2

T2DM is an insulin resistant state. Hence the consequences of the metabolic-endocrine stress response to surgery seen in patients without diabetes will likely be amplified in these patients. A study of patients undergoing CRCsx revealed that patients with T2DM are more catabolic than patients without T2DM under similar conditions (Schricker, Gougeon et al. 2005) It was shown by Shaw et al. that benefits of PNS are directly proportional to the state of catabolism (Shaw 1988) making patients with T2DM ideal candidates for the same. However there are some concerns with the application of nutritional support in this group of patients. In addition to IR of glucose metabolism which defines T2DM, IR of protein metabolism has also been demonstrated in this condition (Pereira, Marliss et al. 2008) In this scenario any attempts at nutritional

supplementation would not only fail in turning the protein balance positive but would also lead to increased blood glucose levels. Hyperglycemia which is the hallmark of diabetes mellitus is often exacerbated with use of nutritional support especially in the background of surgically induced stress response. Hyperglycemia during the perioperative period is known to profoundly impact the convalescence in patients with T2DM (Estrada, Young et al. 2003) and increase the incidence of infectious complications (Golden, Peart-Vigilance et al. 1999). Hence avoidance of hyperglycemia while providing adequate nutritional support is of essence in this category of patients. Significant hyperglycemia occurs with the perioperative use of glucose in patients with T2DM (Schricker, Gougeon et al. 2005). Nonetheless, this class of patients can be rendered anabolic using a short term feeding regimen of amino acids following CRCsx without the occurrence of severe hyperglycemia (Lugli, Donatelli et al. 2008). Lugli et al. compared nutritional regimens, using either dextrose or amino acids in patients with T2DM undergoing CRCsx. Only patients in the amino acid group entered positive nitrogen balance while maintaining normoglycemia while the patients in the dextrose group continued to sustain net catabolism (Lugli, Donatelli et al. 2012). Also the anabolic response to short term PAA was stronger in patients with type 2 diabetes (Lugli, Donatelli et al. 2010). However the effect of a prolonged administration of moderate dose of amino acids has not been studied.

2.5. EFFECT OF COMPOSITION OF NUTRITION SUPPORT

In the acute phase of surgical stress, i.e. intra and immediate postoperative periods, glucose and protein metabolisms are depressed. This is followed by a period of reduced uptake of glucose; and increased protein breakdown and amino acid oxidation for gluconeogenesis and energy supply (Schricker, Lattermann et al. 2001; Lattermann, Carli et al. 2002; Lattermann, Carli et al. 2003). In this acute phase, the use of epidural blockade can only suppress the endogenous glucose production while having no effects on glucose utilization or the suppressed protein metabolism (Lattermann, Carli et al. 2002). Hypo-caloric glucose administered in the immediate perioperative period suppresses endogenous glucose production. However is incapable of sparing proteins (Lattermann, Carli et al. 2003). Schricker et al. demonstrated that the inhibitory influence of exogenous glucose administration on endogenous glucose production is reduced in the presence of acute surgical stress (Schricker, Lattermann et al. 2004), while nutritional support with hypo caloric glucose in the presence of epidural analgesia 2 days after abdominal surgery is capable of shifting metabolism from protein to a glucose dominated substrate utilization (Schricker, Wykes et al. 2000). It is thus evident that the surgical stress response is least modifiable in the acute phase.

In the postoperative period, glucose providing 50% of REE does not favorably modify leucine kinetics, and leucine balance remains negative (Schricker, Meterissian et al. 2004).The effort of reversing the catabolic state associated with surgery has prompted significant manipulations of the compositions of nutritional support. It has

been postulated that an anabolic response requires provision of both energy in the form of glucose as well as anabolic substrates in the form of amino acids given the biochemical link between protein and glucose metabolism. Schricker et al. demonstrated an anabolic response in patients without T2DM with combined PAA and glucose infusions in hypo-caloric amounts in contrast to persistent negative balance with glucose only regimen (Schricker, Wykes et al. 2005). Mimura et al. compared different peripheral PNS regimens in gastrectomy patients. They found that the cumulative nitrogen balance was significantly less negative in patients receiving combined glucose and amino acids as compared to glucose alone (Mimura, Yamakawa et al. 1997). Thus provision of amino acid, which are building blocks of proteins, maybe the key to inducing an improvement in protein balance in postsurgical patients.

Proteins contain varying amounts of glucogenic amino acids, generating 50-80 g of glucose per 100 g of protein ingested (Janney 1915). Thus hyperglycemia can be a concern with use of amino acid solutions. Studies looking at amino acid supplementation of perioperative hypocaloric carbohydrate infusions did not note any worsening of hyperglycemia. In perioperative colorectal cancer patients without T2DM amino acids at 1 $g \cdot kg^{-1} \cdot d^{-1}$ along with hypocaloric glucose did not worsen hyperglycemia (Schricker, Wykes et al. 2007). Behrendt et al. compared different carbohydrate infusions supplemented with a similar dose of amino acids in the postoperative period and found no aggravation of hyperglycemia (Behrendt, Raumanns et al. 1988). Data on the effects of prolonged perioperative amino acid administration in patients with T2DM are unavailable. Nonetheless, a short term feeding study in postoperative patients with

T2DM employing high dose amino acids at 2.9 $g \cdot kg^{-1} \cdot day^{-1}$ demonstrated no significant perturbations of blood glucose levels (Lugli, Donatelli et al. 2012). Thus it may be safe to assume that a moderate dose of amino acids maybe employed in perioperative parenteral nutrition without the risk of hyperglycemia.

Meanwhile significant hyperglycemia continues to be associated with nutritional support regimens containing glucose leading to speculation over its role in perioperative nutrition (Mimura, Yamakawa et al. 1997; Schricker, Wykes et al. 2002; Lattermann, Carli et al. 2003; Schricker, Gougeon et al. 2005). Short term PAA with or without glucose elicited similar net anabolic responses in patients without T2M yet hyperglycemia was avoided only when glucose was omitted from the regimen (Schricker, Meterissian et al. 2007). Patients with T2DM experienced hyperglycemia with short term glucose regimen but not with high dose amino acid regimen, while only the amino acid regimen induced a positive leucine balance (Lugli, Donatelli et al. 2012). Thus glucose may be dispensable in nutrition regimens aimed at an anabolic response.

2.6. STABLE ISOTOPE TRACER TECHNIQUE FOR ASSESSMENT OF PROTEIN METABOLISM

The large variety of methods available for assessing malnutrition (McLaren and Meguid 1983; Pisters and Pearlstone 1993) are broadly classified as those used clinically and those used for research purposes. The first category includes methods like anthropometric measurements, biochemical parameters, functional measurements and nitrogen balance studies. The latter includes methods for measuring body composition

or assessment of protein and amino acid metabolism using stable isotopes or measurements of metabolites (Pisters and Pearlstone 1993). Methods that are simple to record such as anthropometric measurements or recent weight loss based on patient's perception, maybe subject to errors due to factors such as variability of the measurement in the population, lack of appropriate standards or errors in patient's recall of initial weight etc. (McLaren and Meguid 1983). Levels of transport proteins e.g. albumin and prealbumin are influenced by multiple factors such as malnutrition, trauma, infection, liver disease, micronutrient deficiency amongst others and hence are not reliable markers of malnutrition (Golden 1982). Handgrip strength which is a functional measure of skeletal muscle mass, is influenced by motivation or presence of disease conditions e.g. arthritis and paralysis, limiting its reliability (Klidjian, Foster et al. 1980; McLaren and Meguid 1983) Nitrogen balance studies come very close to measuring the protein balance. However they are very expensive and time consuming and are prone to incorrect estimations due to errors in measuring intake or output. Also they provide only an overall picture of anabolic or catabolic state with no possibility of exploring the underlying mechanisms (Kopple 1987).

Whole body leucine kinetics have been measured using primed continuous infusions of a stable leucine isotope L-[1-^{13}C]leucine (Matthews, Motil et al. 1980). Leucine is an essential amino acid and is metabolized primarily in skeletal muscle. It plays an important role in oxidative metabolism and is the principle source of alpha-amino nitrogen of glucogenic amino acids released from muscle. It has regulatory influence on the other two branched chain amino acids (BCAA) and on muscle

breakdown and synthesis (Odessey, Khairallah et al. 1974). Hence estimates of body protein breakdown, oxidation and protein synthesis can be obtained from measured leucine kinetics. This method thus enables an understanding of the underlying dynamics of protein metabolism that leads to shifts in the overall balance, which is vital to planning effective intervention strategies for correction of malnutrition.

Whole body protein balance assessed using leucine kinetics provides the weighted average of the synthetic and breakdown processes taking place throughout the whole body. It does not offer any additional information on the changes taking place at organ level (Wagenmakers 1999). The direction of change at organ level may not be the same as that at whole body level. In order to assess tissue level responses, fractional synthesis rates of plasma proteins can be measured using primed continuous infusion of L-[$^{2}H_{5}$] phenylalanine from the rate of incorporation of the tracer into the particular protein (Wagenmakers 1999). For the purpose of this calculation the isotopic enrichment of VLDL Apo lipoprotein B100 (VLDL Apo B100), at steady state, is taken to represent the isotopic enrichment of the precursor pool from which the plasma proteins are synthesized (amino-acyl t-RNA). VLDL Apo B100 is a very rapidly turning over hepatically-derived lipoprotein that is synthesized from the same amino acid precursor pool as the plasma proteins. Due to its rapid turnover rate, phenylalanine isotopic enrichment of VLDL Apo B100 equilibrates with that of the precursor pool very rapidly and thus phenylalanine enrichment of VLDL Apo B100 can be taken to represent the precursor pool enrichment for plasma protein synthesis (Reeds, Hachey et al. 1992; Mackenzie, Warren et al. 2003). There are reports of a reduction in secretion of Apo B100 with

surgical stress with increase in turnover time (Kesteloot, Cobbaert et al. 1992). Yet, we were able to demonstrate a plateau in the enrichment of phenylalanine tracer in the VLDL Apo B100 indicating that the isotopic plateau was reached in the lipoprotein molecules.

3. RATIONALE

Insulin resistance (IR) due to surgical stress affects both glucose and protein metabolism leading to a catabolic state and hyperglycemia. Epidural analgesia can modify these metabolic changes only in the presence of nutrient supply. Greater benefits are achieved with avoidance of preoperative fasting and individualization of nutritional support. In the setting of CRCsx, hypocaloric parenteral nutrition is commonly used given the problems associated with hyper or isocaloric PNS and the brief interruption of oral intake. Patients with T2DM maybe more depleted before surgery and may also be more susceptible to experiencing an exaggerated catabolic response to surgical stress given their inherent IR. They may also be resistant to the protein anabolic effects of insulin. High dose PAA administered in isolation over a short time have been successful in establishing a positive protein balance in both patients with and without T2DM, as measured by stable isotope tracer techniques. Similarly use of a moderate dose of PAA with glucose in subjects without T2DM was successful in attaining positive balance. But glucose administration is consistently associated with hyperglycemia, which can adversely affect outcomes. This is the rationale for introducing a PNS regimen based on a moderate dose of PAA alone, at 1 $g \cdot kg^{-1} \cdot d^{-1}$, administered from 20 hours preoperatively to the second postoperative day. Few studies evaluating the effects of short PNS regimens in patients with and without T2DM exist. The present study was undertaken in the absence of a direct fasted and fed state comparison of the two groups looking at the effect of a prolonged nutrition support regimen.

4. HYPOTHESIS AND OBJECTIVES

4.1. HYPOTHESIS

The purpose of the present study was to investigate the differences in the effects of a prolonged PNS regimen based on a moderate dose PAA alone in patients with (T2DM) and without T2DM (Non-T2DM) undergoing CRCsx. We hypothesized that all patients would be in negative leucine balance (Leu Bal) in the preoperative overnight fasted state; however insulin resistance of patients with T2DM would be evident as higher plasma glucose and endogenous glucose rate of appearance (Endo Glu Ra). Nutrition would improve Leu Bal to neutral in T2DM group and positive in Non T2DM group. Normoglycemia would be maintained in both groups despite higher Endo Glu Ra in T2DM. Nutrition would also support the APR as demonstrated by increased fractional synthesis rates (FSR) of hepatic secretory proteins postoperatively.

4.2. OBJECTIVES

The objective of the present study was to assess the response of T2DM vs. non-T2DM to an individualized iso-nitrogenous hypo-caloric PNS regimen based on a moderate dose of PAA alone (nearly $1g \cdot kg^{-1} \cdot d^{-1}$) initiated 20 hours before surgery and administered continuously over 72 hours. In patients scheduled for trans-abdominal resection of colorectal cancer the following were measured:

- Whole body glucose kinetics with primed continuous infusion of [6,6-$^{2}H_2$]glucose,
- Whole body leucine kinetics with primed continuous infusion of L-[1-^{13}C]leucine,
- Fractional and absolute synthesis rates of hepatic secretory proteins with primed continuous infusion of L-[$^{2}H_5$] phenylalanine,

first on the day prior to surgery after an overnight fast to characterize baseline and again on the second POD while receiving PNS to assess the response to nutrition

5. MANUSCRIPT: Parenteral Amino Acids Improve Leucine Balance Without Aggravating Hyperglycemia In Patients With Type 2 Diabetes Undergoing Colorectal Cancer Surgery.

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5.1. ABSTRACT

BACKGROUND: Insulin resistance of type 2 diabetes mellitus (T2DM) is accentuated by the surgical stress response and nutrition worsens hyperglycemia. Thus balancing anabolic and euglycemic strategies is vital to improving outcomes. **STUDY DESIGN:** Patients with T2DM (n=8) or without (non T2DM, n=11) undergoing colorectal cancer surgery received perioperative nutrition support (NS) based on 20% of measured resting energy expenditure as parenteral amino acids (PAA) in the form of Travasol® starting 20h preoperatively. Whole body glucose and protein kinetics and synthetic rates of hepatic secretory proteins were measured using infusions of D-[6,6-$^{2}H_2$]glucose, L-[1-^{13}C]leucine and L-[ring-$^{2}H_5$]phenylalanine in preoperative fasted and 2nd postoperative day fed state. **RESULTS:** Preoperatively, all subjects were in negative leucine balance, with T2DM (-18±7 μmol· kg^{-1}·h^{-1}) being more negative than Non-T2DM (-13±4 μmol· kg^{-1}·h^{-1}) (p=0.027). PAA improved leucine balance in both groups (p=0.008), but balance remained negative in T2DM (-12±12 vs. -5±8 μmol· kg^{-1}·h^{-1}). Surgical acute phase response (APR) increased fibrinogen concentration (p<0.0001) and FSR (p=0.0007). Albumin FSR was maintained postoperative. However, it is unclear if the fall in albumin concentration (p<0.0001) was mediated by extravasation or hemodilution. Plasma glucose was higher in T2DM (p=0.005) but was unchanged by nutrition. Plasma amino acid profile showed changes typical of the postoperative state. **CONCLUSION:** PAA based PNS supports the APR in T2DM but with moderate hyperglycemia and without attainment of positive leucine balance. Studies with higher doses of amino acids or improved amino acid composition are needed to explore the possibility of achieving an anabolic response in patients with T2DM.

5.2. INTRODUCTION

Patients undergoing major surgery experience derangements of protein and carbohydrate metabolism due to the surgical stress response. This is comprised of activation of the hypothalamo-pituitary-adrenal axis by afferent neuronal impulses from the site of surgical injury (neuronal component). As a result, the hormonal profile is altered with an increase in secretion of counter-regulatory hormones like cortisol, glucagon, growth hormone and catecholamines; and a decrease in anabolic hormones like insulin, T3 and T4. Finally, activated immune cell-derived cytokines mediate local immunity and inflammation and propagate the systemic APR (immune-cytological component). The metabolic consequence of these responses is a catabolic state characterized by IR; accelerated glycogenolysis and hepatic gluconeogenesis; and, peripheral protein breakdown and lipolysis. These act in concert to provide substrates for energy supply and synthesis of proteins or glucose (Desborough 2000). The resulting hyperglycemia and protein depletion may compromise surgical outcome through protracted ventilatory support, susceptibility to infection, poor wound healing among other causes and result in prolonged hospital stay, morbidity or mortality (Kyle, Genton et al. 2005; Noordzij, Boersma et al. 2007; Vilar-Compte, Alvarez de Iturbe et al. 2008; Park, Hsu et al. 2009).

The combination of EDA to modify the neurohumoral component of the stress response and judicious use of glucose to spare proteins has achieved partial success in limiting catabolism in the postoperative period (Schricker, Wykes et al. 2000). Patients

undergoing CRCsx are subject to an obligate perioperative period of insufficient or absent oral intake (Schricker, Meterissian et al. 2008). Even short interruptions of nutrient intake may worsen malnutrition (Kinney, Long et al. 1968) which can be avoided by parenteral nutrition. The low grades of malnutrition (Garth, Newsome et al. 2010) and brief interruption of oral intake prevalent in a large proportion of patients presenting for CRCsx does not warrant the risks associated with overfeeding (Montalvo-Jave, Zarraga et al. 2007). Hence hypocaloric PNS is favored in these patients (Group 1991; Sena, Utter et al. 2008; Jiang, Sun et al. 2011). However the ideal composition of such hypocaloric PNS is under evolution. Anabolism in postoperative patients was reported to be maximal with combined administration of amino acids and glucose (Gazzaniga, Day et al. 1976). In postoperative CRCsx patients without T2DM, co-administration of high dose PAA with moderate dose of glucose over a short period simulating postprandial conditions yield a positive balance, but with significant hyperglycemia (Schricker, Wykes et al. 2002). Moderate doses of PAA and glucose, still allow attainment of a reasonable positive balance accompanied by moderate hyperglycemia but only when preoperative fasting is avoided through prolonged perioperative administration of nutrition support (Schricker, Meterissian et al. 2008). It remains to be seen if similar improvement in balance can be attained by avoiding glucose while providing a moderate dose of PAA alone.

The early or mild cachexia associated with colorectal cancer may impair glucose metabolism (Fredrix, Soeters et al. 1991; Ehrmann-Josko, Sieminska et al. 2006). This may compound the IR of T2DM increasing the likelihood of a severe catabolic response

in patients with T2DM presenting for CRCsx. This group of patients may be “resistant to protein synthesis“ (Pereira, Marliss et al. 2008) while protein loss following surgery is 50% higher as compared to patients without T2DM (Schricker, Gougeon et al. 2005). IR of protein metabolism suggests the need for aggressive nutritional support. However IR of glucose metabolism, aggravated by surgical stress decreases tolerance for exogenous glucose and increases the risk of hyperglycemia (Schricker, Gougeon et al. 2005) and related adverse outcomes (Golden, Peart-Vigilance et al. 1999; Estrada, Young et al. 2003). Short term high dose PAA (nearly $3g \cdot kg^{-1} \cdot d^{-1}$) in patients with T2DM after CRCsx allow attainment of a positive protein balance while avoiding hyperglycemia thus overcoming the blocks in protein and glucose metabolism (Lugli, Donatelli et al. 2010). The impact of a prolonged administration of moderate dose of PAA has not yet been studied.

Thus the objective of the present study was to assess the response of patients with and without T2DM to an isonitrogenous hypocaloric PNS regimen tailored to individual requirement, assessed using RMR measurement. The nutrition regimen was based on a moderate dose of PAA alone initiated 20 hours before surgery and administered continuously over 72 hours. Patients scheduled for transabdominal resection of colorectal cancer were studied on the day prior to surgery in the fasted state and again on the second postoperative day while receiving PNS. Whole body protein and glucose kinetics and FSRs of hepatic secretory proteins were studied with primed continuous infusion of L-[1-^{13}C]leucine, [6,6-$^{2}H_2$]glucose and L-[$^{2}H_5$]phenylalanine respectively. We hypothesized that all patients would be in negative Leu Bal in the

preoperative fasted state. However patients with T2DM would show evidence of IR in higher plasma glucose levels and Endo Glu Ra. PAA would improve Leu Bal in both groups. Normoglycemia would be maintained in both groups despite higher Endo Glu Ra in T2DM. Nutrition would also support the APR as demonstrated by increased FSR of hepatic secretory proteins.

5.3. METHODS.

5.3.1. STUDY DESIGN AND PATIENTS

Patients with (T2DM)or without (non–T2DM) type 2 diabetes mellitus undergoing CRCsx received nutritional support in the form of PAA based on 20% of measured RMR from 20h before surgery until the second postoperative day(POD). Whole body glucose and protein kinetics; and FSR of hepatic secretory proteins were measured by stable isotope tracer technique with primed continuous infusions of L-[1-^{13}C]leucine, D-[6,6-$^{2}H_2$]glucose and L-[ring-$^{2}H_5$]phenylalanine respectively, while fasting on the preoperative day, and again while receiving PNS on 2nd POD. The study was approved by the Ethics Committee of the Royal Victoria Hospital (RVH) and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient before enrollment.

In all 8 T2DM and 11 non-T2DM subjects with American Society of Anesthesiologists class <3 (Appendix 1) and age >18 undergoing CRCsx at RVH were recruited after screening to exclude patients with evidence of metastatic disease, body mass index (BMI) >30 $Kg \cdot m^{-2}$, severe anemia (hemoglobin <10$g \cdot dL^{-1}$), type I diabetes mellitus, significant cardio respiratory, hepatic, renal, neurological, musculoskeletal or neuromuscular disease or receiving drugs known have metabolic effects (e.g. steroids, beta-blockers). The protocol was slightly different for the initial patients recruited in the study (one T2DM and two non-T2DM). These patients received only [6,6-$^{2}H_2$]glucose and

L-[1-^{13}C]leucine during the preoperative infusion study and hence were excluded from analysis of hepatic secretory protein data.

5.3.2. EXPERIMENTAL PROTOCOL

Figure 1: Overall Study Protocol

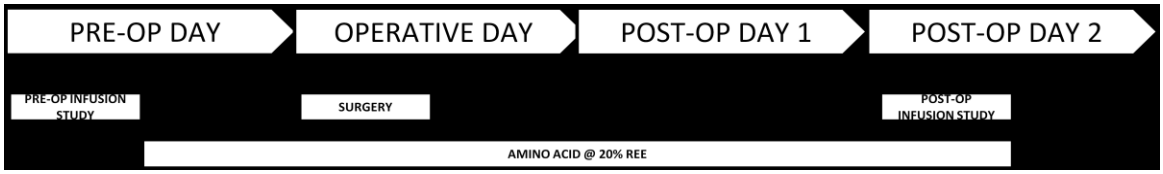
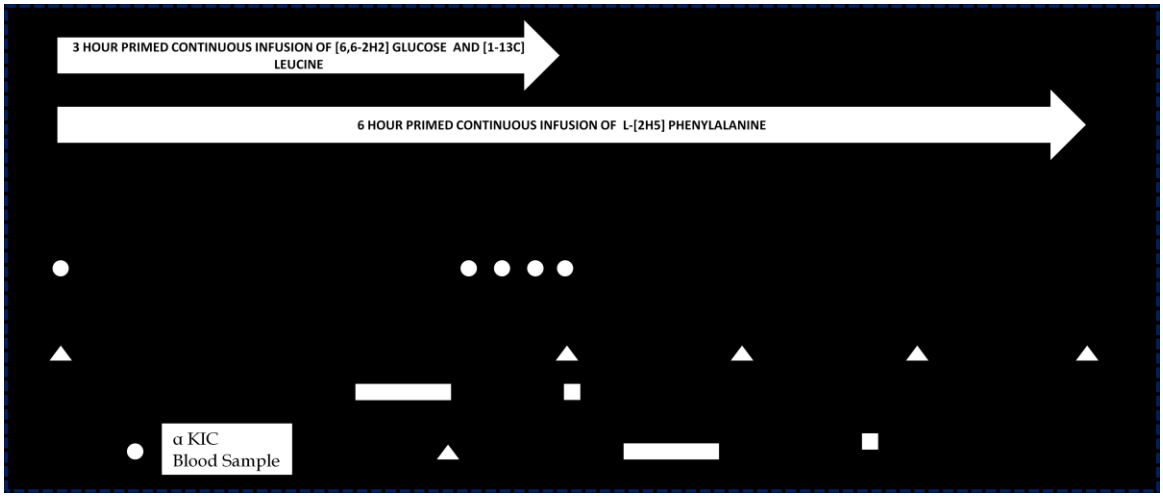


Figure 2: Pre and Post-operative Infusion and Sampling Protocol



5.3.2.1. NUTRITION SUPPORT: COMPOSITION AND ADMINISTRATION

Patients were given one last meal at 7.00 PM, 2 days before surgery. On the morning of the preoperative day age, sex, weight and height were recorded. Parenteral nutrition was administered continuously through a cannula placed in a superficial forearm vein, beginning from the end of the preoperative infusion study to the end of the postoperative infusion study. It supplied 20% of the patient`s measured RMR (equivalent to approximately 1 g/kg/day) as a 10% AA solution (Travasol™, Baxter, Montreal, Canada). This amount was chosen to provide proteins at a rate 20% higher than the RDA for metabolically normal healthy adults (Stein, Boehles et al. 2009).

The composition of Travasol™ (µmol/ml) is as follows: proline 35, threonine 34, glycine 217, alanine 207, valine 36, methionine 37, isoleucine 34, leucine 56, tyrosine 2, phenylalanine 35, tryptophan 9, lysine 38, histidine 26, arginine 57, serine 5.

5.3.2.2. ANAESTHESIA AND PERIOPERATIVE CARE

The patients received no pre-medication and were operated on by the same surgeon. General anesthesia was performed by one of two anesthesiologists using fentanyl, propofol, rocuronium, nitrous oxide, isoflurane. Normothermia was maintained by covering the patient with a warming blanket. An epidural catheter was inserted between T9-T11 vertebral levels. 15-20 ml of 0.5% Bupivacaine was injected to ensure bilateral segmental sensory block to pin prick from T6-L1 dermatomal levels which was maintained with bolus doses of 0.25% Bupivacaine (approximately 5-10 $ml \cdot hour^{-1}$). All patients received a bolus of 10 $ml \cdot kg^{-1}$ i.v. normal saline before induction

followed by 5 $ml \cdot kg^{-1} \cdot hr^{-1}$ i.v. during surgery. Blood losses were replaced with Pentaspan (Bristol-Myers, Squibb, Montreal, Quebec, Canada). Postoperative analgesia was maintained using continuous epidural infusion of 0.1% Bupivacaine supplemented with 3 $\mu g \cdot ml^{-1}$ fentanyl adjusted to keep pain scores at rest below 4 (numerical scale from 0= no pain to 10= worst imaginable pain).

5.3.2.3. STABLE ISOTOPE TRACER INFUSION

Sterile solutions of tracers, purchased from Cambridge isotope Labs, (Cambridge, MA) were prepared by Galenova Inc., St. Hyacinthe, QC and underwent bacterial and fungal sterility culture testing (RVH Microbiology Lab), and pyrogen testing (Nucro-Technics Inc. Scarborough, ON). They were stored at 4^{o}C until use. Each study began with administration of priming doses of $NaH^{13}CO_3$ (1 $\mu mol \cdot kg^{-1}$, PO) and L-[1-^{13}C]leucine (4 $\mu mol \cdot kg^{-1}$ i.v.), [6,6-$^{2}H_2$]glucose (19 $\mu mol \cdot Kg^{-1}$, i.v.) and L-[$^{2}H_5$]phenylalanine (12.35 $\mu mol \cdot Kg^{-1}$, i.v.) to hasten the plateau. This was followed by continuous, i.v. infusion of L-[1-^{13}C]leucine (0.06 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and [6,6-$^{2}H_2$]glucose (0.22 $\mu mol \cdot Kg^{-1} \cdot min^{-1}$) for 3 hours and L-[$^{2}H_5$]phenylalanine (0.15 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) for 6 hours using a cannula inserted in a superficial forearm vein.

5.3.2.4. SAMPLING

Blood was sampled from a dedicated cannula placed in a superficial vein of the hand contra lateral to the forearm used for isotope infusion. The cannula was kept patent with heparinised saline. Breath samples were collected by asking the patient to breath into a 2L latex bag and transferring the expired air to vacutainers using a syringe

and needle. Samples were collected before isotope administration for measuring baseline enrichments of [1-^{13}C] α-ketoisocaproate ([1-^{13}C] α-KIC) and [6, 6-$^{2}H_2$] glucose in blood and $^{13}CO_2$ in expired air. Those collected at 150, 160, 170 and 180 minutes of isotope infusion were used to assess plateau enrichments. Blood samples were transferred immediately to heparinised tubes and placed on ice, followed by centrifugation at 4°C and aliquots of plasma stored at -70°C. For measurement of phenylalanine enrichment in plasma proteins, blood samples were collected hourly from baseline to six hours, transferred into pre-chilled sodium EDTA tubes containing protease inhibitor cocktail (P2714, Sigma-Aldrich, St. Louis, MO), centrifuged at 4°C and plasma stored at -70°C for later analysis (Schricker, Meterissian et al. 2008). Blood samples were also collected at 180 min of pre and post-operative infusion periods to measure plasma levels of glucose, amino acids, insulin, cortisol, total proteins, albumin and fibrinogen.

5.3.3. ANALYTICAL METHODS

5.3.3.1. WHOLE BODY LEUCINE KINETICS.

Plasma α-KIC was derivatized to its pentafluorobenzyl ester (Hachey, Patterson et al. 1991; Schricker, Meterissian et al. 2008). Isotopic enrichment was analyzed on a 5975C gas chromatograph mass spectrometer (GC/MS, Agilent Technologies Canada, Mississauga, ON), using methane negative chemical ionization and selected-ion monitoring at 129 m/z and 130 m/z after isolation in the 7890 GC (Agilent) equipped with a SP 2380 column (Supelco, Bellfonte, PA). The $^{13}CO_2$ enrichments were analyzed by

isotope ratio-mass spectrometry (IRMS Analytical Precision AP2003, Manchester, UK) (Schricker, Meterissian et al. 2008). Isotopic enrichment was calculated as atoms percent excess (APE).

5.3.3.2. GLUCOSE KINETICS

Plasma glucose was derivatized to its penta-acetate compound, and the [6,6-$^{2}H_{2}$]glucose enrichment determined by GC/MS using electron-impact-ionization and selected ion monitoring at 200m/z and 202m/z after separation on a J&W DB-1701 (15mx0.25mmx0.24um) column (Schricker, Meterissian et al. 2008).

5.3.3.3. PLASMA PROTEINS KINETICS

Total proteins (TP) in plasma were isolated by precipitation with trichloroacetic acid. Albumin and fibrinogen were isolated by differential solubility in ethanol and purified by gel electrophoresis using 8% SDS-PAGE on a Mini-Protean II System (Bio-Rad Laboratories, Hercules, CA). VLDL-apo-B100, which represents the precursor pool enrichment, was isolated by ultracentrifugation followed by isopropanol precipitation. Following hydrolysis of proteins, amino acids were isolated by cation exchange chromatography (Dowex-50W-X8, Bio-Rad Laboratories, Hercules, CA). After esterification and derivatisation to the n-propyl ester heptafluorobutyramide, Phenylalanine enrichment was analyzed by GC/MS equipped with J&W DB 5MS (30mx0.25mmx1.0um) by monitoring under methane negative chemical ionization conditions the $[M\text{-}FH]^{-}$ ions at mass to charge ratio 383 to 388. Tracer: tracee ratios were

determined using raw ion abundances, and analysis of a standard curve incorporating the tracer and natural abundance of phenylalanine.

5.3.3.4. RESTING METABOLIC RATE BY INDIRECT CALORIMETRY

Indirect calorimetry was performed (True One 2400 Metabolic Measurement System, Parvo Medics, Inc., Utah, USA) with subjects lying in a semi-recumbent position (20°) and breathing room air in a ventilated hood for 30 minutes. Measurements were performed on two occasions, first on preoperative day (10.00 AM) and next on the 2^{nd}POD (10.00 AM). Oxygen consumption (VO_2) and carbon dioxide (VCO_2) production were measured and RMR and respiratory quotient (RQ) calculated there from. Average values were taken, with a coefficient of variation (CV) less than 10%. The preoperative RMR measurement was used to guide amino acid infusion rate.

5.3.3.5. METABOLIC SUBSTRATES, HORMONES AND INFLAMMATORY MARKERS.

Plasma glucose, serum insulin, cortisol, TP, albumin, and fibrinogen were measurements were carried out in the hospital central laboratory.

Plasma glucose was determined by a glucose oxidase method using a glucose analyzer 2 (Beckman Instruments, Fullerton, CA).

Plasma amino acids underwent precolumn OPA derivatisation analyzed by UV absorbance using an Agilent 1290 UHPLC equipped with a Poroshell 120 EC-C18 (4.6mx150mmx2.7um) column (Agilent). Analysis was performed by Masshunter software (version B.04).

Serum insulin and cortisol were determined by chemiluminescent microparticle immunoassay (CMIA, Access Immunoassay Systems).

TP (biuret method) and albumin (bromocresol dye technique) were measured using the VP Super System (Abbott Laboratories, Irving, TX).

Fibrinogen was measured by the modified Clausse method using Multifibrin U reagent (BCS, Seimens).

5.3.4. CALCULATIONS

Whole body glucose and leucine kinetics were calculated by conventional isotope dilution technique using a two-pool stochastic model during steady-state conditions. At isotopic steady state, the rate of appearance (Ra) of unlabeled substrate can be derived from plasma enrichment (APE). It is calculated by $R_a = (APE_{inf}/APE_{pl}-1)\cdot F$, where F is the infusion rate of the labeled tracer ($\mu mol\cdot kg^{-1}\cdot min^{-1}$), APE_{inf} is the tracer enrichment in the infusate, and APE_{pl} is the tracer enrichment in plasma at steady state. The APE value used in this calculation represents the mean of APE values determined during each isotopic plateau. The accuracy of the isotopic enrichments at isotopic plateau was tested by evaluating the scatter of the APE values around their mean, expressed as the CV. A CV < 5% for 4 values was used as a confirmation of a valid plateau.

5.3.4.1. WHOLE BODY LEUCINE KINETICS

In the measurement of leucine kinetics, plasma enrichment of α-[1-^{13}C]KIC is used to represent the intracellular precursor pool enrichment (Schwenk, Beaufrere et al. 1985) (Schwenk, Tsalikian et al. 1985) .

The Ra of leucine (Ra Leu) represents the total movement of leucine into and from the plasma pool. Leucine kinetics are measured from the flux as described previously (Schricker, Meterissian et al. 2008). In the calculation of leucine oxidation (Leu Ox), a factor of 0.76 during the fasted state and a factor of 0.8 during the fed state were applied to account for the fraction of ^{13}C- carbon dioxide released from leucine but retained within slow turnover rate pools. (Matthews, Motil et al. 1980).

5.3.4.2. GLUCOSE KINETICS.

Under post-absorptive conditions, the rate of appearance of glucose (Ra Glu) is the sum of varying proportions of glycogenolysis and gluconeogenesis. In the postabsorptive state, gluconeogenesis accounts for progressively higher proportions of endogenous glucose production over time, accounting for just about 50% of endogenous glucose production after an overnight fast to almost 100% after 42 hours of fasting (Landau, Wahren et al. 1996; Chandramouli, Ekberg et al. 1997). In the present study, patients received amino acids providing 20% of the RMR leading to average blood glucose levels of 8 mmol/L or less. Feeding glucogenic precursors is expected to contribute to hepatic gluconeogenesis both by direct and indirect pathways. Hoewever, assuming that in the absence of enteral feeding the splanchnic blood glucose levels would not be higher than

the systemic levels, hepatic gluconeogenesis would be sufficiently low to be considered insignificant (Radziuk and Pye 2001). From this we can infer that, in the postoperative state gluconeogenesis accounted for almost all of the endogenous glucose production.

At steady state, whole body glucose uptake i.e. glucose rate of disappearance equals Ra Glu (Jacquez 1992). However the capacity of tissues to take up glucose depends on and changes proportionally with the prevalent glucose concentration. Thus the glucose clearance (Glu Cl) which is the Ra Glu divided by glucose concentration, serves as a measure of capacity of tissues to take up glucose.

5.3.4.3. FRACTIONAL PLASMA PROTEIN SYNTHESIS RATES.

FSR of plasma proteins (the total pool, albumin, or fibrinogen) was calculated using the flowing equation:

$$FSR\ (\%\cdot d^{-1}) = \frac{(Et_2 - Et_1) \times 24 \times 100}{E_{free} \times (t2 - t1)}$$

Where Et_2-Et_1 is the increase in the tracer: tracee ratio of phenylalanine incorporated in the particular plasma protein during the final three hours of the infusion (t2-t1) measured by linear regression of the enrichment in samples from hours three to six of the infusion and expressed in moles per cent excess; and E_{free} is the precursor pool L-[$^2H_5$] phenylalanine enrichment. For the purpose of this calculation the isotopic enrichment of VLDL Apo B100 at steady state is taken to represent the isotopic

enrichment of the precursor pool (Reeds, Hachey et al. 1992). Absolute intravascular synthesis rate was calculated as the product of FSR, plasma concentration of the protein and the estimated plasma volume (Schricker, Meterissian et al. 2008).

5.3.5. STATISTICS

Data are presented as means ± SD. The principal outcome was leucine balance. Based on an expected difference in leucine balance of 10 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ between the two groups and a SD of 6 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ in each group, a sample size of 6 patients per group would provide a power of 80% with a type I error of 5%. (Schricker, Wykes et al. 2005; Lugli, Donatelli et al. 2010). However, due to the high variability observed in T2DM group, recruitment was continued beyond the original calculated sample size. Analyses of dependent variables were performed using two factorial analysis of variance for repeated measures. Normality of data was tested using the normal distribution curves (skewness between -1 and +1, Kurtosis between-3 and +3). Non–normal data were transformed as shown in APPENDIX 2 to achieve normality. Significant effects induced by nutrition were assumed when *P* values for time dependency (comparing the preoperative fasted and postoperative fed states) were less than 0.05. Influences by the feeding regimen were accepted as significant when the interaction term of the analysis of variance were less than 0.05. Demographic data were compared by Student's t-test. All analyses were performed using the general linear model in SAS version 9.2.

5.4. RESULTS

Patient characteristics were similar between the two groups (Table 1), except that HbA_1C, as expected was higher in the T2DM group. Oral hyperglycemic agents were effective in maintaining patients in the T2DM group in relatively good glycemic control; however prescription details were not available.

Isotopic steady states in [6, 6-$_2H^2$]glucose , [1-^{13}C]α- KIC and ^{13}C-CO_2 were achieved in all patients (CV<5%) permitting the use of steady state equations. Blood glucose concentration (Table 2) was higher inT2DM group both pre and post-operatively (p=0.005); however glucose kinetics were comparable in both groups and remained unchanged by nutrition and surgery.

In the preoperative fasted state, Leu Bal (Table 3) was negative in both groups, while being more negative in the T2DM group (p=0.030). Leu Ox showed a tendency to be higher in T2DM group (p=0.060). In the postsurgical fed state, Leu Ra and Leu Ox increased in both groups (P=0.005 and P<0.0001, respectively). Nutrition led to improved Leu Bal in both groups (p=0.008). However because of absence of group difference, balance remained significantly more negative in T2DM group.

Albumin concentration (Table 4) was lower following surgery in all patients (P<0.0001); however neither FSR nor ASR was changed. The classic acute phase protein, fibrinogen, showed dramatic increases in concentration, FSR and ASR (P<0.0001, P=0.0007, P<0.0001, respectively), following surgery. TP concentration in plasma decreased after surgery (P<0.0001), reflecting the change in its largest constituent

protein; however FSR doubled in both groups reflecting the APR (P<0.0001). Differences between patient groups did not reach statistical significance.

BCAA and EAA (Table 5) increased in the postoperative fed state in both groups (P<0.0001) but to a lesser extent in T2DM (P<0.04, P<0.03 respectively) along with an increased Phe/ Tyr (P<0.0001). Concentrations of the following AA decreased in both patient groups with nutrition support after surgery: glutamine, glutamate, asparagine and lysine; while concentrations of phenylalanine, methionine, arginine and glycine increased.

VO2, VCO2 and RMR (Table 6) were higher in T2DM group (P=0.01) and were unchanged by surgery and nutrition. In contrast, RQ was comparable in the two groups and was reduced by nutrition support (P=0.005).

There were no differences observed in hormones or HOMA-IR (Table 7). Using simple linear regression, HOMA-IR was negatively associated with Leu Bal and positively associated with whole body proteolysis only in T2DM (APPENDIX3).

TABLE 1. Characteristics of Patients

Characteristic	NON-T2DM	T2DM
Age (y)	65 ± 15	70 ± 11
Gender (M/F)	6/5	5/3
Weight on admission (kg)	75 ± 11	75 ± 12
Height (m)	1.70 ± 0.09	1.68 ± 0.08
BMI ($kg \cdot m^{-2}$)	26± 2.1	27 ± 3.8
HbA_1C (%)*	5.7 ± 0.4	6.8 ± 1.3
Type of surgery		
Low Anterior Resection	2	4
Hemi/ Transverse colectomy	5	4
Sigmoid resection	3	0
Small Bowel Resection	1	0

Values indicate mean ± SD; N=11/9 (NON-T2DM /T2DM)

* different between groups P=0.05 N=9/8 (NON-T2DM /T2DM)

TABLE 2. Whole Body Glucose Kinetics

	NON-T2DM		T2DM		*P*		
	Preop fasted	**Postop fed**	**Preop fasted**	**Postop fed**	**Group ***	**Time †**	**Interaction ±**
Glucose rate of appearance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	11.6 ±1.9	11.2 ±2.4	13.5 ±3.6	14.8±5.8	**0.10**	0.57	0.55
Glucose clearance ($ml \cdot kg^{-1} \cdot min^{-1}$)	2.2 ±2.0	2.2 ±0.6	2.0 ±0.3	2.0 ±0.4	0.13	0.75	0.96
Glucose concentration ($mmol \cdot L^{-1}$)	5.4 ±0.9	5.1 ±1.0	6.8 ±1.0	8.0 ±4	**0.005**	0.69	0.77

Values indicate mean ± SD N = 11/7 (NON-T2DM /T2DM)

† Probability that values change from preoperative fasted to postoperative fed state

* Probability the values are different between groups

± Probability that the change over time is different between groups

TABLE 3. Whole Body Leucine Kinetics

	NON-T2DM		T2DM		*P*		
	Preop fasted	**Postop fed**	**Preop fasted**	**Postop fed**	**Group ***	**Time †**	**Interaction‡**
Leucine rate of appearance	99±17	129±24	111±22	123 ±27	0.670	**0.005**	0.140
(μmol·kg^{-1}·h $^{-1}$)							
Endogenous leucine rate of appearance	99±17	107±22	111±22	99±24	0.980	0.630	0.100
(μmol·kg^{-1}·h $^{-1}$)							
Leucine oxidation	13±4	27±10	18±7	28±5	0.060	**<.0001**	0.350
(μmol·kg^{-1}·h $^{-1}$)							
Non-oxidative leucine disposal	86 ±18	103±19	92± 16	87 ± 25	0.450	0.490	0.060
(μmol·kg^{-1}·h $^{-1}$)							
Leucine balance	-13±4	-5±8	-18±7	-12±12	**0.030**	**0.008**	0.720
(μmol·kg^{-1}·h $^{-1}$)							

Values indicate mean ± SD

N = 11/8 (NON-T2DM /T2DM)

* Probability the values are different between groups

† Probability that values change from preoperative fasted to postoperative fed state

± Probability that the change over time is different between groups

TABLE 4. Plasma Protein Synthesis Rates and Concentrations

	NON-T2DM		T2DM		*P*		
	Preop Fasted	**Postop Fed**	**Preop Fasted**	**Postop Fed**	**Group***	**Time†**	**Interaction ±**
Albumin FSR ($\%\cdot d^{-1}$)	17 ± 3.6	16± 3.6	10± 2.5	18± 14.8	0.06	0.33	0.16
Albumin ASR ($mg\cdot kg^{-1}\cdot day^{-1}$)	199± 46	139± 24	126± 33	156± 112	0.06	0.25	0.33
Albumin concentration ($g\cdot L^{-1}$)	36± 4.2	26 ± 4.9	37± 1.6	27 ± 5	0.53	**<.0001**	0.85
Fibrinogen FSR ($\%\cdot d^{-1}$)	38 ± 11	56± 11	32± 16	53 ± 20	0.08	**0.0007**	0.45
Fibrinogen ASR ($mg\cdot kg^{-1}\cdot day^{-1}$)	40± 11	104± 24	32 ± 16	92± 47	0.07	**<.0001**	0.69
Fibrinogen concentration ($g\cdot L^{-1}$)	3.3 ± 0.3	5.2 ± 0.5	3.0 ± 0.4	5.0 ± 1.1	0.19	**<.0001**	0.67
Total proteins FSR ($\%\cdot d^{-1}$)	22± 4	39 ± 10	18 ± 7	36 ± 9	0.19	**<.0001**	0.85
Total proteins ASR ($mg\cdot kg^{-1}\cdot day^{-1}$)	417 ± 83	625 ± 145	354 ± 123	581 ± 122	0.17	**<.0001**	0.48
Total protein concentration ($g\cdot L^{-1}$)	60 ± 6	47 ± 7	62 ± 5	48± 5	0.39	**<.0001**	0.77

Values indicate mean ± SD ; Albumin

N (NON-T2DM /T2DM) = 8/6;

Fibrinogen N = 8/6; Total Proteins N = 9/7

* Probability the values are different between groups

† Probability that values change from preoperative fasted to postoperative fed state

± Probability that the change over time is different between groups

TABLE 5. Plasma Amino Acid Concentrations (µmol·L^{-1})

	NON-T2DM		T2DM		*P*		
	Preop Fasted	Postop Fed	Preop Fasted	Postop Fed	Group *	Time†	Interaction ±
Isoleucine	51±15	208 ± 51	77±24	183± 24	0.95	**<.0001**	**0.04**
Leucine	110± 23	271± 58	156±33	248± 35	0.07	**<.0001**	**0.008**
Valine	191±37	502± 110	274±47	484± 62	**0.02**	**<.0001**	**0.008**
BCAA	352±68	980± 214	508± 99	916± 117	0.37	**<.0001**	**0.04**
Threonine	93 ± 24	96 ± 21	108 ± 33	94± 27	0.58	0.63	0.39
Lysine	161 ±30	156± 18	205 ±43	158± 27	**0.04**	**0.02**	0.06
Histidine	54 ± 10	69 ± 10	59 ±8	63 ± 11	0.96	**0.01**	0.13
Phenylalanine	77± 10	130± 20	78 ± 7	126± 21	0.81	**<.0001**	0.66
Tryptophan	46± 7	52±13	50± 7	53± 16	0.61	0.35	0.69
Methionine	22± 3	43± 9	24± 6	42± 8	0.60	**<.0001**	0.45
EAA	805±129	1526 ± 232	1030± 155	1451± 177	0.25	**<.0001**	**0.03**
Cysteine	16± 2	15± 3	17±3	16± 3	0.23	0.35	0.99
Glutamate	66±19	37 ± 14	58± 18	33± 13	0.32	**<.0001**	0.72
Glutamine	795 ± 111	549 ± 54	848± 143	556 ± 82	0.41	**<.0001**	0.54
Asparagine	28± 8	17± 5	36± 13	18± 4	0.11	**<.0001**	0.48
Serine	83±17	110± 29	106± 23	114 ± 26	0.1	**0.05**	0.21
Glycine	191 ± 39	248 ± 50	231 ±91	258 ± 70	0.39	**0.02**	0.52
Arginine	65 ± 15	91 ± 20	74 ±25	90 ± 19	0.55	**0.004**	0.52
Alanine	390 ±136	377± 110	430±228	336± 104	0.82	0.46	0.54
Tyrosine	58± 9	62± 18	57± 5	60± 10	0.93	0.46	0.99
Total	2497 ± 344	3030 ± 351	2888 ± 555	2932± 423	0.33	0.06	0.11
Phe/Tyr	1.36 ± 0.16	2.20 ± 0.47	1.38 ± 0.18	2.16 ± 0.56	0.93	**<.0001**	0.79

Values indicate mean ± SD ;
N = 9/7(NON-T2DM /T2DM)

* Probability the values are different between groups

† Probability that values change from preoperative fasted to postoperative fed state

± Probability that the change over time is different between groups

TABLE 6. Gaseous Exchange and Resting Metabolic Rate

	NON-T2DM		T2DM		*P*		
	Preop Fasted	Postop Fed	Preop Fasted	Postop Fed	Group*	Time†	Interaction±
VO_2 ($ml \cdot min^{-1}$)	189 ±34	201 ±42	225 ±55	239 ±45	**0.01**	0.38	0.96
VCO_2 ($ml \cdot min^{-1}$)	164 ±25	152 ±27	187 ±43	177 ±24	**0.02**	0.29	0.9
RQ	0.88 ±0.13	0.76 ±0.06	0.83 ±0.07	0.76 ±0.10	0.42	**0.005**	0.54
RMR ($kcal \cdot d^{-1}$)	1319 ±219	1368 ±274	1558 ±374	1695 ±469	**0.01**	0.40	0.69
RMR ($kcal \cdot kg^{-1} \cdot d^{-1}$)	17 ±2.4	18 ±4.3	21 ±3.5	23 ±5	**0.008**	0.28	0.67

Values indicate mean ± SD ; N = 11/8 (NON-T2DM /T2DM)

* Probability the values are different between groups

† Probability that values change from preoperative fasted to postoperative fed state

± Probability that the change over time is different between groups

TABLE 7. Metabolic Hormones and HOMA-IR

	NON-T2DM		T2DM		*P*		
	Preop Fasted	Postop Fed	Preop Fasted	Postop Fed	Group *	Time†	Interaction ±
Insulin concentration (pM)	40 ±30	28 ±19	30 ±14	26 ±14	0.56	0.27	0.88
Cortisol Concentration (nM)	213 ±69	318 ±243	243 ±72	379 ±167	0.18	0.11	0.57
HOMA-IR	1.5 ±1.3	1.0 ±0.8	1.3 ±0.7	1.2 ±0.7	0.49	0.30	0.72

Values indicate mean ± SD ; N = 11/8 (NON-T2DM /T2DM)

* Probability the values are different between groups

† Probability that values change from preoperative fasted to postoperative fed state

± Probability that the change over time is different between groups

5.5. DISCUSSION

In patients without T2DM, PAA maintained normoglycemia postoperatively, unchanged from fasting levels. Glycemia was similar to fasting values previously seen in colorectal cancer patients (Schricker, Meterissian et al. 2008; Lugli, Schricker et al. 2010) as well as normal volunteers (Schricker, Berroth et al. 1996; Schricker, Klubien et al. 2000) and lower than values achieved with glucose containing regimens (Schricker, Meterissian et al. 2008; Lugli, Donatelli et al. 2010). Moderate doses of glucose have not been observed to modify the increased proteolysis typically seen postoperatively (Schricker, Wykes et al. 2005; Lugli, Schricker et al. 2010). A short course of high dose amino acids, in contrast, effectively suppressed proteolysis while maintaining normoglycemia in patients without T2DM (Donatelli, Schricker et al. 2006).

Patients with T2DM were well controlled with oral hypoglycemic agents as seen from the moderate HbA_1C levels as seen in TABLE 1 (2012). PAA administration in the present protocol avoided severe hyperglycemia that typically accompanies even judicious glucose regimens in the state of increased IR, triggered by surgical stress (Lugli, Donatelli et al. 2012). Short term high dose PAA infusion maintains similar normoglycemia but additionally suppresses proteolysis compared to fasting or dextrose administration (Lugli, Donatelli et al. 2012). Thus continuously supplied moderate dose of PAA in the present study may have provided gluconeogenic precursors to achieve normoglycemia in controls and the slightly increased glucose concentration in patients with T2DM while preventing the typical postoperative increase in proteolysis seen with glucose only regimens. The higher plasma glucose levels in these patients may be

explained by the trend of increased endo Glu Ra (P=0.10) together with decreased glucose clearance (P=0.13). Surprisingly, no group differences in either HOMA or insulin levels were seen.

Preoperative leucine kinetics were similar to values seen in normal volunteers (Schricker, Klubien et al. 2000) contrary to a previous report of high protein turnover in cancer-bearing patients (Fearon, Hansell et al. 1988). As expected, Leu Bal was negative in the fasted state. In patients without T2DM, Leu Ox doubled with PAA administration. Oxidation typically increases with high dose amino acids (Donatelli, Schricker et al. 2006) but not with infusions of glucose-only or moderate dose AA regimens containing glucose. (Schricker, Meterissian et al. 2004; Schricker, Meterissian et al. 2008). This response pattern suggests that in the absence of glucose, glucogenic AAs are diverted to gluconeogenesis, which minimized their availability for protein metabolism. In the absence of significant changes in synthesis or breakdown, average Leu Bal remained negative after surgery with only 33 per cent of control patients attaining positive balance. So far, significant net positive balance has been achieved only with a high dose of PAA (Donatelli, Schricker et al. 2006). The greater improvement in balance over the fasted state seen with moderate dose PAA regimen containing glucose compared to the current amino acid-only regimen (Schricker, Meterissian et al. 2008) again supports the notion that co-administration of glucose may improve the anabolic response to PAAs.

Patients with T2DM were in more negative Leu Bal before surgery primarily due to higher Leu Ox. Evidence is divided as to whether oxidation is generally higher (Gougeon, Styhler et al. 2000; Woerle, Szoke et al. 2006) or unaffected (Staten, Matthews et al.

1986; Schricker, Gougeon et al. 2005; Bell, Volpi et al. 2006) in T2DM . The increase in Leu Ox with PAA administration was more moderate in T2DM patients compared to the controls. Despite this, the improvement in Leu Bal was modest and lower than that achieved in controls, with only 13 percent patients in T2DM group realizing net anabolism after surgery. When leucine kinetics with high dose PAA administration are compared to the present results, we see that the higher balance in the former was accompanied by increased synthesis and reduction in breakdown while oxidation was comparable (Lugli, Donatelli et al. 2012). Resistance to the anabolic effect of insulin has been substantiated in patients with T2DM even remote to the state of surgical stress (Pereira, Marliss et al. 2008). It was subsequently demonstrated that hyper aminoacidemia can overcome this resistance (Bassil, Marliss et al. 2011). Thus our results probably suggest that in control patients Leu Bal can probably benefit from a moderate dose of glucose or higher dose of PAA. Patients with T2DM may have catabolism triggered by IR which is not modifiable by a moderate dose of PAA suggesting that protein requirements maybe significantly higher in this group.

Fibrinogen synthesis rate in the present study was higher compared to non-cancer-bearing patients (Barber, Fearon et al. 2000). Thus cancer induced inflammatory state may have up regulated fibrinogen synthesis (Barber, Fearon et al. 2000) and may have prevented emergence of the anticipated higher fibrinogen synthesis in patients with T2DM (Barazzoni, Kiwanuka et al. 2003; Tessari, Kiwanuka et al. 2006). Fibrinogen concentration and rate of synthesis increased dramatically in both groups after surgery. This rise is typically seen in response to surgical injury (Desborough 2000) as well as to

balanced nutritional support in cancer bearing patients (Barber, Fearon et al. 2000). However the augmentation in synthesis observed in the present study was lower than that obtained with supplementation of PAA with a moderate dose of glucose (Schricker, Meterissian et al. 2008). This suggests that glucose included in PNS may better support APR by sparing AA.

Albumin concentration and synthesis were similar in both groups. Similar observations were made by Tessari et al. in non-surgical normoalbuminuric patients with and without T2DM (Tessari, Kiwanuka et al. 2006). The postoperative drop in albumin levels is common and occurs despite increased synthesis (Rittler, Jacobs et al. 2007). The decrease may result from hemodilution given the postoperative drop of hematocrit (NonT2DM preop v/s. postop-35 v/s.30; T2DM preop v/s. postop-34 v/s.31). Other plausible explanations maybe transcapillary escape (Fleck, Raines et al. 1985) or the effect of anesthesia (Carli, Ronzoni et al. 1993). The present protocol providing nearly 1 $g \cdot kg^{-1} \cdot d^{-1}$ of PAA alone did not stimulate albumin synthesis in contrast to increased synthetic rates seen with addition of glucose to PAA (Schricker, Meterissian et al. 2008). Even a higher dose of PAA alone (1.6 $g \cdot kg^{-1} \cdot d^{-1}$) is ineffective in stimulating albumin synthesis (Rittler, Kuppinger et al. 2009) suggesting higher protein requirement in the absence of glucose administration.

Synthesis of total plasma proteins represents the weighted average of synthesis of all plasma proteins and was increased significantly in both groups following the APR. Given that albumin is the most abundant intravascular protein, the low postoperative total protein concentration probably follows the drop in albumin concentration. Taken

together these results suggest that APR, in particular albumin synthesis, is sensitive to and modifiable by nutritional intake. Inclusion of glucose in PAA regimens may results in protein sparing, making greater proportions of administered PAA available for APR and peripheral tissue anabolism. Moderate dose of PAA alone result in both lower stimulation of APR as well as lower anabolic response as compared to the former regimen.

VO_2 and VCO_2 and hence RMR were significantly higher in patients with T2DM. Higher RMR in patients with T2DM is thought to be associated with the higher energy cost of protein turnover with RMR predicting leucine flux and synthesis. (Gougeon, Morais et al. 2008). However these last two were not different between groups in the present study. Another study identified fat free mass and not diabetic status or glycemia as a determinant of RMR (Ryan, Salle et al. 2006). However, FFM was not measured in the present study. RMR remained unchanged in response to nutrition contrary to reports of increased metabolic rate with feeding (Pacy, Garrow et al. 1988; Fredrix, Soeters et al. 1991).

The baseline amino acid profile of patients without T2DM was similar to that of healthy controls studied in the fasted state (Jackson, Phillips et al. 2001).Patients with T2DM had significantly higher valine levels and a trend to higher leucine in the fasted state. Insulin is known to suppress skeletal muscle proteolysis while insulin resistance of T2DM may reverse this suppression increasing BCAA release (Wijekoon, Skinner et al. 2004; Adeva, Calvino et al. 2012). The resolution of these differences with feeding in the postoperative period, however, cannot be explained. Levels of BCAA, EAA,

phenylalanine, methionine and the phenylalanine to tyrosine ratio (phe/tyr) were increased in the postoperative period, while levels of glutamine, glutamate, asparagine and lysine decreased. These changes are common in postoperative aminograms (Askanazi, Furst et al. 1980; Vinnars, Holmstrom et al. 1983; Ebeling, Tuominen et al. 2001). Postoperative increases in BCAA are a consistent finding seen in surgical patients and may result from increased proteolysis releasing AAs into the plasma pool (Askanazi, Furst et al. 1980; Holecek 2002). Evidence shows greater increases in BCAA levels with PAA-only regimen as compared to glucose containing PNS (Askanazi, Furst et al. 1980). This is supported in the present study with a greater postoperative increase in BCAA as compared to that seen with the inclusion of glucose in PAA regimen (Schricker, Meterissian et al. 2008). Glutamine, the most abundant amino acid, serves important immune and regulatory functions. BCAA act as nitrogen donors in synthesis of glutamine (Holecek 2002) which may become conditionally essential in stress (Melis, ter Wengel et al. 2004). Drop in postoperative glutamine levels, similar to present results has been demonstrated in the absence of its supplementation (Mittendorfer, Gore et al. 1999; Viggiano, Passavanti et al. 2012). Supplementation, on the other hand, is known to improve balance through protein sparing (Lin, Kung et al. 2002). Thus we can infer that in the absence of glutamine supplementation, proteolysis remains stimulated to provide BCAA as precursors. However, levels still drop due to inability to meet the high glutamine demands. The resultant imbalance of amino acid profile coupled with the lack of proteolytic suppression could have limited the anabolic response with the present protocol. An interesting pattern was the relative accumulation of EAA in plasma in the

fed state. This was evident by the increase in ratio of total EAA to total AA in both groups from the fasted (0.32/ 0.35: nonT2DM/T2DM) to fed (0.5/0.49: nonT2DM/T2DM) state. Similar changes in ratio have been seen in healthy volunteers administered PAA solutions without energy supply, while the ratio remained stable with glucose co-administration. These changes in amino acid patterns are related to the accelerated use of NEAA for gluconeogenesis and energy supply at the expense of the nitrogen balance , in the absence of an exogenous energy source (Tweedle, Fitzpatrick et al. 1977).This imbalance with respect to relative proportions of essential and non-essential AAs could also have limited protein synthesis and the anabolic response. The increase in phe/tyr is normally seen in the postoperative setting (Holbrook, Gross et al. 1979) and may be attributed to increased catabolism increasing phenylalanine flux, transient hepatic dysfunction preventing phenylalanine to tyrosine conversion or unbalanced nutrition support (Herndon, Wilmore et al. 1978). No change in baseline protein breakdown was demonstrated in the present study. The hepatic dysfunction theory has been refuted by Wannemacher et al. (Wannemacher, Klainer et al. 1976). However, the low level supplementation of tyrosine through Travasol may account for the change observed. These findings highlight the need for rethinking the composition of amino acid solution to better suit the requirements of the perioperative subjects. With the avoidance of glucose for better glycemic control, the imbalance of amino acid profile may take on even greater significance, by hindering improvement in leucine balance.

The present study had some limitations. First, the small sample size may have prevented emergence of significant differences between groups. Second, changes in the

postoperative fed state were evaluated against a baseline preoperative fasted state. This study design may have precluded clear separation of the influence of surgery or nutrition on the parameters of interest. Yet, the design allowed effective testing of the hypothesis and results are specific to the conditions of the study. Lastly, [6,6-$^{2}H_2$]glucose measures endogenous glucose production and does not differentiate between glycogenolysis and hepatic gluconeogenesis. Nonetheless, evidence suggests that prolonged fasting leads to progressive diminution of glycogenolysis over time (Landau, Wahren et al. 1996; Chandramouli, Ekberg et al. 1997). Thus we infer that glycogenolysis accounted minimally to endogenous glucose production in the postoperative state.

In conclusion, in the preoperative fasted state, patients with T2DM are more catabolic and hyperglycemic than patients without T2DM. In patients without T2DM, PAA maintain preoperative levels of normoglycemia and improve leucine balance without reaching neutral balance. Moreover stimulation of hepatic protein synthesis is less than that observed with co-administration of glucose. Thus, in these patients, a moderate dose of glucose may enhance protein sparing of PAA only regimen, achieving a higher anabolic response coupled with greater stimulation of albumin synthesis without loss of glycemic control. However, in patients with T2DM, the trend towards a higher endogenous glucose production maintains moderate levels of hyperglycemia with PAA in the postoperative period. Here, avoidance of glucose is vital to glycemic control, yet at the expense of a greater improvement in leucine balance. These findings taken together with speculations over the sufficiency of current protein allowance

recommendations in critical illness (Hoffer and Bistrian 2012) suggest that the present protocol may be protein deficient especially in T2DM. Additionally the amino acid profiles point towards depletion of non essential glucogenic amino acids like glutamine. Thus future trials providing higher doses of amino acids or improved composition of amino acid solution are necessary to determine if an amino acid based PNS regimen can institute positive leucine balance in patients with T2DM and ultimately answer the question “do we really need glucose?”

5. CONCLUSION

5.1. INTERPRETATION OF RESULTS

The present study compared the effect of a prolonged PAA only regimen in patients with and without T2DM undergoing colorectal cancer surgery. Whole body glucose and protein kinetics and synthesis of hepatic secretory proteins were measured with the help of stable isotope tracers.

We found that in the fasted state all patients were in negative leucine balance. Patients with T2DM were characterized by lower leucine balance and moderately higher glucose levels while normoglycemia was maintained in patients without T2DM. PAA infusion saw continuation of the same theme postoperatively despite similar improvements in balance in both groups. Thus patients without T2DM benefitted from maintenance of normoglycemia along with improvements in balance. Patients with T2DM avoided severe hyperglycemia seen with glucose but displayed persistent negative leucine balance. The postoperative plasma amino grams were suggestive of diversion of AAs from protein synthesis into gluconeogenesis and possible imbalance of the composition of AA solution used. These results may be suggestive of insufficiency of moderate doses of PAA alone to induce neutral balance in the state of surgical stress. Patients without T2DM may benefit from co-administration of a judicious dose of glucose or higher dose of PAAs. However due to the IR of T2DM, strategies aimed at improving protein status should avoid glucose and focus on exploring the effect of either a higher dose or improved composition of amino acid solution administered.

5.2. FUTURE RESEARCH

The results of the present study confirm the potential of administration of PAA alone as an anabolic strategy in the perioperative period due to the favorable effects on glycemic control. However, due to inability to attain the target positive leucine balance in both groups, this strategy needs further refining.

The present protocol provided amino acids at approximately $1g \cdot kg^{-1} \cdot h^{-1}$, which is slightly above the current recommendations in hospitalized metabolically normal adults of $0.76g \cdot kg^{-1} \cdot hr^{-1}$. There is growing evidence of the insufficiency of these recommendations in critically ill patients (Hoffer and Bistrian 2012). Our results back up this belief through the sustained negative balance observed in the fed state. Future studies could target defining the optimal level of amino acids or proteins in perioperative nutrition through trials of higher doses of PAA than used in this study.

Analysis of the postoperative plasma aminograms brought out patterns of change which were suggestive of amino acid imbalance arising from the pattern of supplementation or utilization. The pattern of amino acid utilization is relatively unmodifiable as compared to supplementation. Thus attempts to match the composition of amino acid solutions to the requirements of perioperative subjects could include trials of supplementation of amino acids such as glutamine. There is mounting evidence of benefits of glutamine supplementation in the perioperative period (Wilmore 2001). Future studies could explore the benefits obtained in patients with T2DM in

terms of leucine balance and glycemic control, with stable isotope tracers affording analysis of the mechanisms of change.

Insulin resistance of T2DM affecting protein metabolism has been validated by the results of the present study in addition to numerous prior studies (Tessari, Cecchet et al. 2011) . However, research supporting insulin resistance of protein metabolism in T2DM is scant (Pereira, Marliss et al. 2008). Whole body leucine kinetic studies in patients with T2DM under insulin clamp may help identify and measure insulin resistance of protein metabolism, if it exists. Studies in the fed state may also help development of effective anabolic strategies.

Finally, the enteral route of feeding is considered the most efficient and cost-effective. Additionally there is accumulating evidence of the benefits of early enteral nutrition after surgery (2012). Studies can be undertaken to look for any potential benefit of enteral nutrition on the APR or glycemic control in patients with T2DM.

6. BIBLIOGRAPHY

Adeva, M. M., J. Calvino, et al. (2012). "Insulin resistance and the metabolism of branched-chain amino acids in humans." Amino Acids **43**(1): 171-181.

Akhtar, S., P. G. Barash, et al. (2010). "Scientific principles and clinical implications of perioperative glucose regulation and control." Anesth Analg **110**(2): 478-497.

Aki, S. Z., E. Suyani, et al. (2012). "Prognostic role of pre-transplantation serum C-reactive protein levels in patients with acute leukemia undergoing myeloablative allogeneic stem cell transplantation." Clin Transplant **26**(5): E513-521.

Al-Joudi, F. S. (2005). "Prognostic value of an index for serum globulin compensation in colon and breast cancers." Singapore Med J **46**(12): 710-713.

Aoki, Y., M. Iwamoto, et al. (2009). "Prognostic indicators related to death in patients with Pneumocystis pneumonia associated with collagen vascular diseases." Rheumatol Int **29**(11): 1327-1330.

Askanazi, J., P. Furst, et al. (1980). "Muscle and plasma amino acids after injury: hypocaloric glucose vs. amino acid infusion." Ann Surg **191**(4): 465-472.

Association., A. D. (2012). "Executive summary: Standards of medical care in diabetes--2012." Diabetes Care **35 Suppl 1**: S4-S10.

Bagry, H. S., S. Raghavendran, et al. (2008). "Metabolic syndrome and insulin resistance: perioperative considerations." Anesthesiology **108**(3): 506-523.

Barazzoni, R., E. Kiwanuka, et al. (2003). "Insulin acutely increases fibrinogen production in individuals with type 2 diabetes but not in individuals without diabetes." Diabetes **52**(7): 1851-1856.

Barber, M. D., K. C. Fearon, et al. (2000). "Liver export protein synthetic rates are increased by oral meal feeding in weight-losing cancer patients." Am J Physiol Endocrinol Metab **279**(3): E707-714.

Bartlett, R. H., R. E. Dechert, et al. (1982). "Measurement of metabolism in multiple organ failure." Surgery **92**(4): 771-779.

Bassil, M., E. B. Marliss, et al. (2011). "Postprandial hyperaminoacidaemia overcomes insulin resistance of protein anabolism in men with type 2 diabetes." Diabetologia **54**(3): 648-656.

Behrendt, W., J. Raumanns, et al. (1988). "Glucose, fructose, and xylitol in postoperative hypocaloric parenteral nutrition." Infusionstherapie **15**(4): 170-175.

Bell, J. A., E. Volpi, et al. (2006). "Skeletal muscle protein anabolic response to increased energy and insulin is preserved in poorly controlled type 2 diabetes." J Nutr **136**(5): 1249-1255.

Boullata, J., J. Williams, et al. (2007). "Accurate determination of energy needs in hospitalized patients." J Am Diet Assoc **107**(3): 393-401.

Braga, M., O. Ljungqvist, et al. (2009). "ESPEN Guidelines on Parenteral Nutrition: surgery." Clin Nutr **28**(4): 378-386.

Brennan, M. F., P. W. Pisters, et al. (1994). "A prospective randomized trial of total parenteral nutrition after major pancreatic resection for malignancy." Ann Surg **220**(4): 436-441; discussion 441-434.

Brillon, D. J., B. Zheng, et al. (1995). "Effect of cortisol on energy expenditure and amino acid metabolism in humans." Am J Physiol **268**(3 Pt 1): E501-513.

Brownlee, M. (2005). "The pathobiology of diabetic complications: a unifying mechanism." Diabetes **54**(6): 1615-1625.

Carli, F., G. Ronzoni, et al. (1993). "The independent metabolic effects of halothane and isoflurane anaesthesia." Acta Anaesthesiol Scand **37**(7): 672-678.

Carli, F., J. Webster, et al. (1991). "Protein metabolism after abdominal surgery: effect of 24-h extradural block with local anaesthetic." Br J Anaesth **67**(6): 729-734.

Chandramouli, V., K. Ekberg, et al. (1997). "Quantifying gluconeogenesis during fasting." Am J Physiol **273**(6 Pt 1): E1209-1215.

Desborough, J. P. (2000). "The stress response to trauma and surgery." Br J Anaesth **85**(1): 109-117.

Donatelli, F., T. Schricker, et al. (2006). "Postoperative infusion of amino acids induces a positive protein balance independently of the type of analgesia used." Anesthesiology **105**(2): 253-259.

Douglas, R. G. and J. H. Shaw (1989). "Metabolic response to sepsis and trauma." Br J Surg **76**(2): 115-122.

Ebeling, P., J. A. Tuominen, et al. (2001). "Carbohydrate depletion has profound effects on the muscle amino acid and glucose metabolism during hyperinsulinaemia." Diabetes Obes Metab **3**(2): 113-120.

Ehrmann-Josko, A., J. Sieminska, et al. (2006). "Impaired glucose metabolism in colorectal cancer." Scand J Gastroenterol **41**(9): 1079-1086.

Estrada, C. A., J. A. Young, et al. (2003). "Outcomes and perioperative hyperglycemia in patients with or without diabetes mellitus undergoing coronary artery bypass grafting." Ann Thorac Surg **75**(5): 1392-1399.

Fearon, K. C., D. T. Hansell, et al. (1988). "Influence of whole body protein turnover rate on resting energy expenditure in patients with cancer." Cancer Res **48**(9): 2590-2595.

Fleck, A. (1989). "Clinical and nutritional aspects of changes in acute-phase proteins during inflammation." Proc Nutr Soc **48**(3): 347-354.

Fleck, A., G. Raines, et al. (1985). "Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury." Lancet **1**(8432): 781-784.

Frankenfield, D., L. Roth-Yousey, et al. (2005). "Comparison of predictive equations for resting metabolic rate in healthy nonobese and obese adults: a systematic review." J Am Diet Assoc **105**(5): 775-789.

Frankenfield, D. C. and C. M. Ashcraft (2011). "Estimating energy needs in nutrition support patients." JPEN J Parenter Enteral Nutr **35**(5): 563-570.

Frankenfield, D. C., W. A. Rowe, et al. (2003). "Validation of several established equations for resting metabolic rate in obese and nonobese people." J Am Diet Assoc **103**(9): 1152-1159.

Fredrix, E. W., P. B. Soeters, et al. (1991). "Resting energy expenditure in cancer patients before and after gastrointestinal surgery." JPEN J Parenter Enteral Nutr **15**(6): 604-607.

Frisch, A., P. Chandra, et al. (2010). "Prevalence and clinical outcome of hyperglycemia in the perioperative period in noncardiac surgery." Diabetes Care **33**(8): 1783-1788.

Gandhi, G. Y., G. A. Nuttall, et al. (2005). "Intraoperative hyperglycemia and perioperative outcomes in cardiac surgery patients." Mayo Clin Proc **80**(7): 862-866.

Garth, A. K., C. M. Newsome, et al. (2010). "Nutritional status, nutrition practices and post-operative complications in patients with gastrointestinal cancer." J Hum Nutr Diet **23**(4): 393-401.

Gazzaniga, A. B., A. T. Day, et al. (1976). "Endogenous caloric sources and nitrogen balance: regulation in postoperative patients." Arch Surg **111**(12): 1357-1361.

Golden, M. H. (1982). "Transport proteins as indices of protein status." Am J Clin Nutr **35**(5 Suppl): 1159-1165.

Golden, S. H., C. Peart-Vigilance, et al. (1999). "Perioperative glycemic control and the risk of infectious complications in a cohort of adults with diabetes." Diabetes Care **22**(9): 1408-1414.

Gougeon, R., J. A. Morais, et al. (2008). "Determinants of whole-body protein metabolism in subjects with and without type 2 diabetes." Diabetes Care **31**(1): 128-133.

Gougeon, R., K. Styhler, et al. (2000). "Effects of oral hypoglycemic agents and diet on protein metabolism in type 2 diabetes." Diabetes Care **23**(1): 1-8.

Griesdale, D. E., R. J. de Souza, et al. (2009). "Intensive insulin therapy and mortality among critically ill patients: a meta-analysis including NICE-SUGAR study data." CMAJ **180**(8): 821-827.

Groblewska, M., B. Mroczko, et al. (2008). "Serum interleukin 6 (IL-6) and C-reactive protein (CRP) levels in colorectal adenoma and cancer patients." Clin Chem Lab Med **46**(10): 1423-1428.

Group, T. V. A. T. P. N. C. S. (1991). "Perioperative total parenteral nutrition in surgical patients. The Veterans Affairs Total Parenteral Nutrition Cooperative Study Group." N Engl J Med **325**(8): 525-532.

Gustafsson, U. O., M. J. Scott, et al. (2012). "Guidelines for perioperative care in elective colonic surgery: Enhanced Recovery After Surgery (ERAS(R)) Society recommendations." Clin Nutr **31**(6): 783-800.

Hachey, D. L., B. W. Patterson, et al. (1991). "Isotopic determination of organic keto acid pentafluorobenzyl esters in biological fluids by negative chemical ionization gas chromatography/mass spectrometry." Anal Chem **63**(9): 919-923.

Herndon, D. N., D. W. Wilmore, et al. (1978). "Abnormalities of phenylalanine and tyrosine kinetics. Significance in septic and nonseptic burned patients." Arch Surg **113**(2): 133-135.

Hill, A. G. (2000). "Initiators and propagators of the metabolic response to injury." World J Surg **24**(6): 624-629.

Hoffer, L. J. and B. R. Bistrian (2012). "Appropriate protein provision in critical illness: a systematic and narrative review." Am J Clin Nutr **96**(3): 591-600.

Holbrook, I. B., E. Gross, et al. (1979). "Response of plasma amino acids to elective surgical trauma." JPEN J Parenter Enteral Nutr **3**(6): 424-426.

Holecek, M. (2002). "Relation between glutamine, branched-chain amino acids, and protein metabolism." Nutrition **18**(2): 130-133.

Jackson, A. A., G. Phillips, et al. (2001). "Synthesis of hepatic secretory proteins in normal adults consuming a diet marginally adequate in protein." Am J Physiol Gastrointest Liver Physiol **281**(5): G1179-1187.

Jacquez, J. A. (1992). "Theory of production rate calculations in steady and non-steady states and its application to glucose metabolism." Am J Physiol **262**(6 Pt 1): E779-790.

Janney, N. W. (1915). "The metabolic relationship of proteins to glucose." J Biol Chem **20**: 321-350.

Jiang, H., M. W. Sun, et al. (2011). "Efficacy of hypocaloric parenteral nutrition for surgical patients: a systematic review and meta-analysis." Clin Nutr **30**(6): 730-737.

Karunakar, M. A. and K. S. Staples (2010). "Does stress-induced hyperglycemia increase the risk of perioperative infectious complications in orthopaedic trauma patients?" J Orthop Trauma **24**(12): 752-756.

Kehlet, H. (1984). "Does regional anaesthesia reduce postoperative morbidity?" Intensive Care Med **10**(4): 165-167.

Kesteloot, H., C. Cobbaert, et al. (1992). "Time course of serum lipid and lipoprotein levels after coronary bypass surgery: modification by pravastatin." Acta Cardiol **47**(6): 519-528.

Kinney, J. M., C. L. Long, et al. (1968). "Tissue composition of weight loss in surgical patients. I. Elective operation." Ann Surg **168**(3): 459-474.

Klidjian, A. M., K. J. Foster, et al. (1980). "Relation of anthropometric and dynamometric variables to serious postoperative complications." Br Med J **281**(6245): 899-901.

Kopple, J. D. (1987). "Uses and limitations of the balance technique." JPEN J Parenter Enteral Nutr **11**(5 Suppl): 79S-85S.

Kyle, U. G., L. Genton, et al. (2005). "Hospital length of stay and nutritional status." Curr Opin Clin Nutr Metab Care **8**(4): 397-402.

Landau, B. R., J. Wahren, et al. (1996). "Contributions of gluconeogenesis to glucose production in the fasted state." J Clin Invest **98**(2): 378-385.

Lattermann, R., F. Carli, et al. (2002). "Epidural blockade modifies perioperative glucose production without affecting protein catabolism." Anesthesiology **97**(2): 374-381.

Lattermann, R., F. Carli, et al. (2003). "Perioperative glucose infusion and the catabolic response to surgery: the effect of epidural block." Anesth Analg **96**(2): 555-562, table of contents.

Lin, M. T., S. P. Kung, et al. (2002). "The effect of glutamine-supplemented total parenteral nutrition on nitrogen economy depends on severity of diseases in surgical patients." Clin Nutr **21**(3): 213-218.

Liposky, J. M. and L. D. Nelson (1994). "Ventilatory response to high caloric loads in critically ill patients." Crit Care Med **22**(5): 796-802.

Ljungqvist, O., J. Nygren, et al. (2002). "Modulation of post-operative insulin resistance by pre-operative carbohydrate loading." Proc Nutr Soc **61**(3): 329-336.

Ljungqvist, O., M. Soop, et al. (2007). "Why metabolism matters in elective orthopedic surgery: a review." Acta Orthop **78**(5): 610-615.

Lu, C. Y., H. Y. Chuang, et al. (2010). "Hypocaloric peripheral parenteral nutrition with lipid emulsion in postoperative gastrointestinal cancer patients." World J Gastrointest Oncol **2**(1): 51-55.

Lugli, A. K., F. Donatelli, et al. (2010). "Protein balance in nondiabetic versus diabetic patients undergoing colon surgery: effect of epidural analgesia and amino acids." Reg Anesth Pain Med **35**(4): 355-360.

Lugli, A. K., F. Donatelli, et al. (2012). "Parenteral amino acids v. dextrose infusion: an anabolic strategy to minimise the catabolic response to surgery while maintaining normoglycaemia in diabetes mellitus type 2 patients." Br J Nutr **107**(4): 573-580.

Lugli, A. K., F. Donatelli, et al. (2008). "Epidural analgesia enhances the postoperative anabolic effect of amino acids in diabetes mellitus type 2 patients undergoing colon surgery." Anesthesiology **108**(6): 1093-1099.

Lugli, A. K., T. Schricker, et al. (2010). "Glucose and protein kinetics in patients undergoing colorectal surgery: perioperative amino acid versus hypocaloric dextrose infusion." Metabolism **59**(11): 1649-1655.

Mackenzie, M. L., M. R. Warren, et al. (2003). "Colitis increases albumin synthesis at the expense of muscle protein synthesis in macronutrient-restricted piglets." J Nutr **133**(6): 1875-1881.

Matthews, D. E., K. J. Motil, et al. (1980). "Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-3C]leucine." Am J Physiol **238**(5): E473-479.

McClave, S. A. and H. L. Snider (1992). "Use of indirect calorimetry in clinical nutrition." Nutr Clin Pract **7**(5): 207-221.

McClave, S. A., H. L. Snider, et al. (1999). "Preoperative issues in clinical nutrition." Chest **115**(5 Suppl): 64S-70S.

McLaren, D. S. and M. M. Meguid (1983). "Nutritional assessment at the crossroads." JPEN J Parenter Enteral Nutr **7**(6): 575-579.

Melis, G. C., N. ter Wengel, et al. (2004). "Glutamine: recent developments in research on the clinical significance of glutamine." Curr Opin Clin Nutr Metab Care **7**(1): 59-70.

Mimura, Y., M. Yamakawa, et al. (1997). "Efficacy of amino acid infusion for improving protein metabolism after surgery: a prospective randomized study in patients undergoing subtotal gastrectomy." J Am Coll Surg **185**(2): 163-171.

Mittendorfer, B., D. C. Gore, et al. (1999). "Accelerated glutamine synthesis in critically ill patients cannot maintain normal intramuscular free glutamine concentration." JPEN J Parenter Enteral Nutr **23**(5): 243-250; discussion 250-242.

Montalvo-Jave, E. E., J. L. Zarraga, et al. (2007). "Specific topics and complications of parenteral nutrition." Langenbecks Arch Surg **392**(2): 119-126.

Muller, T. F., A. Muller, et al. (1995). "Immediate metabolic effects of different nutritional regimens in critically ill medical patients." Intensive Care Med **21**(7): 561-566.

Noordzij, P. G., E. Boersma, et al. (2007). "Increased preoperative glucose levels are associated with perioperative mortality in patients undergoing noncardiac, nonvascular surgery." Eur J Endocrinol **156**(1): 137-142.

Odessey, R., E. A. Khairallah, et al. (1974). "Origin and possible significance of alanine production by skeletal muscle." J Biol Chem **249**(23): 7623-7629.

Pacy, P. J., J. S. Garrow, et al. (1988). "Influence of amino acid administration on whole-body leucine kinetics and resting metabolic rate in postabsorptive normal subjects." Clin Sci (Lond) **75**(3): 225-231.

Park, C., C. Hsu, et al. (2009). "Severe intraoperative hyperglycemia is independently associated with surgical site infection after liver transplantation." Transplantation **87**(7): 1031-1036.

Pereira, S., E. B. Marliss, et al. (2008). "Insulin resistance of protein metabolism in type 2 diabetes." Diabetes **57**(1): 56-63.

Pisters, P. W. and D. B. Pearlstone (1993). "Protein and amino acid metabolism in cancer cachexia: investigative techniques and therapeutic interventions." Crit Rev Clin Lab Sci **30**(3): 223-272.

Price, S. R., B. K. England, et al. (1994). "Acidosis and glucocorticoids concomitantly increase ubiquitin and proteasome subunit mRNAs in rat muscle." Am J Physiol **267**(4 Pt 1): C955-960.

Radziuk, J. and S. Pye (2001). "Hepatic glucose uptake, gluconeogenesis and the regulation of glycogen synthesis." Diabetes Metab Res Rev **17**(4): 250-272.

Raman, M. and J. P. Allard (2007). "Parenteral nutrition related hepato-biliary disease in adults." Appl Physiol Nutr Metab **32**(4): 646-654.

Reeds, P. J., C. R. Fjeld, et al. (1994). "Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states?" J Nutr **124**(6): 906-910.

Reeds, P. J., D. L. Hachey, et al. (1992). "VLDL apolipoprotein B-100, a potential indicator of the isotopic labeling of the hepatic protein synthetic precursor pool in humans: studies with multiple stable isotopically labeled amino acids." J Nutr **122**(3): 457-466.

Richardson, A. P. and J. A. Tayek (2002). "Type 2 diabetic patients may have a mild form of an injury response: a clinical research center study." Am J Physiol Endocrinol Metab **282**(6): E1286-1290.

Rittler, P., R. Jacobs, et al. (2007). "Dynamics of albumin synthesis after major rectal operation." Surgery **141**(5): 660-666.
Rittler, P., D. Kuppinger, et al. (2009). "Differential regulation of protein synthesis in hepatic and intestinal tissues by amino acids: studies in patients recovering from major abdominal operations." Surgery **146**(1): 113-121.
Ryan, M., A. Salle, et al. (2006). "Resting energy expenditure is not increased in mildly hyperglycaemic obese diabetic patients." Br J Nutr **96**(5): 945-948.
Samuel, V. T. and G. I. Shulman (2012). "Mechanisms for insulin resistance: common threads and missing links." Cell **148**(5): 852-871.
Sandstrom, R., C. Drott, et al. (1993). "The effect of postoperative intravenous feeding (TPN) on outcome following major surgery evaluated in a randomized study." Ann Surg **217**(2): 185-195.
Schricker, T., A. Berroth, et al. (1996). "Influence of vaginal versus abdominal hysterectomy on perioperative glucose metabolism." Anesth Analg **83**(5): 991-995.
Schricker, T., R. Gougeon, et al. (2005). "Type 2 diabetes mellitus and the catabolic response to surgery." Anesthesiology **102**(2): 320-326.
Schricker, T., K. Klubien, et al. (2000). "Effect of epidural blockade on protein, glucose, and lipid metabolism in the fasted state and during dextrose infusion in volunteers." Anesthesiology **92**(1): 62-69.
Schricker, T., R. Lattermann, et al. (2001). "Integrated analysis of protein and glucose metabolism during surgery: effects of anesthesia." J Appl Physiol **91**(6): 2523-2530.
Schricker, T., R. Lattermann, et al. (2004). "Effect of i.v. dextrose administration on glucose metabolism during surgery." JPEN J Parenter Enteral Nutr **28**(3): 149-153.
Schricker, T., S. Meterissian, et al. (2007). "Parenteral nutrition and protein sparing after surgery: do we need glucose?" Metabolism **56**(8): 1044-1050.
Schricker, T., S. Meterissian, et al. (2008). "Anticatabolic effects of avoiding preoperative fasting by intravenous hypocaloric nutrition: a randomized clinical trial." Ann Surg **248**(6): 1051-1059.
Schricker, T., S. Meterissian, et al. (2004). "Postoperative protein sparing with epidural analgesia and hypocaloric dextrose." Ann Surg **240**(5): 916-921.
Schricker, T., L. Wykes, et al. (2000). "Epidural blockade improves substrate utilization after surgery." Am J Physiol Endocrinol Metab **279**(3): E646-653.
Schricker, T., L. Wykes, et al. (2007). "Perioperative amino acid supplementation of hypocaloric glucose does not impair glucose metabolism after surgery." Metabolism **56**(11): 1508-1513.
Schricker, T., L. Wykes, et al. (2005). "Randomized clinical trial of the anabolic effect of hypocaloric parenteral nutrition after abdominal surgery." Br J Surg **92**(8): 947-953.
Schricker, T., L. Wykes, et al. (2002). "The anabolic effect of epidural blockade requires energy and substrate supply." Anesthesiology **97**(4): 943-951.
Schwenk, W. F., B. Beaufrere, et al. (1985). "Use of reciprocal pool specific activities to model leucine metabolism in humans." Am J Physiol **249**(6 Pt 1): E646-650.
Schwenk, W. F., E. Tsalikian, et al. (1985). "Recycling of an amino acid label with prolonged isotope infusion: implications for kinetic studies." Am J Physiol **248**(4 Pt 1): E482-487.
Sena, M. J., G. H. Utter, et al. (2008). "Early supplemental parenteral nutrition is associated with increased infectious complications in critically ill trauma patients." J Am Coll Surg **207**(4): 459-467.

Shaw, J. H. (1988). "Influence of stress, depletion, and/or malignant disease on the responsiveness of surgical patients to total parenteral nutrition." Am J Clin Nutr **48**(1): 144-147.

Staten, M. A., D. E. Matthews, et al. (1986). "Leucine metabolism in type II diabetes mellitus." Diabetes **35**(11): 1249-1253.

Stein, J., H. J. Boehles, et al. (2009). "Amino acids - Guidelines on Parenteral Nutrition, Chapter 4." Ger Med Sci **7**: Doc24.

Svanfeldt, M., A. Thorell, et al. (2006). "Postoperative parenteral nutrition while proactively minimizing insulin resistance." Nutrition **22**(5): 457-464.

Tessari, P., D. Cecchet, et al. (2011). "Insulin resistance of amino acid and protein metabolism in type 2 diabetes." Clin Nutr **30**(3): 267-272.

Tessari, P., S. Inchiostro, et al. (1996). "Hyperglucagonemia stimulates phenylalanine oxidation in humans." Diabetes **45**(4): 463-470.

Tessari, P., E. Kiwanuka, et al. (2006). "Albumin and fibrinogen synthesis and insulin effect in type 2 diabetic patients with normoalbuminuria." Diabetes Care **29**(2): 323-328.

Thorell, A., J. Nygren, et al. (1996). "The metabolic response to cholecystectomy: insulin resistance after open compared with laparoscopic operation." Eur J Surg **162**(3): 187-191.

Thorell, A., J. Nygren, et al. (1999). "Insulin resistance: a marker of surgical stress." Curr Opin Clin Nutr Metab Care **2**(1): 69-78.

Tijerina, A. J. (2004). "The biochemical basis of metabolism in cancer cachexia." Dimens Crit Care Nurs **23**(6): 237-243.

Tisdale, M. J. (2001). "Cancer anorexia and cachexia." Nutrition **17**(5): 438-442.

Tweedle, D. E., G. F. Fitzpatrick, et al. (1977). "Intravenous amino acids as the sole nutritional substrate. Utilization and metabolism in fasting normal human subjects." Ann Surg **186**(1): 60-73.

Uchida, I., T. Asoh, et al. (1988). "Effect of epidural analgesia on postoperative insulin resistance as evaluated by insulin clamp technique." Br J Surg **75**(6): 557-562.

Viggiano, E., M. B. Passavanti, et al. (2012). "Plasma glutamine decreases immediately after surgery and is related to incisiveness." J Cell Physiol **227**(5): 1988-1991.

Vilar-Compte, D., I. Alvarez de Iturbe, et al. (2008). "Hyperglycemia as a risk factor for surgical site infections in patients undergoing mastectomy." Am J Infect Control **36**(3): 192-198.

Vinnars, E., B. Holmstrom, et al. (1983). "Metabolic effects of four intravenous nutritional regimens in patients undergoing elective surgery II.--Muscle amino acids and energy-rich phosphates." Clin Nutr **2**(1): 3-11.

Wagenmakers, A. J. (1999). "Tracers to investigate protein and amino acid metabolism in human subjects." Proc Nutr Soc **58**(4): 987-1000.

Wallace, T. M. and D. R. Matthews (2002). "The assessment of insulin resistance in man." Diabet Med **19**(7): 527-534.

Wang, C. Y., M. J. Hsieh, et al. (2009). "Higher serum C-reactive protein concentration and hypoalbuminemia are poor prognostic indicators in patients with esophageal cancer undergoing radiotherapy." Radiother Oncol **92**(2): 270-275.

Wannemacher, R. W., Jr., A. S. Klainer, et al. (1976). "The significance and mechanism of an increased serum phenylalanine-tyrosine ratio during infection." Am J Clin Nutr **29**(9): 997-1006.

Watters, J. M., S. M. Clancey, et al. (1993). "Impaired recovery of strength in older patients after major abdominal surgery." Ann Surg **218**(3): 380-390; discussion 390-383.

Wijekoon, E. P., C. Skinner, et al. (2004). "Amino acid metabolism in the Zucker diabetic fatty rat: effects of insulin resistance and of type 2 diabetes." Can J Physiol Pharmacol **82**(7): 506-514.

Wilmore, D. W. (2001). "The effect of glutamine supplementation in patients following elective surgery and accidental injury." J Nutr **131**(9 Suppl): 2543S-2549S; discussion 2550S-2541S.

Woerle, H. J., E. Szoke, et al. (2006). "Mechanisms for abnormal postprandial glucose metabolism in type 2 diabetes." Am J Physiol Endocrinol Metab **290**(1): E67-E77.

Yuill, K. A., R. A. Richardson, et al. (2005). "The administration of an oral carbohydrate-containing fluid prior to major elective upper-gastrointestinal surgery preserves skeletal muscle mass postoperatively--a randomised clinical trial." Clin Nutr **24**(1): 32-37.

7 APPENDIX

APPENDIX 1. ASA Physical Status Classification System	
ASA Physical Status 1	A normal healthy patient
ASA Physical Status 2	A patient with mild systemic disease
ASA Physical Status 3	A patient with severe systemic disease
ASA Physical Status 4	A patient with severe systemic disease that is a constant threat to life
ASA Physical Status 5	A moribund patient who is not expected to survive without the operation
ASA Physical Status 6	A declared brain-dead patient whose organs are being removed for donor purposes

APPENDIX 2. Transformations of Variables	
Variables	**Transformation**
Rate of appearance of leucine	inverse
Leucine balance	untransformed
Non-oxidative leucine disposal	log
Leucine oxidation	log
Glucose concentration	inverse
Rate of appearance of glucose	inverse square
Glucose clearance	inverse
Albumin FSR	log
Albumin ASR	log
Albumin concentration	inv log
Fibrinogen FSR	inverse
Fibrinogen ASR	log
Fibrinogen concentration	log10
Total proteins FSR	untransformed
Total proteins ASR	log
total proteins concentration	inverse square
Insulin concentration	log
Cortisol Concentration	log
HOMA index	log
Hematocrit	inverse
VCO2	log
VO2	untransformed
Rest metabolic rate	untransformed
RQ	untransformed

APPENDIX 3. Exploratory analysis using simple linear regression		
	P	R^2
T2DM: Preop Endo Glu Ra= 6.55 + 0.38 (Preop Leu Ox)	0.0126	0.67
Preop: Leucine balance= 2.48- 0.17 (preop Endo Leu Ra)	0.01	0.3
T2DM Preop: Leucine balance= 14.83-0.3(preop Endo Leu Ra)	0.001	0.82
T2DM Preop: Leucine balance= -7.24-8.3 (HOMA-IR)	0.008	0.71
T2DM Preop Endo Leu: Ra=80.64 + 23.31 (HOMA-IR)	0.03	0.57
Postop Leucine balance=12.23-0.72 (Postop Leu Ox)	0.01	0.32
NonT2DM Postop: Leucine balance=15.3-0.75 (Postop Leu Ox)	<0.0 001	0.83

Preoperative Leu Bal was negatively correlated with Endo Leu Ra overall, as expected; however, in patients with T2DM the relation explained more than 80% of the changes in balance. Additionally in T2DM preoperative Glu Ra was related to Leu Ox.