Perioperative protein sparing in diabetes mellitus type 2 patients

An integrated analysis of perioperative protein and glucose metabolism using stable isotope kinetics

Andrea Kopp Lugli, MD
School of Dietetics and Human Nutrition
McGill University, Montreal
September 2006

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Your file Votre référence
ISBN: 978-0-494-32731-9
Our file Notre référence
ISBN: 978-0-494-32731-9

Andrea Rita Kopp Lugli
Perioperative Protein Sparing in Diabetes Mellitus
Type 2 Patients
School of Dietetics and Human Nutrition
McGill University
Master of Science
Copy # 1

Table of contents

Table of figures and tables

Abstract

The potential effects of nutritional support with amino acids or dextrose and epidural blockade on the catabolic response to surgery were investigated in diabetic patients undergoing colorectal surgery. Protein and glucose metabolism were assessed with a stable isotope infusion technique using the two stable isotopes L-[1-^{13}C]leucine and [6,6-$^{2}H_2$]glucose.

1. The first intervention of a postoperative infusion of amino acids avoided pronounced hyperglycaemia in diabetic patients after colorectal surgery and achieved a positive protein balance compared to dextrose.

2. The second intervention of a short term infusion of amino acids postoperatively blunted protein breakdown and stimulated protein synthesis. This resulted in a positive protein balance in patients with epidural blockade compared to patient controlled analgesia with intravenous morphine. With regard to glucose metabolism, amino acid supply after surgery decreased glucose clearance and endogenous glucose production independent from type of analgesia.

Résumé

Les effets potentiels de l'approvisionnement d'acides aminés et de glucose et d'un bloc épidurale à la réponse catabolique au stress autour d'une chirurgie abdominale a été évaluée chez des patients diabétiques. La cinétique du métabolisme protéique et du glucose a été mesuré avec deux isotopes stables L-[1-^{13}C]leucine et [6,6-$^{2}H_2$]glucose.

1. La première intervention d'une infusion d'acides aminés après l'opération a empêché une élévation prononcée du niveau glucidique chez des patients diabétiques après une opération colorectale et a réalisé une balance protéique positive comparée à l'infusion de glucose.

2. La deuxième intervention d'une infusion d'acides aminés après l'opération a émoussé le catabolisme protéique et a stimulé la synthèse protéique. Cela résultait en une balance protéique positive pour les patients avec un bloc épidural comparé à la pompe avec morphine. En ce qui concerne du métabolisme du glucose, l'infusion d'acides aminés après l'intervention a diminué la prise périphérique et la production endogène du glucose indépendant du type d'analgésie.

Contribution of authors

Two manuscripts entitled "*The effect of amino acids on the catabolic response to surgery in diabetes mellitus type 2 patients in comparison to dextrose*" and "*Can epidural analgesia mitigate the catabolic response to surgery in diabetes mellitus type 2 patients receiving amino acid infusion?*" are included in this thesis.

Andrea Kopp Lugli was involved in developing the study design of both manuscripts. In collaboration with Dr. Carli and Dr. Donatelli she recruited the study patients and performed the study sessions according to the protocol. She assessed the samples and derivatized them. Dr. Wykes and the candidate carried out gas chromatography and mass spectrometry analysis.

As primary author the candidate was responsible for the calculations, analyzing the data, writing the papers and creating figures and tables. Dr. Carli, Dr. Wykes and Dr. Schricker provided direction and expertise to the study and assisted with the manuscripts.

Acknowledgements

Technical support was provided by Mrs. Mazza (derivatization of the samples), Mr. Nitschmann (gas chromatography and mass spectrometry analysis) and Ms. Zlobec (statistical analysis). The candidate was supported by a grant of the Swiss National Science Foundation and the Kantonsspital Aarau, Switzerland.

A. Introduction

Surgical injury provokes a stress response consisting of an endocrine-metabolic and inflammatory reaction.[1] These pathways mediated by stress hormones and cytokines cause a catabolic state including stereotypical metabolic alterations such as hyperglycemia, enhanced lipolysis, increased muscle protein breakdown and amino acid oxidation.[2] The loss of lean tissue promotes immunosuppression, delayed wound healing and decreased muscle strength which may result in prolonged convalescence and increased morbidity.[3-5] Patients with type 2 diabetes mellitus (DM2) show an even higher protein catabolism than non-diabetic patients, as reflected by an increased oxidative protein loss.[6]

Managing perioperative care has therefore aimed to develop and evaluate techniques to modify surgical stress responses:

Epidural anaesthesia and analgesia with local anaesthetics inhibits afferent neural stimuli and offers excellent pain relief, earlier recovery and decreased postoperative morbidity.[7, 8] Furthermore, neuraxial blockade influences the endocrine-metabolic stress response by reducing the secretion of catabolic hormones and decreasing insulin resistance.[9] But it does not influence protein catabolism in the fasted state, as the anabolic effect of epidural blockade requires energy and substrate supply.[10-12]

Intravenous provision of glucose suppresses gluconeogenesis and blunts amino acid oxidation, thereby saving lean body mass.[13] This regimen causes hyperglycemia, which is associated with adverse clinical effects[14-16], and does not stimulate whole body protein synthesis.[11] In contrast, postoperative infusion of amino acids inhibits protein breakdown

and increases protein synthesis resulting in positive protein balance independent of the pain-relief technique applied.[17]

Recent studies have shown that the control of perioperative metabolism influences postoperative outcome not only in diabetic but in all patients subjected to surgery and that patients with DM2 are at higher risk for complications.[18] Therefore, the two proposed studies investigate the benefits achieved by manipulating the stress response with epidural blockade and perioperative nutritional support in diabetic patients. Dynamic changes in protein and glucose metabolism will be assessed by determining endogenous glucose production, protein breakdown, protein oxidation and protein synthesis using a stable isotope tracer technique (L-[1-^{13}C]leucine and [6,6-$^{2}H_2$]glucose).

B. Literature Review

B. 1. The stress response to surgery

The impact of surgery causes profound alterations in body physiology and leads to changes in haemodynamic, endocrine-metabolic and immune functions.[19-23] These effects begin with the initiation of surgery and last up to 10 days postoperatively.[24] The principal release mechanisms of the surgical stress response include afferent neural stimuli and inflammatory mediators, which may be amplified by other factors such as infection, hypovolaemia, anxiety, hypothermia, starvation and immobilization.[19-23]

Afferent neural stimuli from traumatized tissues activate the hypothalamopituitary and sympathoadrenergic system which induces the endocrine-metabolic response: the secretion of stress hormones is increased (most importantly cortisol, epinephrine, norepinephrine and glucagon), while the release and potential effect of anabolic hormones like insulin is decreased.

Polypeptides and glycoproteins, so-called cytokines are produced by diverse cell types at the site of the surgical injury and by systemic immune cells, forming the immunologic response. Cytokines like interleukins, interferon-γ or tumor necrosis factor-α (TNF-α) influence immune cell activity, differentiation and proliferation by acting on cell surface receptors to stimulate gene transcription.[21] Additionally, cytokines direct the inflammatory response to sites of injury, revealing cytokines to be essential supporters of proper wound healing.[21]

The evolutionary purpose of the stress response to injury may be explained as promoter of immediate survival by mediating catabolism of the body fuels. However, these mechanisms are suggested to be redundant in current surgical and anaesthetic practice.[20]

Furthermore, an aggravated and prolonged stress response can lead to a systemic inflammatory response syndrome (SIRS), which contributes to increased perioperative morbidity and death.[1]

The stress response aims at provision of nutrients, thus manifesting in increased turnover of carbohydrate, fat and protein:

Carbohydrate metabolism

Blood glucose concentration increases with the start of the operation and is related to the intensity of the surgical injury. The persisting hyperglycaemia is due to the following interactions: First, catabolic hormones boost glucose production by raising hepatic glycogenolysis and gluconeogenesis. Second, peripheral use of glucose is decreased arising from a relative lack of insulin and peripheral insulin resistance due to increased levels of stress hormones.[20] Insulin interacts not only with the glucose metabolism, but also with other hormones, such as insulinlike growth factor-I and cortisol, or with the inflammatory response, which underlines its intriguing role on the perioperative state of metabolism.[18]

Glucose toxicity is a known feature in patients with diabetes mellitus and seems to be even more aggressive when occurring in the postoperative state.[18] This may be explained by the glucose overloading in tissues with noninsulin-dependent glucose uptake, since an increased inflow of glucose into noninsulin-dependent cells has recently been suggested to provoke higher levels of oxidative stress.[25]

Protein metabolism

Increased cortisol and TNF-α concentrations are major inducers of muscle catabolism.[20, 21] Primarily the breakdown of skeletal muscle has to be noted, but there is also a small amount of visceral muscle protein catabolized to release the constituent amino acids. These amino acids may be oxidized, used in the liver to form new proteins (particularly acute-phase proteins) or converted into glucose, fatty acids, ketone bodies and other substrates (new protein tissue in wounds, for proliferation of macrophages and other cellular components involved in the healing process). Protein catabolism results in marked weight loss and muscle wasting in patients after major surgical and traumatic injury. Thus, up to 1500g of lean tissue can be lost.[22, 26] Since proteins represent structural and functional components, erosion of lean body mass can initiate immunosuppression, impaired wound healing, decreased muscle strength and fatigue which may result in prolonged convalescence and increased morbidity.[3-5, 27]

Lipid metabolism

Lipolytic activity is stimulated by cortisol, catecholamines and growth hormone and is inhibited by insulin. Perioperatively the net balance results in increased mobilization of triglycerides. The glycerol produced by lipolysis is a substrate for gluconeogenesis in the liver. Fatty acids enter a pool, from which they may be oxidized in the liver and the muscle, converted to ketone bodies or re-esterified.[20]

Acute stimulation of lipolysis, resulting in elevated fatty acid plasma levels, has been associated with a number of adverse metabolic effects, such as impaired glucose utilization[28], decreased insulin sensitivity[29], and increased gluconeogenesis with potential

impact on protein catabolism.[30, 31] Additionally, fatty acids provoke cardiac arrhythmias, which may cause myocardial ischemic injury.[31, 32]

B. 1.1. Catabolic response to surgery: Impact of diabetes mellitus type 2

A major phenomenon of the stress response to surgery is the development of insulin resistance, which causes similar metabolic derangements seen in patients with DM2.[6, 33] Therefore, it has been hypothesized that patients with DM2 suffer from marked catabolic responses.[34] But few studies have been conducted in this field to support that assumption. A study in patients undergoing cataract surgery showed pronounced hyperglycaemia in the group of noninsulin-dependent patients with DM2 compared to the non-diabetic control group.[35] A recent study of Schricker et al. investigated patients with DM2 undergoing colon surgery on the second postoperative day compared to non-diabetic patients. Epidural analgesia was maintained postoperatively for at least 48h in both groups. Therefore, the observed effects allow not to distinguish between the impact of analgesia and nutritional support on glucose and protein metabolism. DM2 patients showed a higher leucine rate of oxidation, but similar results for leucine rate of appearance and non-oxidative leucine disposal. Infusing glucose (at 4 $mg*kg^{-1}*min^{-1}$) did not affect leucine metabolism in both groups. In diabetic patients, glucose rate of appearance and plasma glucose levels were higher. In contrast, endogenous glucose production was suppressed to a smaller extent. The authors concluded that protein catabolism is elevated in diabetic patients as reflected by an increased oxidative protein loss.[6] However, whether this increased loss can be attributed to increased proteolysis in muscle, plasma proteins or some other pool is unclear or has not been determined.

Taking into account these impacts of the stress response, several interventions blunting the metabolic alterations have been established: pain-relieving techniques, nutritional support, use of high-dose glucocorticoid and use of minimally invasive surgery. The following explanations will concentrate on the effects achieved by manipulating the stress response with nutritional support and epidural blockade.

B. 2. Effect of epidural analgesia on the stress response to surgery

The potential benefits of epidural blockade become intensively apparent in gastrointestinal surgery. Epidural analgesia provides not only excellent pain-relief but affects also positively the cardiovascular, coagulation, pulmonary and gastrointestinal physiology as well as the surgical stress response and immune function resulting in improved surgical outcome.[7]

The principal site of action for neuraxial blockade is the nerve root. Analgesics are injected into the epidural space, through which dorsal and ventral nerve roots at each spinal level travel, thus, blocking motor, sensory and autonomic stimuli.[36]

Since afferent neural stimuli initiate the endocrine response, blocking these impulses may result in a reduced stress reaction and influence postoperative organ dysfunctions.[1] However, analgesics inserted into the epidural space may develop also systemic effects arising from their reabsorption from the epidural space.[36] The impact of this systemic pathway on the stress reaction cannot be easily distinguished from the neuraxial blockade.[37]

Verifying the effects of epidural blockade on the endocrine-metabolic response to surgery involves the duration and the level of epidural blockade as well as the type of epidural analgesics injected: Single injection as used for intraoperative epidural anaesthesia has

only a limited potential to attenuate the stress response, lacking a beneficial endocrine or metabolic effect beyond the operation.[38] Therefore, continuous epidural blockade for 24h postoperatively rewards a higher endocrine-metabolic benefit, which may be further prolonged with a 48h application.[38, 39]

An appropriate level of epidural blockade is crucial for an effective attenuation of the stress response. Continuous lumbar epidural blockade modifies efficiently endocrine-metabolic responses after lower body procedures but lacks an effect in the setting of abdominal surgery as the thoracic segments remain unblocked.[1] In contrast, endocrine-metabolic responses after abdominal surgery are only partially inhibited by continuous thoracic epidural blockade due to inadequate afferent neural blockade[38], concomitant hypothalamic stimulation from inflammatory mediators[19, 22] and the reflexes of the phrenic nerves. Since the diaphragm's embryonal descent from the C3-5 myotomes the corresponding phrenic nerves are not covered by a standard epidural block level up to thoracic dermatome 4.[40]

The choice of epidural analgesic drugs contributes to the extent of the stress response blockade. Local anaesthetics act on sodium channels in nerves leading to a reduced action potential depolarization, thus, reducing nerve stimulus propagation.[36] This non-selective effect acts on autonomic and somatic nerves and is a function of nerve diameter.[36] Opioids bind to opioid receptors blocking selectively pain without impact on motor function or sense of touch.[36] As a result of these different performances an accurate attenuation of stress response can only be achieved by local anaesthetics[1, 41], suggesting that pain-related pathways are only partly responsible.[42] Epidural blockade results in lower postoperative plasma levels of adrenocorticotrophic hormone, cortisol, aldosterone and glucose as well as a blunted increase of stress-related oxygen consumption.[9, 43, 44]

According to a review of Moraca et al.[7], epidural anaesthesia and analgesia provides not only an improved postoperative pain-control, but has also the potential to enhance surgical outcome and faster recovery: a significant reduction in perioperative cardiac morbidity, pulmonary infections, pulmonary embolism, postoperative ileus, acute renal failure and blood loss can be noted. In contrast, a review of epidural analgesia in gastrointestinal surgery by Fotiadis et al.[36] found no reduction in intraoperative blood loss, transfusion requirement, risk of thromboembolism or cardiac morbidity compared to conventional analgesia in unselected patients. However, in patients at high risk of cardiac or pulmonary complications thoracic epidural analgesia lowered hospital costs and length of stay.

Potential complications such as transient paresthesias or potentially devastating epidural haematomas should not be left unstated.[7] Nevertheless, the positive impact of epidural blockade on endocrine-metabolic response is stated undoubtedly.[1, 7, 36]

B. 2.1. Epidural blockade and protein metabolism

The stress response to surgery shifts protein metabolism to a catabolic state.[1] Several studies on the effect of continuous epidural blockade (initiated before abdominal surgery and maintained postoperatively) and protein metabolism showed a significant reduction in postoperative nitrogen excretion[45-47], an attenuation of increased amino acid oxidation[48, 49] and whole body protein breakdown as well as a blunted decrease in muscle protein synthesis.[11, 50] These results were obtained in patients receiving continuous parenteral nutritional support, and therefore it is not possible to distinguish between the effect of epidural blockade and nutrition on the stress response.[11]

During the postoperative setting of abdominal surgery and in a fasted state, four studies reported consistently no difference between epidural blockade and patient-controlled analgesia regarding whole body protein synthesis, breakdown, leucine oxidation and glucose clearance[10-12, 17], but only two trials detected a lower endogenous glucose production in patients with epidural blockade.[12, 17] The infusion of glucose (4 mg*kg^{-1}*min^{-1}) on the second day after abdominal surgery increased glucose clearance and decreased leucine oxidation in the presence of epidural blockade. Whole body protein synthesis was not altered independent of the pain control technique. Therefore, the protein-sparing effect of epidural blockade was suggested to require adequate energy supply, whereas energy supply alone does not stimulate whole body protein synthesis.[10] These conclusions were adapted in a subsequent trial, during which glucose (4 mg*kg^{-1}*min^{-1}) and amino acids (0.02 ml*kg^{-1}*min^{-1}; Travasol™) were administered on the second postoperative day in order to stimulate whole body protein synthesis.[11] This regimen decreased endogenous protein breakdown and glucose production to the same extent in both groups, but increased whole body protein synthesis to a greater degree in the epidural group.[11] Although glucose was infused at a low dose, a hyperglycaemic response with concentrations near 10 mmol*l^{-1} was found in both studies.[10, 11]

Hyperglycaemia affects the immune system leading to an increased risk of infection and is related to an increased risk of postoperative complications in cardiac surgery.[51] Consequently, in a following study, amino acids have been provided (0.02 ml*kg^{-1}*min^{-1} Travasol™, equivalent to 2.9 g*kg^{-1}*day^{-1}) alone on the second postoperative day after abdominal surgery.[17] This infusion inhibited protein breakdown and stimulated protein synthesis, resulting in positive protein balance independent from the pain-control technique applied.[17]

B. 2.2. Epidural blockade and glucose metabolism

In the postoperative phase epidural blockade modifies glucose metabolism including decreased plasma glucose levels, reduced endogenous glucose production as well as higher glucose tolerance due to lower insulin resistance.[1] These findings may be explained by the inhibition of the endocrine-metabolic response due to epidural blockade, thus, reducing the levels of catabolic hormones (cortisol and catecholamines) which leads to an improved glucose utilization. Studies performing perioperative intravenous glucose tolerance tests in patients who underwent inguinal herniotomy or transabdominal hysterectomy, showed normal values for the epidural group, while patients with inhalational anaesthesia had an impaired glucose tolerance.[52, 53] A study on patients undergoing open cholecystectomy examined the effect of a constant infusion of glucose. The increase in blood glucose levels was suppressed in patients with epidural blockade compared to the group of general anaesthesia suggesting a relation between a reduced splanchnic release of glucose combined with an increased peripheral uptake.[54]

Two studies showed, as stated above, that epidural blockade had no significant impact on glucose production and glucose clearance in the fasted state.[10, 11] However, between protein breakdown and endogenous glucose production a weak inversion correlation was detected.[10] The effects of the infusion of glucose alone and glucose combined with amino acids had been noted in the preceding chapter. Provision of amino acids alone (at 0.02 $ml*kg^{-1}*min^{-1}$ Travasol™, equivalent to 2.9 $g*kg^{-1}*day^{-1}$) on the second day after abdominal surgery resulted in a decreased endogenous glucose production. Furthermore, a decreased glucose clearance suggesting a state of insulin resistance was observed. These results were all independent of the pain-relief technique applied.[17]

B. 2.3. Potential benefits of epidural blockade in diabetes mellitus type 2 patients

So far, no studies on the specific effects of type and quality of perioperative analgesia on the catabolic response to abdominal surgery in DM2 patients have been performed. As stated above, the only trial including DM2 patients could not elucidate the specific role of epidural blockade due to the study setting.[6]

B. 3. Implications of perioperative nutrition

Patients undergoing gastrointestinal surgery are at a high risk of nutritional depletion from inadequate nutritional intake, surgical stress and the subsequent increase in metabolic rate.[55] The occurrence of postoperative complications, mainly of mechanical and infectious nature, depends to a high extent on the nutritional status of patients.[56] The traditional strategy of fasting and intravenous fluids until passage of flatus was based on concerns regarding possible postoperative ileus and anastomosis leakage. However, it has been shown that small intestinal motility recovers within 6-8 hours after surgical trauma and moderate absorptive capacity exists even in the absence of normal peristalsis.[57] According to several trials, postoperative feeding in patients undergoing gastrointestinal resection is safe and well tolerated even when started within 12 hours of surgery.[55, 58, 59] These findings have been adapted to a various extent in clinical practice ranging from an early feeding as described to a conservative approach including starvation until passage of feces. The standard management of perioperative nutrition at the Montreal General Hospital provides a minimal amount of calories for 2-3 days: The nutritional support consists of starvation with administration of intravenous fluids until the passage of flatus and a following step-wise introduction to enteral food starting with a liquid diet (fruit juice, protein supplements, and cremes).

Enteral feeding entails clinical benefits including reduced incidence of postoperative infections and improved wound healing.[55, 60, 61] Further analyses have to show whether enteral nutrition truly affects gut function or tolerance of enteral nutrition is just an indicator of healthy organ function.[62] Recent studies have reported that total parenteral nutrition in the perioperative phase reduces significantly morbidity and mortality, but only in severely malnourished patients with gastrointestinal malignancy.[55] According to meta-analyses enteral nutrition is associated with fewer septic complications compared to parenteral support, lower costs and a shorter hospital stay.[55]

Exogenous glucose suppresses gluconeogenesis, thereby reducing the need for muscle protein breakdown to supply gluconeogenic amino acids.[63] The amount of nitrogen spared by decreasing the rate of gluconeogenesis from amino acids is accessible for reincorporation into protein rather than for excretion as urea. However, the inhibitory impact of exogenous glucose on gluconeogenesis depends on the dose of glucose and the physiologic state of the patient. Endogenous glucose production can be completely suppressed by infusing glucose at a rate of 4 $mg*kg^{-1}*min^{-1}$ in healthy volunteers. In patients with sepsis or after trauma a higher rate of glucose infusion is necessary to gain nitrogen-sparing effects. [64, 65] The doubled rate of glucose infusion in healthy volunteers achieved a further decrease in urea production, even though gluconeogenesis from amino acids was already suppressed by the lower infusion rate. These findings can be explained by an increased glucose oxidation.[64]

Consequently, it can be proposed that exogenous glucose should be administered at an amount not higher as where endogenous glucose production is suppressed and that a supplementation with exogenous amino acids may counteract further the oxidation of

endogenous amino acids. This pathway has been suggested to be responsible for additional protein preservation in critically ill patients.[17]

B. 3.1. Perioperative nutrition in diabetes mellitus type 2 patients

In non-diabetic patients the nutritional support aims to achieve a protein sparing effect by providing balanced amounts of exogenous glucose and amino acids: by supplying enough energy and suppressing endogenous glucose production with exogenous glucose, the exogenous amino acids are directed towards an anabolic pathway. This regimen must be particularly promoted in diabetic patients since protein catabolism appears to be even higher.[6] In contrast, glucose infusion causes hyperglycaemia, a non-desirable side-effect especially in diabetic patients. However, the possible effects of exogenous amino acids have been neglected so far in diabetic patients.

B. 3.2. Limitations of nutritional support: alternative approaches

The elevated rate of protein catabolism in critically ill patients remains unaltered by the administration of exogenous glucose and amino acids, since these infusions attenuate protein losses by increasing whole body protein synthesis, but do not change protein breakdown.[65] The lacking influence of exogenous glucose on protein catabolism arises from the not suppressible gluconeogenesis and the impaired capacity of glucose oxidation in stressed patients.[64, 65]

In order to shift protein metabolism to an anabolic state, another approach involving the hormonal pathway has been attempted. Catabolic hormones are released in association with stress and surgery, opposing the effects of insulin.[66] Infusion of cortisol[67], adrenaline[68], glucagon[69] and growth hormone[70] in healthy volunteers caused several of

the metabolic derangements seen in relationship with trauma including insulin resistance. A study by Brandi et al. in patients undergoing elective colorectal surgery reported a marked increase of insulin needed to maintain normoglycemia during parenteral nutrition after surgery compared with preoperative need.[71] Additionally, the provision of insulin was associated with a normalization of fatty acid concentration, urea excretion and respiratory quotient.[71] These findings strongly suggest that insulin has not only effects on glucose metabolism in the postoperative patient, but regulates also lipolysis and protein breakdown, which is sustained by other studies.[72, 73] However, insulin has to be administered in high doses to overcome insulin resistance[72], whereas excessive amounts of exogenous glucose need to be applied to achieve normoglycemia. But exorbitant carbohydrate and caloric intake may provoke fatty infiltration of the liver[74] and stimulates carbon dioxide production.[14]

B. 4. Assessment of protein and glucose metabolism

B. 4. 1. Stable isotope methodology

Turnover rates of amino acids and glucose can be followed in vivo by using stable isotope tracers, i.e. substrates, which have been labelled with stable, non radioactive isotopes (^{2}H, ^{13}C) and the subsequent measurement of isotope enrichments in plasma. In contrast, even though generally adequate for clinical decision making, observations based on plasma levels of metabolites alone provide no information on the different interactions resulting from this. An increased blood glucose concentration might be the consequence of accelerated release, diminished glucose uptake or a combination of both. Any conclusion derived from static observation of blood glucose levels produces, therefore, erroneous inferences.

B. 4.2. Protein kinetics

The degree of protein catabolism in surgical patients is frequently characterized by the calculation of net nitrogen balances retrieved from the difference between the nutritive nitrogen intake and the excretion of nitrogen. However, this method cannot distinguish the contribution from alterations in protein synthesis and protein degradation. For example, negative nitrogen balance results from two different procedures: Either protein breakdown and amino acid oxidation increase and synthesis remains equal or breakdown and oxidation rates remain unchanged and the rate of protein synthesis decreases.

In contrast, the measurement of protein turnover, amino acid oxidation and protein synthesis with labelled stable isotopes reveals a dynamic picture of protein metabolism. In the fasted state, breakdown of endogenous proteins constitutes the only source of the essential amino acid "leucine" for protein synthesis and oxidation.[75] The isotope dilution technique offers an in vivo estimation of whole body protein metabolism by using a labelled essential amino acid, L-[1-^{13}C] leucine.[75] The flux of L-[1-^{13}C] leucine represents the total amount of leucine withdrawn from and provided to the plasma pool. Oxidation of labelled leucine produces ^{13}C-carbon dioxide. The rate of protein synthesis can be calculated indirectly by subtracting leucine oxidation from leucine flux. Protein balance is defined as the difference between whole body protein synthesis and endogenous protein breakdown, thus, positive values indicate a state of anabolism.

The general principal described for leucine can be applied for any other essential amino acid. However, only isoleucine and valine have intracellular ketoacids comparable to the leucine/KIC system.

Another method to measure protein kinetics uses ^{15}N-glycine.[76] This technique bases its calculation on a metabolic pool of nitrogen to which the inflow consists of amino acids

from the diet or from body protein breakdown and the outflow of amino acids being incorporated into protein synthesis or being used as energy source and excreted in the urine mainly as urea.

B. 4.3. Glucose kinetics

The kinetics of whole body glucose metabolism can be assessed by infusing the isotope labelled tracer [6,6-$^2H_2$] glucose.[77] Whole body glucose production depends on the extent of glycogenolysis and gluconeogenesis. The use of deuterated glucose does not allow differentiating between these two metabolic pathways. However, it has been reported that in surgical patients, who are fasting and receiving a small amount of glucose, postoperative glucose production is presumably of gluconeogenesis origin.[78, 79]

In the physiologic steady state, the rate of endogenous glucose production equals whole body glucose uptake. Most glucose is incorporated into non-insulin sensitive tissues. Therefore, the rate of glucose uptake is determined to a large extent by the diffusion gradient for glucose: glucose uptake increases proportionally to rising blood glucose concentrations. Consequently, changes in whole body glucose uptake do not necessarily reflect corresponding alterations in the tissue ability for glucose uptake and the rate of glucose uptake has to be corrected for the prevailing plasma glucose concentration.[63] The glucose clearance rate is calculated by dividing endogenous glucose production by the corresponding plasma glucose concentration and represents an index for glucose take-up ability of tissues. This methodology differs from the glucose clamp technique since the metabolic milieu is not altered by the small amount of glucose tracer infused during the study.

The effect of amino acids on the catabolic response to surgery in diabetes mellitus type 2 patients in comparison to dextrose

Andrea Kopp Lugli, M.D.[1-3], Francesco Donatelli, M.D.[1,2], Thomas Schricker, M.D., Ph.D.[1], Linda Wykes, Ph.D.[2], Franco Carli, M.D., M.Phil.[1]

[1] Department of Anesthesia, McGill University, Montreal

[2] School of Dietetics and Human Nutrition, McGill University, Montreal

[3] Department of Anesthesia and Perioperative Intensive Care Medicine, Kantonsspital, Aarau, Switzerland

Correspondence should be sent to:

Dr. Andrea Kopp Lugli

Department of Anesthesia

McGill University Health Centre

1650 Avenue Cedar, D10-144

Montreal, Quebec, Canada, H3G 1A4

Phone: 514 934 1934 (ext.: 43261)

Fax: 514 934 8249

Email: andrea.kopplugli@mail.mcgill.ca

C. 1. Abstract

Background: Loss of body protein and hyperglycemia represent typical features of the stress response to surgery. In patients with type 2 diabetes mellitus (DM2) protein catabolism after colorectal surgery is increased as reflected by a higher oxidative protein loss and an aggravated state of insulin resistance. The study tests the hypothesis that amino acid infusion blunts protein breakdown and maintains blood glucose homeostasis, therefore having the potential to reduce perioperative risk in the especially vulnerable population of DM2 patients. The results were compared with the values of a previously published study[6] with the same setting and technique but providing dextrose (4 $mg*kg^{-1}*min^{-1}$; DEX group) instead of amino acids (AA group) in 6 diabetic patients.

Methods: 6 diabetic patients scheduled for colorectal surgery underwent a 5 h stable infusion study (2 h fasted, 3 h fed) on the second postoperative day. They received an infusion of amino acids (0.02 $ml*kg^{-1}*min^{-1}$ (equivalent to 2.9 $g*kg^{-1}*day^{-1}$) during the fed state. Glucose and protein kinetics were determined by L-[1-^{13}C]leucine and [6,6-$^{2}H_2$]glucose and circulating concentrations of glucose, insulin and cortisol were assessed.

Results: The effect of feeding caused a statistically significant decrease in endogenous Ra glucose ($p<0.0001$) and glucose clearance ($p=0.0002$). In the fed state, endogenous Ra glucose was lower in the DEX group compared to the AA group ($p<0.0001$), whereas Ra glucose was lower in the AA group compared to DEX group ($p<0.0001$) reflecting the different impact of the type of feeding.

Results for fasting protein kinetics were similar in both groups except for leucine oxidation (DEX group: 30 ± 10 $\mu mol*kg^{-1}*h^{-1}$; AA group: 15 ± 5 $\mu mol*kg^{-1}*h^{-1}$; $p=0.009$), which resulted in a less negative protein balance in the AA group compared to the DEX group (DEX group: -30 ± 10 $\mu mol*kg^{-1}*h^{-1}$; AA group: -15 ± 5 $\mu mol*kg^{-1}*h^{-1}$;

p=0.010). The effect of feeding caused a statistically significant decrease in net protein breakdown (p=0.014) and was reduced by a higher amount in the AA group compared to the DEX group (p=0.022). Close analysis indicated that this effect of type of nutrition was greater in the AA group compared to the DEX group (p=0.030). The type of nutrition has a greater impact on leucine oxidation in the AA group than in the DEX group (p=0.005). For Protein balance a statistically significant difference for the transition from the fasted to the fed state was found (p=0.022), but an even more pronounced difference when comparing the two groups (p<0.0001), resulting in a greater effect of nutrition in the AA group (p=0.032 for interaction).

The hyperglycaemic effect of feeding was pronounced in the DEX group, with mean values by 4.8 $mmol*l^{-1}$ higher than in the AA group (DEX: 12.7 ± 1.5 $mmol*l^{-1}$; AA group: 7.9 ± 1.7 $mmol*l^{-1}$).

Conclusion: The infusion of amino acids avoids pronounced hyperglycemia in diabetic patients after colorectal surgery and achieves a positive protein balance compared to dextrose infusion alone.

C. 2. Introduction

Surgical injury induces an endocrine-metabolic stress response via the activation of the hypothalamo-pituitary and sympathoadrenergic system.[1] As a consequence, catabolic stress hormones such as cortisol, epinephrine, norepinephrine and glucagon increase, whereas the secretion and peripheral action of insulin decreases.[68, 80] This pathway causes a catabolic state including stereotypical metabolic alterations such as hyperglycemia, enhanced lipolysis, increased muscle protein breakdown and amino acid oxidation.[2] Since insulin resistance appears to play an intriguing role in these metabolic changes and similar derangements are seen in patients with DM2, it has been suggested, that this population suffers from marked catabolic stress responses to surgery.[6, 33, 34] But few studies have been conducted in this field to support the assumption. A trial in patients undergoing cataract surgery showed pronounced hyperglycemia in the group of noninsulin-dependent patients with DM2 compared to non-diabetic counterparts.[35] A recent study by Schricker et al. investigated patients with DM2 on the second day after colon surgery compared to non-diabetic patients.[6] Leucine rate of oxidation was higher in DM2 patients, whereas similar results were found for leucine rate of appearance and non-oxidative leucine disposal. Infusing dextrose (4 $mg*kg^{-1}*min^{-1}$) did not affect leucine metabolism in both groups. In contrast, glucose rate of appearance and plasma glucose levels were higher, while endogenous glucose production was suppressed to a smaller extent in diabetic patients. The authors concluded that protein catabolism is elevated in diabetic patients as reflected by an increased oxidative protein loss. However, since epidural analgesia was maintained postoperatively for at least 48 h in both groups, the results did not allow to distinguish between the impact of analgesia and nutrition support on glucose and protein metabolism.

Nutrition support aims to achieve a protein sparing effect by providing balanced amounts of exogenous energy and substrates. By supplying an amount of dextrose which suppresses endogenous glucose production, the exogenous amino acids are directed towards an anabolic pathway. This regimen deserves special attention in diabetic patients, since protein catabolism appears to be even higher.[6] However, exogenous glucose provokes hyperglycaemia close to 10 $mmol*l^{-1}$, even when infused at a low dose.[10, 11] Recent studies have shown that the control of perioperative metabolism influences postoperative outcome not only in diabetic but in all patients subjected to surgery.[18]

The aim of the present study was to determine the effect of exogenous amino acids on postoperative protein and glucose metabolism in patients with DM2 undergoing colorectal surgery. It is hypothesized that amino acid infusion maintains blood glucose homeostasis, therefore having the potential to reduce perioperative risk in the especially vulnerable population of DM2 patients.

C. 3. Methods

C. 3.1. Patients

Six patients undergoing elective colorectal surgery were recruited between November 2005 and June 2006. The study protocol was approved by the ethics committee of the Montreal General Hospital (GEN#04-060) and written informed consent was obtained from all patients. Inclusion criteria were age >18 years, DM2 (controlled by diet, oral hypoglycaemic medication or insulin) and colorectal surgery for non-metastatic disease (including right, transverse, left, sigmoid, subtotal, total and hemicolectomy and low anterior resection). Exclusion criteria were severe cardiac, hepatic, renal or metabolic disorders, diabetes mellitus type 1, plasma albumin concentration $<35 g*l^{-1}$, more than 10% weight loss over the preceding three months, anaemia (hematocrit <30%), use of steroids, previous spine surgery limiting the use of an epidural catheter and pregnancy. The patients (n=6; AA group) received general anaesthesia combined with perioperative epidural analgesia.

As group of comparison the data of previously published study with the same setting and technique but providing dextrose ($4 mg*kg^{-1}*min^{-1}$; DEX group) instead of amino acids in 6 diabetic patients were kindly provided by Dr. Schricker.[6]

C. 3.2. Anaesthesia and perioperative care

Patients underwent bowel preparation on the day before surgery and were allowed to drink clear fluids until midnight. Patients were operated in the morning hours by two surgeons who collaborated as co-investigators.

Oral hypoglycaemic medications were discontinued on the day of surgery. Perioperative glycemic control was achieved by an insulin sliding scale aiming to maintain blood glucose levels between 5 and 10 $mmol*l^{-1}$. Blood glucose was measured every 6 hours.

On the arrival at the operation room an epidural catheter was inserted between thoracic vertebral levels T8-T11 before the induction of general anaesthesia. Neuraxial blockade was established with 0.5 % bupivacaine to achieve a bilateral sensory block from thoracic dermatome level four (T 4) to sacral dermatome level one (S 1). The blockade was maintained with a constant infusion of 0.25% bupivacaine at a rate of 10 $ml*hr^{-1}$ during surgery.

General anaesthesia was induced by propofol (3 $mg*kg^{-1}$) and fentanyl (1.5 $\mu g*kg^{-1}$) and endotracheal intubation was facilitated by rocuronium (0.6 $mg*kg^{-1}$). General anaesthesia was maintained using 65 % nitrous oxide in oxygen and desflurane at endtidal concentrations required to keep heart rate within 20 % of preoperative values. Falls in mean arterial blood pressure (<60 mmHg were treated with fluid administration (NaCl 0.9 %) and increments of phenylephrine (0.1 mg). Normal saline was infused during surgery at a rate of 4 $ml*kg^{-1}*min^{-1}$ and at a rate of 100 $ml*h^{-1}$ after surgery. On the first day after surgery the infusion was changed to Dextrose 5% in ¼ normal saline (at 100 $ml*h^{-1}$) and patients were allowed to drink clear fluids according to the state of bowel passage. At midnight of the first day after surgery the infusion was changed to normal saline (at 100 $ml*h^{-1}$) and patients were only allowed to drink water.

The sensory block from T 8 to L 3 dermatomes was postoperatively maintained for at least 48 hours by continuous epidural infusion of 0.1% bupivacaine supplemented with 3 $\mu g*ml^{-1}$ fentanyl (at a rate between 6-14 $ml*hr^{-1}$) to provide adequate analgesia for a 10-15 cm paramedian subumbilical incision. Pain levels at rest, during mobilization and

coughing were evaluated in all patients using a 10 point visual analogue score (VAS) every four hours after surgery. Pain treatment was adjusted to achieve a VAS level at rest below two and below five during mobilization or coughing.

C. 3.3. Experimental protocol

On the second day after surgery, starting at 8:00 am and under postabsorptive conditions, patients underwent a 5-hour preoperative tracer kinetic study to characterize baseline protein and glucose metabolism. The study consisted of a 2-hour fasted state, which was followed by a 3-hour fed state (figure C1). Plasma kinetics of leucine and glucose were determined by using tracer quantities of L-[1-^{13}C] leucine (99% ^{13}C) and [6,6-$^{2}H_2$]glucose (99% ^{2}H) obtained from the local pharmacy. Sterile solutions of isotopes were prepared in the hospital pharmacy and kept at 4°C until administration.

A superficial vein in the dorsum of the hand was cannulated and the cannula kept patent with saline. A second superficial vein on the contralateral arm was cannulated to provide access for the infusion of the stable isotopes. Blood and expired air samples were collected to determine baseline enrichments. Each blood sample was transferred immediately to a heparinized tube, centrifuged at 4°C (2400 g x 10 minutes) and stored at –70 °C. Breath samples were collected in a 2 liters latex bag and transferred immediately to 10 ml vacutainers (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ).

Sodium bicarbonate ($NaH^{13}CO_3$) (0.08 mg*kg^{-1}) was administered orally. Priming doses of L-[1-^{13}C]leucine (4 μmol*kg^{-1}), and [6,6-$^{2}H_2$]glucose (22 μmol*kg^{-1}) were injected and followed immediately by continuous infusions of L-[1-^{13}C] leucine 0.06 μmol*kg^{-1}*min^{-1} and [6,6-$^{2}H_2$] glucose 0.22 μmol*kg^{-1}*min^{-1} lasting for 2 hours (fasted state).

Blood and air samples were collected at 90, 100, 110 and 120 min of the study period, when the tracers were assumed to have reached an isotopic steady state, to determine the protein and glucose metabolism in the fasted state. For the subsequent 3-h period of fed state the concentration of L-[1-^{13}C] leucine was increased to 0.12 μmol*kg^{-1}*min^{-1} and that of [6,6-$^{2}H_2$] glucose maintained at 0.22 μmol*kg^{-1}*min^{-1}. During the fed state a 10% amino acid solution without electrolytes (Travasol™, Baxter, Montreal, Canada) was infused at a rate of 0.02 ml*kg^{-1}*min^{-1} (equivalent to 2.9 g*kg^{-1}*day^{-1}) to achieve plasma amino acid concentration at least twofold above the basal.[81] The composition of Travasol™ solution, which was verified before each administration, was as follows: 35 μmol*ml^{-1} proline, 34 μmol*ml^{-1} threonine, 217 μmol*ml^{-1} glycine, 207 μmol*ml^{-1} alanine, 36 μmol*ml^{-1} valine, 37 μmol*ml^{-1} methionine, 34 μmol*ml^{-1} isoleucine, 45 μmol*ml^{-1} leucine, 2 μmol*ml^{-1} tyrosine, 35 μmol*ml^{-1} phenylalanine, 9 μmol*ml^{-1} tryptophan, 38 μmol*ml^{-1} lysine, 26 μmol*ml^{-1} histidine and 57 μmol*ml^{-1} arginine. Blood and air samples were collected at 330, 340, 350 and 360 min of the study period, when the tracers were assumed to have reached an isotopic steady state, to determine the protein and glucose metabolism in the fasted state.

Blood samples were drawn during the last 10 min of the fasted and fed state periods for the analysis of glucose, insulin and cortisol.

Indirect calorimetry (Vmax 29N; SensorMedics, Yorba Linda, Calif) was performed for 15 min in the last hour of the fasted and fed states of tracer kinetic study period. The subjects were lying in a semirecumbent position and breathing room air in a ventilated tent. Whole-body oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were determined.

C. 3.4. Measurements

C. 3.4.1. Isotopic enrichments

Plasma enrichment of [1-^{13}C]α-KIC, representing intracellular leucine, were used as the basis for calculating both flux and oxidation of leucine as described recently.[82, 83] Plasma [1-^{13}C]α-KIC enrichment were analyzed by its pentafluorobenzylester derivative using methane negative chemical ionization gas chromatography-mass spectrometry (GC/MS).[84, 85] Plasma [6,6-$^{2}H_2$]glucose enrichment were determined by electron impact analyses of the pentaacetate derivative as previously described.[86] $^{13}CO_2$ enrichment in expired breath was determined by isotope ratio mass spectrometry (Analytical Precision AP2003, Manchester, United Kingdom).[82]

C. 3.4.2. Plasma metabolites and hormones

Plasma concentrations of glucose were measured by using an enzymatic colorimetric assay (Roche, GLU Glucose GOD-PAP) on automated clinical chemistry analyzers (Roche/Hitachi 904911: CAN 249 or Roche/Hitachi 912/917/MODULAR: CAN 525, Roche Diagnostics, Indianapolis, IN). Insulin concentrations were determined by a solid-phase, two-site chemiluminescent immunometric assay (IMMULITE/IMMULITE 1000 Insulin; Diagnostic Products Corporation, Los Angeles, CA). Plasma concentrations of cortisol were measured by using an immunoassay (Unicell DXI 800; Backman Coulter, Brea, CA).

C. 3.4.3. Gaseous exchange

Average values of VO_2 and VCO_2 as well as the calculated respiratory quotient were retrieved from indirect calorimetry, accepting a coefficient of variation less than 10%.

C. 3.5. Calculation of protein and glucose metabolism

Whole body leucine and glucose kinetics were determined by conventional isotope dilution practice applying a two-pool stochastic model during stead-state conditions of the fasted and fed states before and after surgery.

When a physiological and isotopic steady state exists, the rate of appearance (Ra) of unlabeled substrate in plasma can be derived from the plasma isotope enrichment (APE or atom percentage excess) calculated by: $Ra = (APE_{inf}/APE_{pl}-1)*F$, where F is the infusion rate of labeled tracer, APE_{inf} is the tracer enrichment in the infusate and APE_{pl} equals the tracer enrichment in plasma, respectively. The APE used in this calculation are the mean of the four APE determined during steady state conditions obtained in each phase of the studies. The accuracy of the isotopic enrichments at isotopic plateau were tested by evaluating the scatter of values above their mean, expressed as coefficient of variation. A coefficient of variation less than 5 % was used as a confirmation of a valid plateau.

The kinetics of the amino acid leucine, which makes up for 8% of whole body protein, represent the dynamics of protein metabolism in this study setting. Therefore, the terms "protein synthesis" and "protein balance" are representing leucine kinetics and are used to present and discuss protein metabolism where not labelled expressively "protein balance (whole body protein)".

Under steady state conditions, leucine flux (Q) is defined by the formula: $Q = S + O = B + I$. Where S is the rate at which leucine is incorporated into body protein, O is the rate of

oxidation of leucine, B is the rate at which unlabeled leucine enters the free amino acid pool from endogenous protein breakdown, and I is the rate of leucine intake including tracer (rate of infusion of L-[1-^{13}C]leucine (μmol*kg^{-1}*h^{-1})) and diet. Under conditions of a postabsorptive state, the essential amino acid leucine for protein synthesis and oxidation comes from a sole source: the breakdown of endogenous proteins, thus flux is equal to breakdown. Plasma enrichment of [1-^{13}C]α-KIC were used as the basis for calculating both flux and oxidation of leucine.[87] In the calculation of oxidation, factors of 0.76 for the fasted state and of 0.92 for the fed state (during amino acid infusion) were applied to account for the fraction of ^{13}C-carbon dioxide released from leucine but retained in the bicarbonate pool of the body.[10, 75, 88] Whole body protein breakdown in the fed state during the postoperative tracer kinetic study was calculated by subtracting only the exogenous leucine intake of the amino acid solution over the 3-h infusion from the measured flux.

In the fasted state the Ra glucose is equal to the endogenous production of glucose. In the physiologic steady state, whole body glucose uptake equals the rate of endogenous glucose production. Because glucose uptake increases proportionally as blood glucose concentrations increase, changes in whole body glucose uptake do not necessarily reflect corresponding changes in the tissues' ability to take up glucose. This may be because most glucose after surgery is taken up by the wound and the cells of the immune system, and the rate of uptake by these non-insulin-sensitive tissues is to a large extent determined by the diffusion gradient of glucose. Thus rate of glucose uptake has to be corrected for the prevailing plasma glucose concentration. The resulting value, the glucose clearance rate, represents an index of the ability of tissues to take up glucose. The plasma glucose

clearance rate was calculated as Ra glucose divided by the corresponding plasma glucose concentration.

C. 3.6. Statistical analysis

The primary endpoint of the study was 1) plasma glucose and 2) protein balance (represented by the values of leucine kinetics). On the basis of previous studies a difference in protein balance (leucine) of 7.5 $\mu mol*kg^{-1}*h^{-1}$ [10] and a difference of plasma glucose of 3 $mmol*l^{-1}$ between the two groups were defined as metabolically relevant. Assuming a standard deviation of 5 $\mu mol*kg^{-1}*h^{-1}$, a repeated measure design with 7 patients per group achieves a power of 80% (alpha two-sided 0.05) for protein balance. Assuming a standard deviation of 1.6 $mmol*l^{-1}$, a repeated measure design with 6 patients per group achieves a power of 80% (alpha two-sided 0.05) for plasma glucose. The treatment effect on variables (glucose production, glucose utilization, protein breakdown, oxidation and synthesis) were evaluated by using two factorial analysis for repeated measures. P-values < 0.05 are considered statistically significant. All analyses were performed using the General Linear Model in SPSS 11.0 for windows, SPSS Inc., Chicago, IL.

C. 4. Results

Patients

The demographic characteristics of the patients are shown in table C1. The pain scores measured by visual analog scale never exceeded the value of 4 and no patient complained about severe pain.

We compared our results to the previously published data of 6 diabetic patients, which were kindly provided by Dr. Schricker.[6] Their values were obtained during a similar study setting and analyzed by the same techniques, except that dextrose (4 $mg*kg^{-1}*min^{-1}$) was administered instead of amino acids.

The two groups were similar regarding sex, age, height, weight and duration of surgery (table C1). The pain scores of the DEX group were comparable to the AA group.

Glucose and protein kinetics

The enrichments of plasma [1-^{13}C]α-ketoisocaproate, [6,6-$^{2}H_2$]glucose, and expired ^{13}C-carbon dioxide reached a plateau in all fast and fed states (coefficient of variation < 5%), which enabled to apply the steady state equation.

The results for glucose and protein kinetics are presented in tables C2.1. and C2.2. Protein balance is indicated for leucine (which was chosen as a representative for protein metabolism) as well as for whole body protein.

a. Glucose kinetics. Results for fasting glucose kinetics were comparable in both groups.

The main effect of feeding was to decrease endogenous Ra glucose ($p<0.0001$) and glucose clearance ($p=0.0002$).

In the fed state, endogenous Ra glucose was lower in the DEX group compared to the AA group ($p<0.0001$), whereas Ra glucose was smaller in the AA group compared to DEX

group ($p<0.0001$) reflecting the different impact of the type of feeding. The infusion of dextrose decreased endogenous rate of appearance of glucose dramatically and stimulated rate of appearance of glucose; in contrast the administration of amino acids decreased endogenous rate of appearance of glucose and rate of appearance of glucose ($p= 0.015$ and $p= 0.005$ respectively).Glucose clearance was decreased by dextrose as well as by amino acid infusion ($p= 0.838$).

b. Protein kinetics. Results for fasting protein kinetics were similar in both groups except for leucine oxidation (DEX group: 30 ± 10 $\mu mol*kg^{-1}*h^{-1}$; AA group: 15 ± 5 $\mu mol*kg^{-1}*h^{-1}$; $p=0.009$), which resulted in a less negative protein balance in the AA group compared to the DEX group (DEX group: -30 ± 10 $\mu mol*kg^{-1}*h^{-1}$; AA group: -15 ± 5 $\mu mol*kg^{-1}*h^{-1}$; $p=0.010$).

The main effect of feeding was a decrease in net protein breakdown ($p=0.014$) and was reduced to a higher amount in the AA group compared to the DEX group ($p=0.022$). Close analysis indicated that this effect of type of nutrition was greater in the AA group compared to the DEX group ($p=0.030$).

The type of nutrition had a greater impact on leucine oxidation in the AA group than in the DEX group ($p=0.005$).

For protein balance a statistically significant difference for the transition from the fasted to the fed state was found ($p=0.022$), but an even more pronounced difference when comparing the two groups ($p<0.0001$), resulting in a greater effect of nutrition in the AA group ($p=0.032$ for interaction). Dextrose infusion did not affect protein balance; however AA infusion dramatically increased protein balance from -15 $\mu mol*kg^{-1}*h^{-1}$ to +30 $\mu mol*kg^{-1}*h^{-1}$.

Gaseous Exchange

Gaseous exchange values were comparable between the groups in the fasted state. No statistically significant changes were observed by the effect of nutrition or type of nutrition (table C3).

Plasma Hormones and Metabolites

The results for plasma hormones and metabolites are presented in table C4. The transition from fasting to the fed state was accompanied by increased concentrations for plasma glucose ($p=0.01$) and insulin ($p=0.006$). This hyperglycaemic effect was pronounced in the DEX group, with mean values by 4.8 $mmol*l^{-1}$ higher than in the AA group (DEX: 12.7 ± 1.5 $mmol*l^{-1}$; AA group: 7.9 ± 1.7 $mmol*l^{-1}$).

C. 5. Discussion

Malnutrition in surgical patients is associated with higher rates of morbidity and mortality.[89-93] These concerns are even emphasized in the population of diabetic patients undergoing gastrointestinal surgery, as they suffer from higher risk per se due to their metabolic disorder and the type of surgery.[55, 94] Protein losses induced by the stress response to surgery can be attenuated by total parenteral nutrition, but only starving or malnourished patients with cancer benefit in the sense of a shift to anabolism.[95-97] Unselected iso- or hypercaloric nutrition support in well-nourished surgical patients has no evidence based indication and may even harm since it is associated with increased morbidity.[98] Traditional strategies for patients after gastrointestinal surgery include fasting and intravenous fluids with hypocaloric dextrose until bowel motility is secured. However, hyperglycaemic levels, as possibly induced by dextrose infusion, affect the

immune system leading to an increased risk of infection and is related to an increased risk of postoperative complications in cardiac surgery.[51]

The results of the present study suggest that the infusion of amino acids avoids pronounced hyperglycaemia in diabetic patients after colorectal surgery compared to dextrose. Furthermore, a positive protein balance can be achieved, resulting in a gain of lean body tissue, whereas dextrose infusion does not affect protein metabolism. Leucine kinetics were used to represent protein metabolism in this study. Leucine makes up 8% of whole body protein. Considering the difference of 58 $\mu mol*kg^{-1}*h^{-1}$ between the two study groups for protein balance (leucine) in the fed state the saved lean body mass of a 70 kg person per day can be calculated and results in 317 grams.

The most important difference in glucose metabolism found in this study setting, is the statistically significant plasma glucose level when comparing amino acids with dextrose. This is in accordance with the state of insulin resistance seen in diabetes mellitus, resulting in limited suppressibility of endogenous glucose production and abnormal peripheral glucose utilization.[99-101]

Endogenous glucose supply is based on glycogenolysis and gluconeogenesis. An overnight fasting leads to postabsorptive conditions, where glycogenolysis and gluconeogenesis provide each about half of whole body glucose production.[79] With ongoing fasting conditions, gluconeogenesis increases gradually and contributes up to 90% to glucose production after 42 h.[102] The patients of this study can be considered to be in a postabsorptive state on the second postoperative day taking into account the long perioperative fasting. In patients with DM2 a moderate increase in glucose production and gluconeogenesis under postabsorptive conditions can be detected.[33, 103-105] Higher glucose production rates have also been found in well-nourished patients with malignant

diseases.[106, 107] Our findings are consistent with these reports, as endogenous Ra glucose is about 5 $\mu ml*kg^{-1}*min^{-1}$ higher when compared to non-diabetic patients in postabsorptive conditions.[10, 11]

The inhibitory effect of exogenous glucose on endogenous glucose production depends on the amount of glucose infused and the patient's metabolic state. This is reflected by a reduced suppression of endogenous glucose production in septic or traumatized patients.[64, 65] Infusion of amino acids in the postoperative period in non-diabetic patients has been shown to reduce endogenous glucose production.[108, 109] In the present study, diabetic patients showed a less pronounced inhibitory effect of dextrose infusion on the endogenous glucose production when compared non-diabetic counterparts.[6] In contrast, the decrease of endogenous glucose production in the group with amino acid supply was more pronounced than previously reported in non-diabetics.[108] Therefore, it may be hypothesized that amino acid infusion suppresses endogenous Ra of leucine i.e. proteolysis, so that less amino acids are available for gluconeogenesis independent from the effect of insulin.

Furthermore, it may be hypothesized that diabetic patients have a higher turnover and respond more intensively to exogenous amino acids than non-diabetics due to their metabolic derangements in glucose metabolism.

The anti-catabolic effect of insulin depends from the state of peripheral resistance and exerts mainly through the inhibition of muscle protein breakdown.[110] In patients with diabetes mellitus type 1 protein breakdown and oxidation are increased, whereas in DM2 with good or moderate glucose control Ra of leucine and protein breakdown are normal.[105, 111, 112] Furthermore, in poorly controlled and obese DM2 patients protein breakdown was higher than in their non-diabetic counterparts.[113, 114] and except for two

studies, leucine oxidation has not been shown to increase.[33, 105] In the fasted state of our postoperative study discrepant results regarding leucine oxidation were detectable. In the DEX group a clearly higher oxidation was found compared to previously published results in non-diabetic patients, whereas this value was slightly lower in the AA group than in non-diabetic patients.[10, 11, 108] A possible explanation is the limitation of this study of not having determined HbA1c prior to surgery. The preoperative plasma glucose concentrations indicated good or moderate control in both groups and one patient of the AA group was on insulin treatment. Hence, the glucose control could not be quantified in our study population and might be an explanation for the divergent results. A recent study reported that hyperglucagonemia appears to be primarily responsible for increased leucine oxidation in diabetes type 1 patients.[115] Furthermore, it could be argued that a higher number of patients is needed to assess this difference in leucine oxidation better.

The present study was not designed to primarily investigate the biochemical factors responsible for catabolic stress response to surgery. Furthermore, an assessment of circulating glucagon plasma levels would have provided information, but this analysis was not available due to technical problems. In the previous study by Schricker et al., which compared diabetic to non-diabetic patients after colorectal surgery, the authors found lower circulating insulin concentrations accompanied by higher circulating glucagons concentrations in the diabetic group and furthermore negative correlations between the insulin/glucagon ratio and glucose production as well as protein oxidation. They concluded therefore, that changes in the insulin/glucagon system may be responsible for these changes.[6]

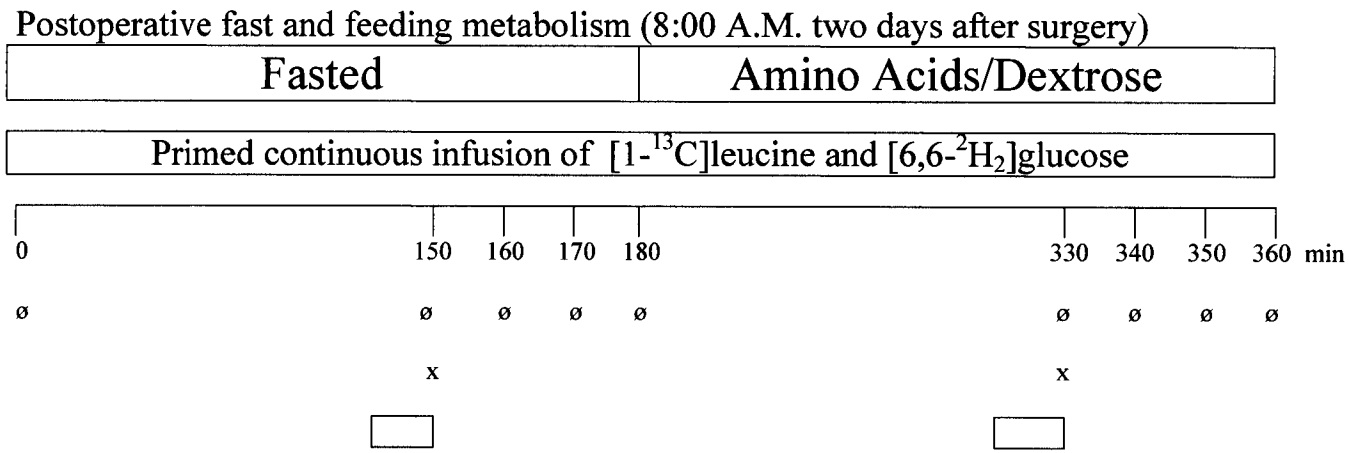
Infusion of amino acids increased Ra of leucine, leucine oxidation, as well as protein synthesis and reduced net protein breakdown, resulting in a positive protein balance. In

contrast, administration of glucose did not affect protein kinetics and patients presented with a unchanged, negative protein balance. These findings are consistent with observations in non-diabetic patients: Infusion of amino acids at a similar rate caused the same alterations, however, protein balance appears to be improved to a higher extent in diabetic patients.[108] Infusion of glucose at a similar rate has been shown not to affect Ra of leucine and oxidation in volunteers[82], but to statistically significant decrease leucine oxidation in non-diabetic patients after colorectal surgery.[10]

RQ values remained unchanged for fast and fed states. Although the insulin resistance of these patients with DM2 contributes to decreased substrate oxidation, one would expect an increase in RQ with feeding. Again, this missing reaction to feeding could be due to the small number of patients assessed. Additionally it may be argued that the infused glucose was not effectively metabolized since glucose clearance remained unchanged also in the state of hyperglycemia.

In summary this was the first study to investigate the effects of different types of feeding in diabetic patients after colorectal surgery. The maintenance of glucose homeostasis and the shift to an anabolic state assured by amino acid infusion suggests reevaluation standard practice in postoperative nutrition support in diabetic patients may be warranted.

Figure C1. Study protocol.



Time course of the infusion of isotopes and collection of plasma and expired air samples (Ø) indirect calorimetry (open rectangles), and collection of plasma for the determination of metabolic substrates and hormones (x) in the fasted state and during the infusion of amino acids

Table C1. Biometric and clinical data of patients

	Amino Acids	Dextrose*
Number	6	6
Age (years)	74 ± 9	68 ± 5
Height (cm)	173 ± 11	168 ± 6
Weight (kg)	87 ± 22	70 ± 6
BMI ($kg*m^{-2}$)	28.9 ± 5.8	24.8 ± 1.0
Sex (male/female)	5/1	4/2
ASA (I/II/III)	0/3/3	0/3/3
Type of surgery		
Hemicolectomy/Colectomy	2	3
Sigmoid resection	1	0
Anterior resection	3	3
Duration of surgery (min)	167 ± 93	204 ± 56

Values are mean ± SD, ASA = American Society of Anesthesiologists classification

* Values are obtained from the previously published study "Type 2 diabetes mellitus and the catabolic response to surgery" by Schricker et al., Anesthesiology, 2005.[6]

Table C2.1. Kinetics of glucose metabolism in the fasted and fed state for the dextrose and amino acid group

Variable	Dextrose[6]		Amino Acids		P-value		
	Fasted	Fed	Fasted	Fed	Feeding state§	Type of nutrition†	Interaction‡
Endogenous rate of appearance of glucose ($\mu mol*kg^{-1}*min^{-1}$)	15 ± 2	4 ± 1	17 ± 3	12 ± 3	**<0.0001**	**0.015**	0.104
Rate of appearance of glucose ($\mu mol*kg^{-1}*min^{-1}$)	15 ± 2	25 ± 2	17 ± 3	12 ± 3	0.352	**0.005**	0.270
Glucose clearance ($ml*kg^{-1}*min^{-1}$)	2.23 ± 0.34	1.96 ± 0.25	2.43 ± 0.36	1.55 ± 0.31	**0.0002**	0.838	0.165

Values are presented as mean ± SD; n=6 per group
§ Probability that values are influenced by parenteral alimentation. † Probability that values are influenced by the type of nutrition.
‡ Probability that the effect of type of nutrition is greater in one group.

Table C2.2. Kinetics of protein metabolism in the fasted and fed state for the dextrose and amino acid group

Variable	Dextrose[6]		Amino Acids		P-value		
	Fasted	Fed	Fasted	Fed	Feeding state§	Type of nutrition†	Interaction‡
Rate of appearance of leucine ($\mu mol*kg^{-1}*h^{-1}$)	137 ± 26	122 ±16	123 ± 19	142 ± 25	0.850	0.539	0.456
Net protein breakdown ($\mu mol*kg^{-1}*h^{-1}$)	137 ± 26	122 ± 16	123 ± 19	88 ± 25	**0.014**	**0.022**	**0.030**
Leucine oxidation ($\mu mol*kg^{-1}*h^{-1}$)	30 ± 10	28 ± 6	15 ± 5	24 ± 8	0.244	**0.005**	0.096
Protein synthesis (leucine) ($\mu mol*kg^{-1}*h^{-1}$)	107 ± 21	94 ± 16	108 ± 19	117 ± 21	0.769	**0.068**	0.905
Protein balance (leucine) ($\mu mol*kg^{-1}*h^{-1}$)	-30 ± 10	-28 ± 6	-15 ± 5	30 ± 8	**0.022**	**<0.0001**	**0.032**
Protein balance (whole body protein) ($\mu mol*kg^{-1}*h^{-1}$)	-49 ± 16	-46 ± 10	-25 ± 8	49 ± 13	**0.022**	**<0.0001**	**0.032**

Values are presented as mean ± SD; n=6 per group
§ Probability that values are influenced by parenteral alimentation. † Probability that values are influenced by the type of nutrition.
‡ Probability that the effect of type of nutrition is greater in one group.

Table C3. Gaseous exchange in the fasted and fed state for the dextrose and amino acid group

Variable	Dextrose[6]		Amino Acids		P-value		
	Fasted	Fed	Fasted	Fed	Feeding state§	Type of nutrition†	Interaction‡
VO_2 ($l*min^{-1}$)	260 ± 55	249 ± 46	262 ± 27	295 ± 78	0.932	0.284	0.933
VCO_2 ($l*min^{-1}$)	195 ± 42	189 ± 35	194 ± 26	209 ± 50	0.881	0.534	0.899
RQ	0.75 ± 0.02	0.76 ± 0.02	0.74 ± 0.05	0.72 ± 0.11	0.745	0.276	0.684

Values are presented as mean ± SD; n=6 per group
§ Probability that values are influenced by parenteral alimentation. † Probability that values are influenced by the type of nutrition. ‡ Probability that the effect of type of nutrition is greater in one group.
RQ = respiratory quotient

Table C4. Plasma concentrations of circulating metabolites and hormones in the fasted and fed state for the dextrose and amino acid group

Variable	Dextrose[6]		Amino Acids		P-value		
	Fasted	Fed	Fasted	Fed	Feeding state§	Type of nutrition†	Interaction‡
Glucose (mmol*l^{-1})	6.9 ± 1.3	12.7 ± 1.5	7.2 ± 1.4	7.9 ± 1.7	**0.010**	**0.028**	0.118
Insulin (pmol*l^{-1})	83 ± 49.8	181 ± 85.3	57 ± 30.2	124 ± 61.4	**0.006**	0.346	0.298
Cortisol (nmol*l^{-1})	390 ± 86	289 ± 109	511 ± 329	503 ± 432	0.593	0.146	0.766

Values are presented as mean ± SD; n=6 per group
§ Probability that values are influenced by parenteral alimentation. † Probability that values are influenced by the type of nutrition. ‡ Probability that the effect of type of nutrition is greater in one group.

C. 6. Summary of manuscript 1 and introduction to manuscript 2

Loss of body protein and hyperglycemia represent typical features of the stress response to surgery. In patients with DM2 protein catabolism after colorectal surgery is increased as reflected by a higher oxidative protein loss and an aggravated state of insulin resistance, resulting in a higher perioperative risk for complications.

The first part of this project investigated the different effects of amino acids and dextrose infusions on the catabolic response on the second day after colorectal surgery in diabetic patients.

Infusion of amino acids maintained glucose homeostasis and shifted protein balance towards an anabolic state compared to dextrose infusion. This suggests to re-evaluate standard practice in postoperative nutritional support in diabetic patients.

However, this study setting did not allow differentiation of the different impacts of analgesia, surgery and feeding on protein and glucose metabolism. The second study aimed to distinguish and quantify these effects on the stress response to surgery in diabetic patients. Since epidural blockade improves substrate utilization after surgery in non-diabetic patients by reducing the intensity of the catabolic response, the hypothesis is tested that in diabetic patients epidural analgesia in comparison with systemic opioids enhances protein balance and, therefore, results in a pronounced anabolic state after colorectal surgery.

Can epidural analgesia mitigate the catabolic response to surgery in diabetes mellitus type 2 patients receiving amino acid infusion?

Andrea Kopp Lugli, M.D.[1-3], Francesco Donatelli, M.D.[1,2], Thomas Schricker, M.D., Ph.D.[1], Linda Wykes, Ph.D.[2], Franco Carli, M.D., M.Phil.[1]

[1] Department of Anesthesia, McGill University, Montreal

[2] School of Dietetics and Human Nutrition, McGill University, Montreal

[3] Department of Anesthesia and Perioperative Intensive Care Medicine, Kantonsspital, Aarau, Switzerland

Correspondence should be sent to:

Dr. Andrea Kopp Lugli

Department of Anesthesia

McGill University Health Centre

1650 Avenue Cedar, D10-144

Montreal, Quebec, Canada, H3G 1A4

Phone: 514 934 1934 (ext.: 43261)

Fax: 514 934 8249

Email: andrea.kopplugli@mail.mcgill.ca

Intended to be sent to Anesthesiology

D. 1. Abstract

Background: Surgical injury induces an endocrine-metabolic stress response, resulting in a catabolic state, for which insulin resistance appears to contribute extensively. It has been suggested, that type 2 diabetes mellitus (DM2) amplifies the stress response, since these patients suffer from similar metabolic derangements.

Methods: 12 diabetic patients undergoing colorectal surgery were randomized to epidural analgesia (EDA group) or patient controlled analgesia (PCA group) for perioperative pain control. On the day before and on the second day after surgery, a 5 h stable isotope infusion study (2 h fasted, 3 h amino acid infusion at $0.02ml*kg^{-1}*min^{-1}$ (equivalent to $2.9g*kg^{-1}*day^{-1}$)) was performed and glucose and protein kinetics measured using L-[1-^{13}C]leucine and [6,6$^{2}H_2$]glucose.

Results: The administration of amino acids provoked a significant increase in protein balance ($p=0.002$), rate of appearance (Ra) of leucine ($p=0.029$) and leucine oxidation ($p=0.014$), whereas net protein breakdown ($p=0.052$) and protein synthesis ($p=0.053$) only changed moderately. Endogenous Ra of glucose ($p=0.007$) and glucose clearance ($p=0.035$) showed both a significant decrease for the transition from the fasted to the fed state. The impact of epidural analgesia was not statistically significant, but close analysis of the interaction between analgesia, feeding and surgery indicated a trend for a higher protein balance ($p=0.062$) and an attenuation in the increase of net protein breakdown ($p=0.06$) postoperatively in the EDA group compared to the PCA group. Postoperative amino acid infusion decreased slightly endogenous rate of glucose ($p=0.05$) when compared to the fasted state.

Conclusion: A short term infusion of amino acids postoperatively blunts protein breakdown and stimulates protein synthesis. This results in a positive protein balance in

patients with epidural blockade with a borderline significance when compared to the PCA group and adjusted for preoperative baseline values. With regard to glucose metabolism, amino acid supply after surgery decreased glucose clearance and endogenous glucose production.

D. 2. Introduction

The nutritional status of surgical patients plays an intriguing role in perioperative management, since malnutrition is associated with higher rates of morbidity and mortality.[89-93, 116] Patients undergoing gastrointestinal surgery are even at higher risk of nutritional depletion due to inadequate nutritional intake, surgical stress and the subsequent increase in metabolic rate.[55]

The stress response to surgery roots on inflammatory mediators produced by diverse cell types at the site of the surgical injury and afferent neural stimuli.[19-23] These afferent neural stimuli from traumatized tissues induce the endocrine-metabolic response resulting in increased secretion of catabolic stress hormones (most importantly cortisol, epinephrine, norepinephrine and glucagon) and decreased release and effect of anabolic hormones like insulin.

The stress response aims at provision of nutrients, thus shifting metabolism to a catabolic state with subsequent hyperglycaemia, muscle protein breakdown and mobilization of triglycerides.[18, 20, 22, 26] Continuous epidural analgesia in a perioperative setting blocks the induction of the stress response and has been shown to reduce significantly postoperative nitrogen excretion[45-47], to attenuate the increased amino acid oxidation[48, 49] and whole body protein breakdown as well as to blunt the decrease in muscle protein synthesis.[11, 50] However, these results were obtained in patients receiving continuous parenteral nutrition support, preventing to distinguish between the effect of feeding and analgesia.

After abdominal surgery and in a fasted state, four studies reported consistently no difference between epidural blockade and PCA with regard to whole body protein synthesis, breakdown, leucine oxidation and glucose clearance[10-12, 108], but only two trials detected a lower endogenous glucose production in patients with epidural blockade.[12, 108]

The infusion of dextrose (4mg*kg^{-1}*min^{-1}) on the second day after abdominal surgery caused an increase in glucose clearance and a decrease in leucine oxidation in the presence of epidural blockade, but did not alter whole body protein synthesis.[10] Concluding that the protein-sparing effect of epidural blockade requires adequate energy and substrate supply, since energy supply alone did not stimulate whole body protein synthesis, The subsequent trial administered dextrose (4mg*kg^{-1}*min^{-1}) and amino acids (0.02ml*kg^{-1}*min^{-1}; Travasol™) during a similar study setting.[11] Endogenous protein breakdown and glucose production were decreased to the same extent in both groups, but whole body protein synthesis increased to a greater degree in the epidural group.[11] Although administered at a low rate, the infusion of dextrose induced hyperglycaemia[10, 11] and suggested to refrain in order to prevent subsequent correlated complications.[51] When administered alone on the second day after abdominal surgery, amino acids (0.02 ml*kg^{-1}*min^{-1}, Travasol™, equivalent to 2.9 g*kg^{-1}*day^{-1}) inhibited protein breakdown and stimulated protein synthesis, resulting in a positive protein balance independent from the pain-control technique applied.[108]

With regard to glucose metabolism, postoperative epidural blockade results in decreased plasma glucose levels, reduced endogenous glucose production and higher glucose tolerance due to lower insulin resistance.[1] These findings may be explained by the inhibition of the endocrine-metabolic response due to epidural blockade, thus, reducing the levels of catabolic hormones which leads to an improved glucose utilization. Studies on glucose tolerance in different surgical settings support this interpretation.[52-54] However, in the study of Donatelli et al., which administered amino acids alone on the second postoperative day, a decreased endogenous glucose production and a decreased glucose

clearance were detected independently from the pain-relief technique applied, suggesting a state of insulin resistance in both groups.[108]

It has been hypothesized that patients with DM2 suffer from marked catabolic responses[34], since a major phenomenon of the stress response is the development of insulin resistance.[6, 33] But few studies have been conducted in this field to support that assumption. Diabetic patients undergoing cataract surgery showed pronounced hyperglycaemia when compared to non-diabetic counterparts.[35] A recent study investigated the effect of epidural blockade and dextrose feeding on the second day after colon surgery comparing diabetic to non-diabetic patients.[6]Diabetic patients showed a higher leucine rate of oxidation and glucose rate of appearance as well as higher plasma glucose levels, but endogenous glucose production was suppressed to a smaller extent. These findings suggest that protein catabolism is elevated in diabetic patients as reflected by an increased oxidative protein loss.[6] However, the observed effects allow not to distinguish between the impact of analgesia and nutrition support on glucose and protein metabolism.

The aim of this study is to determine the effect of perioperative epidural analgesia on protein catabolism (fasted and fed states) in patients with DM2, undergoing colorectal surgery and receiving postoperative feeding with amino acids, the hypothesis being that the catabolic responses are blunted to a greater extent in presence of epidural analgesia.

D. 3. Methods

D. 3.1. Patients

Twelve patients undergoing elective colorectal surgery were recruited between November 2004 and July 2006. The study protocol was approved by the ethics committee of the Montreal General Hospital (GEN#04-060) and written informed consent was obtained from all patients. Inclusion criteria were age >18 years, diabetes mellitus type 2 (controlled by diet, oral hypoglycaemic medication or insulin and colorectal surgery for non-metastatic disease (including right, transverse, left, sigmoid, subtotal, total and hemicolectomy and low anterior resection). Exclusion criteria were severe cardiac, hepatic, renal or metabolic disorders, diabetes mellitus type 1, plasma albumin concentration $<35 g*l^{-1}$, more than 10% weight loss over the preceding three months, anaemia (hematocrit <30%), use of steroids, previous spine surgery limiting the use of an epidural catheter and pregnancy. The patients were randomly allocated by a computer-generated schedule into an epidural group (EDA) receiving general anaesthesia combined with perioperative epidural analgesia (n=6) and a control group (PCA) receiving general anaesthesia combined with postoperative patient controlled analgesia (n=6).

D. 3.2. Anaesthesia and perioperative care

Patients underwent bowel preparation on the day before surgery and were allowed to drink clear fluids until midnight. Patients were operated in the morning hours by two surgeons who collaborated as co-investigators.

Oral hypoglycaemic medications were discontinued on the day of surgery. Perioperative glycemic control was achieved by an insulin sliding scale aiming to maintain blood glucose levels between 5 and 10 $mmol*l^{-1}$. Blood glucose was measured every 6 hours.

In the epidural group an epidural catheter was inserted between thoracic vertebral levels T8-T11 before the induction of general anaesthesia. Neuraxial blockade was established with 0.5% bupivacaine to achieve a bilateral sensory block from thoracic dermatome level four (T4) to sacral dermatome level one (S1). The blockade was maintained with a constant infusion of 0.25% bupivacaine at a rate of 10 $ml*hr^{-1}$ during surgery.

In both groups general anaesthesia was induced by propofol (3 $mg*kg^{-1}$) and fentanyl (1.5 $\mu g*kg^{-1}$ in the epidural group and 3 $\mu g*kg^{-1}$ in the control group) and endotracheal intubation was facilitated by rocuronium (0.6 $mg*kg^{-1}$). General anaesthesia was maintained using 65 % nitrous oxide in oxygen and desflurane at endtidal concentrations required to keep heart rate within 20 % of preoperative values. Falls in mean arterial blood pressure (<60 mmHg) were treated with fluid administration (NaCl 0.9 %) and increments of phenylephrine (0.1 mg). Normal saline was infused during surgery at a rate of 4 $ml*kg^{-1}*min^{-1}$ and at a rate of 100 $ml*h^{-1}$ after surgery. On the first day after surgery the infusion was changed to Dextrose 5% in ¼ normal saline (at 100 $ml*h^{-1}$) and patients were allowed to drink clear fluids according to the state of bowel passage. At midnight of the first day after surgery the infusion was changed to normal saline (at 100 $ml*h^{-1}$) and patients were only allowed to drink water.

In the epidural group the sensory block from T 8 to L 3 dermatomes was postoperatively maintained for at least 48 hours by continuous epidural infusion of 0.1% bupivacaine supplemented with 3 $\mu g*ml^{-1}$ fentanyl (at a rate between 6-14 $ml*hr^{-1}$) to provide adequate analgesia for a 10-15 cm paramedian subumbilical incision. Pain levels at rest, during mobilization and coughing were evaluated in all patients using a 10 point visual analogue score (VAS) every four hours after surgery. Pain treatment was adjusted to achieve a VAS level at rest below two and below five during mobilization or coughing.

In the control group patients received a PCA with intravenous morphine to control pain relief postoperatively. The incremental dose of morphine was 1-2 mg, lockout was 7 min and dose duration was 30 seconds.

D. 3.3. Experimental protocol

On the day before surgery, starting at 8:00 am and under postabsorptive conditions, patients underwent a 5-hour preoperative tracer kinetic study to characterize baseline protein and glucose metabolism (figure D1). The study consisted of a 2-hour fasted state, which was followed by a 3-hour fed state. The study was repeated on the second postoperative day starting at 8:00 am (figure 3). Plasma kinetics of leucine and glucose were determined by using tracer quantities of L-[1-^{13}C] leucine (99% ^{13}C) and [6,6-$^{2}H_2$]glucose (99% ^{2}H) obtained from the local pharmacy. Sterile solutions of isotopes were prepared in the hospital pharmacy and kept at 4°C until administration.

A superficial vein in the dorsum of the hand was cannulated and the cannula kept patent with saline. A second superficial vein on the contralateral arm was cannulated to provide access for the infusion of the stable isotopes. Blood and expired air samples were collected to determine baseline enrichments. Each blood sample was transferred immediately to a heparinized tube, centrifuged at 4°C (2400 g * 10 minutes) and stored at –70 °C. Breath samples were collected in a 2 liters latex bag and transferred immediately to 10 ml vacutainers (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ).

Sodium bicarbonate ($NaH^{13}CO_3$) (0.08 mg*kg^{-1}) was administered orally. Priming doses of L-[1-^{13}C]leucine (4 μmol*kg^{-1}), and [6,6-$^{2}H_2$]glucose (22 μmol*kg^{-1}) were injected and followed immediately by continuous infusions of L-[1-^{13}C] leucine 0.06 μmol*kg^{-}

^{1}*min^{-1} and [6,6-$^{2}H_2$] glucose 0.22 μmol*kg^{-1}*min^{-1} lasting for 2 hours (fasted state). Blood and air samples were collected at 90, 100, 110 and 120 min of the study period, when the tracers were assumed to have reached an isotopic steady state, to determine the protein and glucose metabolism in the fasted state. For the subsequent 3-h period of fed state the concentration of L-[1-^{13}C] leucine was increased to 0.12 μmol*kg^{-1}*min^{-1} and that of [6,6-$^{2}H_2$] glucose maintained at 0.22 μmol*kg^{-1}*min^{-1}. During the fed state a 10% amino acid solution without electrolytes (Travasol™, Baxter, Montreal, Canada) was infused at a rate of 0.02ml*kg^{-1}*min^{-1} (equivalent to 2.9 g*kg^{-1}*day^{-1}) to achieve plasma amino acid concentration at least twofold above the basal.[81] The composition of Travasol^{TM} solution, which was verified before each administration, was as follows: 35 μmol*ml^{-1} proline, 34 μmol*ml^{-1} threonine, 217 μmol*ml^{-1} glycine, 207 μmol*ml^{-1} alanine, 36 μmol*ml^{-1} valine, 37 μmol*ml^{-1} methionine, 34 μmol*ml^{-1} isoleucine, 45 μmol*ml^{-1} leucine, 2 μmol*ml^{-1} tyrosine, 35 μmol*ml^{-1} phenylalanine, 9 μmol*ml^{-1} tryptophan, 38 μmol*ml^{-1} lysine, 26 μmol*ml^{-1} histidine and 57 μmol*ml^{-1} arginine. Blood and air samples were collected at 330, 340, 350 and 360 min of the study period, when the tracers were assumed to have reached an isotopic steady state, to determine the protein and glucose metabolism in the fasted state.

Blood samples were drawn during the last 10 min of the fasted and fed state periods for the analysis of glucose, insulin and cortisol.

Indirect calorimetry (Vmax 29N; SensorMedics, Yorba Linda, Calif) was performed for 15 min in the last hour of the fasted and fed states during the pre- and postoperative tracer kinetic study periods. The subjects were lying in a semirecumbent position and breathing

room air in a ventilated tent. Whole-body oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were determined.

D. 3.4. Measurements

D. 3.4.1. Isotopic enrichments

Plasma enrichment of [1-^{13}C]α-KIC, representing intracellular leucine, were used as the basis for calculating both flux and oxidation of leucine as described recently.[82, 83] Plasma [1-^{13}C]α-KIC enrichment were analyzed by its pentafluorobenzylester derivative using methane negative chemical ionization gas chromatography-mass spectrometry (GC/MS).[84, 85] Plasma [6,6-$^{2}H_2$]glucose enrichment were determined by electron impact analyses of the pentaacetate derivative as previously described.[86] ^{13}CO enrichment in expired breath was determined by isotope ratio mass spectrometry (Analytical Precision AP2003, Manchester, United Kingdom).[82] The rates of appearance (R_a) of leucine and glucose were calculated using the Steele formula as it applies to steady state conditions.[117] Steady state conditions for [6,6-$^{2}H_2$]glucose, [1-^{13}C]α-KIC and ^{13}CO were considered for analysis provided that the coefficient of variation was less than 5 % for the four enrichment measurements during the fasted and fed periods.

D. 3.4.2. Plasma metabolites and hormones

Plasma concentrations of glucose were measured by using an enzymatic colorimetric assay (Roche, GLU Glucose GOD-PAP) on automated clinical chemistry analyzers (Roche/Hitachi 904911: CAN 249 or Roche/Hitachi 912/917/MODULAR: CAN 525, Roche Diagnostics, Indianapolis, IN). Insulin concentrations were determined by a solid-

phase, two-site chemiluminescent immunometric assay (IMMULITE/IMMULITE 1000 Insulin; Diagnostic Products Corporation, Los Angeles, CA). Plasma concentrations of cortisol were measured by using an immunoassay (Unicell DXI 800; Backman Coulter, Brea, CA).

D. 3.4.3. Gaseous exchange

Average values of VO2 and VCO2 as well as the calculated respiratory quotient were retrieved from indirect calorimetry, accepting a coefficient of variation less than 10%.

D. 3.5. Calculation of protein and glucose metabolism

Whole body leucine and glucose kinetics were determined by conventional isotope dilution practice applying a two-pool stochastic model during stead-state conditions of the fasted and fed states before and after surgery.

When a physiological and isotopic steady state exists, the rate of appearance (Ra) of unlabeled substrate in plasma can be derived from the plasma isotope enrichment (APE or atom percentage excess) calculated by: $Ra = (APE_{inf}/APE_{pl}-1)*F$, where F is the infusion rate of labeled tracer, APE_{inf} is the tracer enrichment in the infusate and APE_{pl} equals the tracer enrichment in plasma, respectively. The APE used in this calculation are the mean of the four APE determined during steady state conditions obtained in each phase of the studies. The accuracy of the isotopic enrichments at isotopic plateau were tested by evaluating the scatter of values above their mean, expressed as coefficient of variation. A coefficient of variation less than 5 % was used as a confirmation of a valid plateau.

The kinetics of the amino acid leucine, which makes up for 8% of whole body protein, represent the dynamics of protein metabolism in this study setting. Therefore, the terms "protein synthesis" and "protein balance" are representing leucine kinetics and are used to present and discuss protein metabolism where not labelled expressively "protein balance (whole body protein)".

Under steady state conditions, leucine flux (Q) is defined by the formula: Q = S + O = B + I. Where S is the rate at which leucine is incorporated into body protein, O is the rate of oxidation of leucine, B is the rate at which unlabeled leucine enters the free amino acid pool from endogenous protein breakdown, and I is the rate of leucine intake including tracer (rate of infusion of L-[1-^{13}C]leucine (μmol*kg^{-1}*h^{-1})) and diet. Under conditions of a postabsorptive state, the essential amino acid leucine for protein synthesis and oxidation comes from a sole source: the breakdown of endogenous proteins, thus flux is equal to breakdown. Plasma enrichment of [1-^{13}C]α-KIC were used as the basis for calculating both flux and oxidation of leucine.[87] In the calculation of oxidation, factors of 0.76 for the fasted state and of 0.92 for the fed state (during amino acids infusion) were applied to account for the fraction of ^{13}C-carbon dioxide released from leucine but retained in the bicarbonate pool of the body.[10, 75, 88] Whole body protein breakdown in the fed state during the preoperative tracer kinetic study was calculated by subtracting only the exogenous leucine intake of the amino acid solution over the 3-h infusion from the measured flux.

In the fasted state the Ra glucose is equal to the endogenous production of glucose. In the physiologic steady state, whole body glucose uptake equals the rate of endogenous glucose production. Because glucose uptake increases proportionally as blood glucose concentrations increase, changes in whole body glucose uptake do not necessarily reflect

corresponding changes in the tissues' ability to take up glucose. This may be because most glucose after surgery is taken up by the wound and the cells of the immune system, and the rate of uptake by these non-insulin-sensitive tissues is to a large extent determined by the diffusion gradient of glucose. Thus rate of glucose uptake has to be corrected for the prevailing plasma glucose concentration. The resulting value, the glucose clearance rate, represents an index of the ability of tissues to take up glucose. The plasma glucose clearance rate was calculated as Ra glucose divided by the corresponding plasma glucose concentration.

D. 3.6. Statistical analysis

The primary endpoint of the study was the protein balance (represented by the values of leucine kinetics). On the basis of previous studies[10] a difference of protein balance (leucine) of 7.5 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ between the two groups were defined as metabolically relevant. Assuming a standard deviation of 5 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, a repeated measure design with 2 time 7 patients achieves a power of 80% (alpha two-sided 0.05). The treatment effect as well as differences between pre- and postoperative variables (glucose production, glucose utilization, protein breakdown, oxidation and synthesis) were evaluated by using two factorial analysis for repeated measures. P-values < 0.05 are considered statistically significant. All analyses were performed using the General Linear Model in SPSS 11.0 for windows, SPSS Inc., Chicago, IL.

D. 4. Results

Patients

The two groups were similar regarding sex, age, height, weight and duration of surgery (table D1). The pain scores measured by visual analog scale at rest 12 and 24 h after surgery and during the study on the second postoperative day never exceeded the value of 4 and no patient complained about severe pain in both groups.

Glucose and Protein Kinetics

The steady state equation was applied, since the enrichments of plasma [1-^{13}C]α-ketoisocaproate, [6,6-$^{2}H_2$]glucose, and expired ^{13}C-carbon dioxide reached a plateau in all fast and fed states (coefficient of variation < 5%).

The data on protein and glucose kinetics are shown on tables D2 and D4. Corresponding p-values are presented in tables D3 and D5. Protein balance is indicated for leucine (which was chosen as a representative for protein metabolism) as well as for whole body protein.

Preoperative parameters for glucose and protein kinetics were comparable in both groups in the fasted and fed state (tables D2 and D4).

Effect of surgery. On the second day after surgery no significant differences in glucose and protein metabolism were detectable.

Effect of feeding. The administration of amino acids increased protein balance (p=0.002), Ra of leucine (p=0.029) and leucine oxidation (p=0.014), whereas net protein breakdown (p=0.052) and protein synthesis (p=0.053) changed only moderately. Endogenous rate of glucose appearance (p=0.007) and glucose clearance (p=0.035) showed both a significant decrease for the transition from the fasted to the fed state.

Interactions. The impact of epidural analgesia was not statistically significant. However, close analysis of the interaction between analgesia, feeding and surgery indicates a trend for a higher protein balance (p=0.062) and an attenuation in the increase of net protein breakdown (p=0.06) postoperatively in the EDA group compared to the PCA group. Postoperative endogenous rate of glucose decreased slightly (p=0.05) when comparing fasted to fed state.

Gaseous exchange

Preoperative VO_2 (p=0.004) and VCO_2 (p=0.016) were significantly different in the two groups as well as when comparing between subjects (VO_2: p= 0.021; VCO_2: p=0.034) (tables D6 and D7). In contrast no further significant effect of analgesia, surgery or feeding was detected for VO_2, VCO_2 or RQ.

Plasma hormones and metabolites

Results for hormones and metabolites are presented in tables D8 and D9.

Glucose. Preoperative fasting glucose levels were elevated to a same extent in both groups (EDA: 7.9 ±2.3 $mmol*l^{-1}$; PCA: 7 ± 2.3 $mmol*l^{-1}$). Plasma glucose concentrations were neither significantly altered by feeding, nor by surgery or analgesia. In the postoperative fed state glucose levels were slightly higher, but did not exceed the mean of 8mmol/l (EDA: ± 1.7 $mmol*l^{-1}$; PCA: ± 1.3 $mmol*l^{-1}$) in both groups.

Insulin. Preoperative fasting insulin levels were comparable in the two groups (EDA: 86 ± 61 $pmol*l^{-1}$; PCA 83 ± 63 $pmol*l^{-1}$). The transition from the fasted to the fed state provoked a significant increase (p=0.021). In contrast, analgesia or surgery as well as interactions did not affect insulin levels.

D. 5. Discussion

The present study was the first to investigate the impact of analgesia and feeding on glucose and protein metabolism in diabetic patients undergoing colorectal surgery. Additionally, the design including a preoperative and postoperative study allowed to adjust for within subject variability and to investigate the effect of surgery.

Insulin deficiency is associated with a catabolic state.[118] In type1 diabetes mellitus patients increased protein breakdown and oxidation have been reported.[115, 119] In poorly controlled and obese DM2 patients a higher protein breakdown was revealed when compared to non-diabetic counterparts.[113, 114] In contrast, in DM2 patients under good or moderate glucose control no alteration of leucine appearance or protein breakdown could be found when compared to a non-diabetic population.[105, 111, 112, 120] For leucine oxidation apart from two studies no other trial found increased values.[33, 105] For DM2 patients suffering from colorectal cancer, previous published results for a small control group indicate that protein oxidation and breakdown are not increased before surgery when compared to non-diabetic subjects.[6] These results are consistent with our findings in the preoperative study.

The infusion of amino acids on the second postoperative day shifted protein balance to a positive amount, resulting from an increased protein synthesis and a decreased net protein breakdown. The impact of analgesia on these findings was not statistically significant, even though a trend towards a pronounced effect in the EDA group could be detected. It appears, that amino acids have a stronger influence on protein metabolism than type of analgesia. The infusion of amino acids postoperatively caused an average increase in protein balance of 40.5 $\mu mol*kg^{-1}*h^{-1}$ of which 9 $\mu mol*kg^{-1}*h^{-1}$ was due to type of analgesia. These findings are consistent with results from a previous trial in non-diabetics

with the same postoperative setting (36.7 μmol*kg^{-1}*h^{-1} and 7.3 μmol*kg^{-1}*h^{-1} respectively)[108], indicating that the effect of amino acids on the protein balance were about 5 times stronger than the type of analgesia. Analyzing only the results of our postoperative study, a statistically significant higher protein balance for the epidural group can be detected, whereas the glucose clearance shows no difference for the two groups. This effect of analgesia after surgery can not be shown anymore when adjusting the results with the baseline values gained preoperatively and must be, therefore, due to within subject variability.

In contrast with previous studies, our protocol included a preoperative assessment in order to characterize the effect of the interventions better and to adjust the postoperative results by using the preoperative values as baseline.

The results with interaction for surgery, feeding and analgesia of our study are congruent to a previously published investigation with the same postoperative setting in non-diabetic patients, which found a shift to a positive balance independent from analgesia.[108] In contrast, a recent study with a comparable protocol for the postoperative assessment infused the same amount of amino acids supplemented by dextrose (4 mg*kg^{-1}*min^{-1}) in non-diabetic patients and reported a positive protein balance in both groups. However, the increase in protein balance was slightly higher in the epidural group than in the PCA group. Therefore, the authors suggested that the greater insulin sensitivity in patients with epidural, as reflected by a greater increase in glucose clearance in these patients, might be responsible.[11]In our study, when concentrating only on the postoperative results, even a statistically significant higher protein balance in the epidural group was detectable, but no difference for glucose clearance. Additionally, we found no modifications of hormone

levels between our two groups as Schricker et al. did, which would support their suggestion of greater insulin sensitivity in patients with epidural blockade.

When comparing our postoperative results with values for the same postoperative setting from a previous study in non-diabetic patients[108] it appears that diabetic patients with epidural blockade show a larger shift towards a positive protein balance (41 $\mu mol*kg^{-1}*h^{-1}$) than non-diabetic patients (34 $\mu mol*kg^{-1}*h^{-1}$) for transition from the fasted to the fed state (table D10). This benefit, however, can not be explained by a higher insulin sensitivity in the diabetic group, since glucose clearance changed to a smaller extent in diabetic patients (-0.8 $ml*kg^{-1}*min^{-1}$) compared to non-diabetic patients (-1.2 $ml*kg^{-1}*min^{-1}$) for the transition from the fasted to the fed state (table D11).

Suppression of gluconeogenesis has a protein sparing effect: a diminished need for gluconeogenic amino acids renders this amount of nitrogen available for reincorporation into lean body mass instead being used as an energy source and finally excreted as urea. In the present study the endogenous glucose production was only affected by feeding, whereas surgery and analgesia showed no impact. The transition from the fasted to the fed state postoperatively showed a minor decrease for endogenous glucose production (26-33%). This finding is consistent with a previous study for the same postoperative setting in non-diabetic patients[108], while in another trial in non-diabetic patients, which supplied not only amino acids, but also dextrose, endogenous glucose production was almost completely suppressed (80-90%).[11] Paradoxically, leucine oxidation was comparable in both studies, but protein balance was higher in the trial with amino acid supply only. However, in our study with diabetic patients, leucine oxidation was lower than in these two studies for non-diabetics. Protein balance was higher in the non-diabetics with amino acids and our study population compared to non-diabetics with

amino acids plus dextrose. Therefore, it appears that if the inhibition of endogenous glucose production should spare amino acids, these are not mainly guided towards the synthetic pathway, but the amount of oxidized amino acids is smaller in diabetic patients than in non-diabetics. It may be hypothesized that is difference is due to a higher turnover in glucose metabolism and subsequently a higher sensibility to exogenous amino acids in diabetic patients.

Even though a decrease in endogenous glucose production was observed in both groups, an impaired glucose uptake resulting in a decreased glucose clearance was found. This finding is consistent with results found in volunteers, where the infusion of amino acids reduced the intracellular utilization of glucose.[121]

The infusion of dextrose even at low rates as performed in previous studies[10, 11]caused hyperglycaemic plasma levels with values up to 10 mmol*l^{-1}. Hyperglycaemia affects the immune system leading to an increased risk of infection and is related to an increased risk of postoperative complications in cardiac surgery.[51] Therefore, it has been emphasized that the effects of insulin resistance and tight control of blood glucose levels have to be considered paramount in the perioperative management.[18] Blood glucose levels in our study did not exceed a mean of 8 mmol*l^{-1}. This result should imply to consider peripheral infusion of amino acids as a potential nutrition support in diabetic patients when managing perioperative glucose homeostasis.

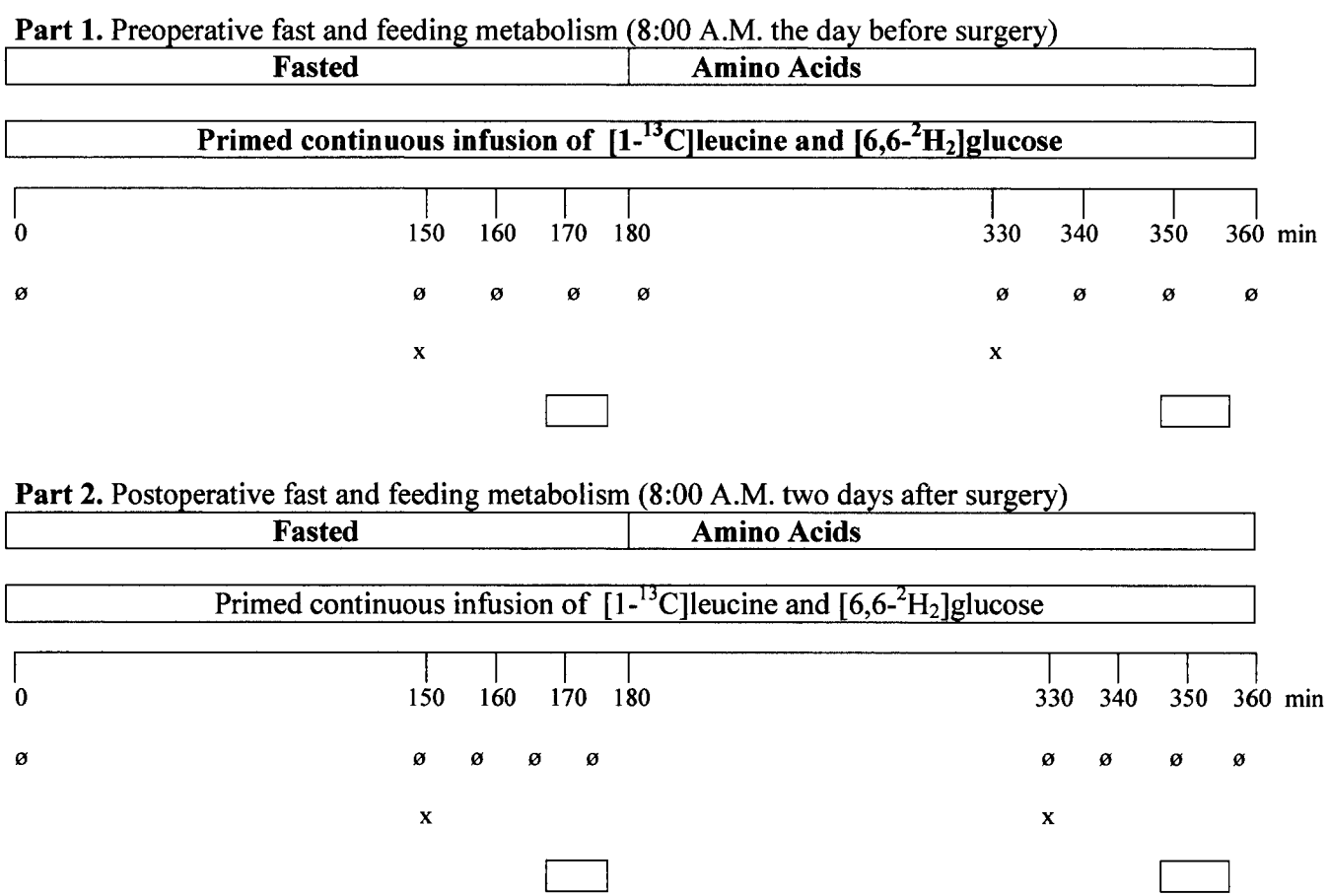
With regard to sample size, the present study failed to enrol the calculated number of patients due to time frame and smaller incidence of diabetic patients than expected. Therefore, it might be argued, that a bigger sample size could have contributed to a more distinct result for protein balance, considering the borderline p-value of 0.062 for interaction of analgesia, feeding and surgery. Another limitation of the study we would

like to acknowledge is the lack of HbA1c measurement. Preoperative plasma glucose concentrations indicated a good or moderate control and the two study groups were comparable regarding their treatment. Additionally, the quality of treatment is suggested to have only a small effect on protein metabolism in DM2 patients.[122]

RQ values remained unchanged for fast and fed states. Although the insulin resistance of these patients with DM2 contributes to decrease substrate oxidation, one would expect an increase in RQ with feeding. Again, this missing reaction to feeding could be due to the small number of patients assessed.

In summary this study is the first to analyze the potential effects of epidural blockade on protein catabolism in diabetic patients after colorectal surgery. A short term infusion of amino acids postoperatively blunts protein breakdown and stimulates protein synthesis, resulting in a positive protein balance with a higher, borderline significant effect in patients with epidural blockade compared to PCA, when adjusting for preoperative baseline values. With regard to glucose metabolism, amino acid supply after surgery decreased glucose clearance and endogenous glucose production. This indicates a state of insulin resistance on which the type analgesia had no effect. Further investigation has to test the hypothesis that diabetic patients benefit more from epidural blockade than non-diabetic counterparts regarding protein balance and glucose clearance.

Figure D1. Study protocol



Time course of the infusion of isotopes and collection of plasma and expired air samples (Ø) indirect calorimetry (open rectangles), and collection of plasma for the determination of metabolic substrates and hormones (x) in the fasted state and during the infusion of aminoacids.

Table D1. Biometric and clinical data of patients

	PCA	EDA
Number	6	6
Age (years)	68 ± 7	74 ± 9
Height (cm)	163 ± 10	173 ± 11
Weight (kg)	76 ±16	87 ± 22
BMI ($kg*m^{-2}$)	28.3 ± 4.5	28.9 ± 5.8
Sex (male/female)	4/2	5/1
ASA (I/II/III)	0/3/3	0/3/3
Type of surgery		
Hemicolectomy/Colectomy	3	2
Sigmoid resection	0	1
Anterior resection	3	3
Duration of surgery (min)	168 ± 78	167 ± 93
Diabetes Treatment (no/diet/OAD/Insulin)	0/2/3/1	1/0/4/1

Values are mean ± SD, PCA = Patient Controlled Analgesia, EDA = Epidural Analgesia, ASA = American Society of Anesthesiologists classification

OAD = oral antidiabetic drug

Table D2. Protein kinetics of the patients receiving epidural blockade or PCA before and after surgery in the fasted and fed state

Protein Kinetics	Before surgery		After surgery		P-value*			
					Before surgery		After surgery	
	Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed
Rate of appearance of leucine ($\mu mol*kg^{-1}*h^{-1}$)								
PCA	112 ± 9	152 ± 16	129 ± 12	165 ± 13	0.531	0.285	0.470	0.067
Epidural	107 ± 19	138 ± 26	123 ± 19	141 ± 25				
Net protein breakdown ($\mu mol*kg^{-1}*h^{-1}$)								
PCA	112 ± 9	98 ± 16	129 ± 12	111 ± 13	0.531	0.285	0.470	0.067
Epidural	107 ± 19	84 ± 26	123 ± 19	88 ± 25				
Leucine oxidation ($\mu mol*kg^{-1}*h^{-1}$)								
PCA	13 ± 2	29 ± 7	16 ± 7	33 ± 4	0.355	0.487	0.726	**0.023**
Epidural	14 ± 3	27 ± 5	15 ± 5	24 ± 8				
Protein synthesis (leucine) ($\mu mol*kg^{-1}*h^{-1}$)								
PCA	99 ± 7	122 ± 10	113 ± 9	132 ± 13	0.404	0.278	0.539	0.178
Epidural	92 ± 18	111 ± 22	108 ± 19	117 ± 21				
Protein balance (leucine) ($\mu mol*kg^{-1}*h^{-1}$)								
PCA	-13 ± 2	25 ± 7	-16 ± 7	20 ± 4	0.355	0.485	0.726	**0.023**
Epidural	-14 ± 2	27 ± 5	-15 ± 5	30 ± 8				
Protein balance (whole body protein) ($\mu mol*kg^{-1}*h^{-1}$)								
PCA	-21 ± 3	41 ± 11	-26 ± 11	33 ± 7	0.355	0.485	0.726	**0.023**
Epidural	-23 ± 3	44 ± 8	-25 ± 8	49 ± 13				

* PCA compared to epidural blockade within each cell
Values are mean ± SD; n= 6 per group

Table D3. P-values for protein kinetics for patients receiving epidural blockade or PCA from table D2

Factor	P-value				
	Rate of appearance of leucine	**Net protein breakdown**	**Leucine oxidation**	**Protein synthesis (leucine)**	**Protein balance (leucine/ whole body protein)**
Between subjects					
Analgesia	0.142	0.113	0.339	0.115	0.665
Within subjects					
Feeding	**0.029**	0.052	**0.014**	0.053	**0.002**
Surgery	0.215	0.182	0.629	0.186	0.833
Analgesia/Feeding	0.353	0.353	0.190	0.126	0.190
Analgesia/Surgery	0.658	0.623	0.452	0.720	0.737
Feeding/Surgery	0.335	0.057	0.108	0.567	**0.039**
Analgesia/Feeding/Surgery	0.746	0.060	0.221	0.789	0.062

Table D4. Glucose kinetics of the patients receiving epidural blockade or PCA before and after surgery in the fasted and fed state

Glucose kinetics	Before surgery		After surgery		P-value*			
					Before surgery		After surgery	
	Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed
Endogenous rate of appearance of glucose ($\mu mol*kg^{-1}*min^{-1}$)								
PCA	16 ± 3	12 ± 2	17 ± 3	14 ± 3	0.250	0.074	0.723	0.393
Epidural	14 ± 3	10 ± 2	17 ± 3	12 ± 3				
Glucose clearance ($ml*kg^{-1}*min^{-1}$)								
PCA	2.5 ± 0.8	1.9 ± 0.7	2.3 ± 0.6	1.7 ± 0.3	0.188	0.247	0.555	0.362
Epidural	1.9 ± 0.7	1.5 ± 0.3	2.4 ± 0.4	1.6 ± 0.3				

* PCA compared to epidural blockade within each cell
Values are mean ± SD; n= 6 per group

Table D5. P-values for glucose kinetics for patients receiving epidural blockade or PCA from table D4

Factor	P-value	
	Endogenous rate of appearance of glucose	**Glucose clearance**
Between subjects		
Analgesia	0.374	0.252
Within subjects		
Feeding	**0.007**	**0.035**
Surgery	0.154	0.733
Analgesia/Feeding	0.678	0.619
Analgesia/Surgery	0.485	0.294
Feeding/Surgery	0.051	0.116
Analgesia/Feeding/Surgery	0.131	0.286

Table D6. Gaseous exchange of the patients receiving epidural blockade or PCA before and after surgery in the fasted and fed state

Gaseous exchange	Before surgery		After surgery		P-value*			
					Before surgery		After surgery	
	Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed
VO_2 ($ml*min^{-1}$)								
PCA	200 ± 21	200 ± 18	200 ± 28	216 ± 41	0.074	**0.040**	**0.003**	0.052
Epidural	269 ± 81	274 ± 74	262 ± 27	295 ± 78				
VCO_2 ($ml*min^{-1}$)								
PCA	157 ± 15	159 ± 16	150 ± 20	168 ± 25	0.175	0.060	**0.008**	0.105
Epidural	187 ± 49	208 ± 55	194 ± 26	209 ± 50				
RQ								
PCA	0.78 ± 0.04	0.79 ± 0.04	0.75 ± 0.04	0.79 ± 0.04	0.175	0.540	0.599	0.180
Epidural	0.71 ± 0.12	0.77 ± 0.1	0.74 ± 0.05	0.72 ± 0.11				

* PCA compared to epidural blockade within each cell
Values are mean ± SD; n= 6 per group
RQ = respiratory quotient

Table D7. P-values for gaseous exchange for patients receiving epidural blockade or PCA from table D6

Factor	P-value		
	VO_2	CO_2	RQ
Between subjects			
Analgesia	**0.021**	**0.034**	0.115
Within subjects			
Feeding	0.517	0.256	0.314
Surgery	0.819	0.885	0.750
Analgesia/Feeding	0.759	0.675	0.891
Analgesia/Surgery	0.696	0.891	0.847
Feeding/Surgery	0.507	0.469	0.728
Analgesia/Feeding/Surgery	0.217	0.171	0.896

RQ = respiratory quotient

Table D8. Hormones and metabolites of patients receiving epidural blockade or PCA before and after surgery in the fasted and fed state

Hormones & metabolites	Before surgery		After surgery		P-value*			
					Before surgery		After surgery	
	Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed
Glucose ($mmol*l^{-1}$)								
PCA	7 ± 2.3	6.7 ± 1.5	7.8 ± 2.4	8 ± 1.3	0.488	0.944	0.588	0.956
Epidural	7.9 ± 2.3	6.6 ± 1.7	7.2 ± 1.4	8 ± 1.7				
Insulin ($pmol*l^{-1}$)								
PCA	83 ± 63	157 ± 140	78 ± 78	177 ± 148	0.946	0.962	0.552	0.437
Epidural	86 ± 61	160 ± 94	57 ± 21	124 ± 61				
Cortisol ($nmol*l^{-1}$)								
PCA	308 ± 87	320 ± 129	435 ± 159	489 ± 204	0.663	0.423	0.620	0.944
Epidural	287 ± 67	273 ± 50	511 ± 329	503 ± 432				

* PCA compared to epidural blockade within each cell
Values are mean ± SD; n= 6 per group

Table D9. P-values for hormones and metabolites for patients receiving epidural blockade or PCA from table D8

Factor	P-value		
	Glucose	**Insulin**	**Cortisol**
Between subjects			
Analgesia	0.677	0.587	0.709
Within subjects			
Feeding	0.863	**0.021**	0.774
Surgery	0.766	0.695	0.114
Analgesia/Feeding	0.845	0.235	0.637
Analgesia/Surgery	0.425	0.652	0.363
Feeding/Surgery	0.743	0.174	0.609
Analgesia/Feeding/Surgery	0.674	0.377	0.730

Table D10. Comparison of protein balance ($\mu mol*kg^{-1}*h^{-1}$) between diabetic and non-diabetic patients in the postoperative study for fasted and fed (amino acids) state receiving epidural blockade or PCA

Analgesia	Diabetic Patients			Non-diabetic Patients[17]		
	Fast	Fed	Difference	Fast	Fed	Difference
PCA	-16 ± 7	20 ± 4	36	- 18 ± 5	23 ± 6	41
Epidural	-15 ± 5	30 ± 8	45	-18 ± 4	16 ± 3	34

Values are mean ± SD; n= 6 per group for diabetic patients, n=8 for non-diabetic patients

Table D11. Comparison of glucose clearance ($ml*kg^{-1}*min^{-1}$) between diabetic and non-diabetic patients in the postoperative study for fasted and fed (amino acids) state receiving epidural blockade or PCA

Analgesia	Diabetic Patients			Non-diabetic Patients[17]		
	Fast	Fed	Difference	Fast	Fed	Difference
PCA	2.3 ± 0.6	1.7 ± 0.3	-0.6	2.9 ± 0.77	1.1 ± 0.24	-1.8
Epidural	2.4 ± 0.4	1.6 ± 0.3	-0.8	2.3 ± 0.3	1.1 ± 0.2	-1.2

Values are mean ± SD; n= 6 per group for diabetic patients, n=8 for non-diabetic patients

E. Final conclusion and summary

The main goal of this project was to investigate different approaches on protein sparing in diabetic patients undergoing colorectal surgery.

Infusion of amino acids avoided pronounced hyperglycaemia in diabetic patients after colorectal surgery and achieved a positive protein balance compared to dextrose.

Our preliminary results should suggest the need to reconsider standard practice for peripheral nutrition support, since traditional dextrose leads to hyperglycaemic blood glucose levels, resulting in a higher perioperative risk for this per se vulnerable patient population.

A short term infusion of amino acids postoperatively blunted protein breakdown and stimulated protein synthesis, resulting in a positive protein balance with a higher, borderline significant effect in patients with epidural blockade compared to PCA, when adjusting for preoperative baseline values. Furthermore, a decreased glucose clearance indicated a state of insulin resistance, which was not altered by analgesia.

However, further studies should investigate the suspected benefit of epidural blockade in diabetic patients compared to non-diabetic counterparts regarding a proportionally higher protein balance and lower decrease glucose clearance as with PCA.

F. References

1. Holte K, Kehlet H. Epidural anaesthesia and analgesia - effects on surgical stress responses and implications for postoperative nutrition. Clin Nutr 2002;21:199-206.
2. Kinney J. Metabolic response to injuries. In: Winters R, Green H (Eds.). Nutritional support of the seriously ill patient. Academic Press, 1983.
3. Smeets HJ, Kievit J, Harinck HI, Frolich M, Hermans J. Differential effects of counterregulatory stress hormones on serum albumin concentrations and protein catabolism in healthy volunteers. Nutrition 1995;11:423-7.
4. Romijn JA. Substrate metabolism in the metabolic response to injury. Proc Nutr Soc 2000;59:447-9.
5. McClave SA, Snider HL, Spain DA. Preoperative issues in clinical nutrition. Chest 1999;115:64S-70S.
6. Schricker T, Gougeon R, Eberhart L, Wykes L, Mazza L, Carvalho G, Carli F. Type 2 diabetes mellitus and the catabolic response to surgery. Anesthesiology 2005;102:320-6.
7. Moraca RJ, Sheldon DG, Thirlby RC. The role of epidural anesthesia and analgesia in surgical practice. Ann Surg 2003;238:663-73.
8. Kehlet H. Multimodal approach to control postoperative pathophysiology and rehabilitation. Br J Anaesth 1997;78:606-17.

9. Smeets HJ, Kievit J, Dulfer FT, van Kleef JW. Endocrine-metabolic response to abdominal aortic surgery: a randomized trial of general anesthesia versus general plus epidural anesthesia. World J Surg 1993;17:601-6; discussion 606-7.

10. Schricker T, Wykes L, Carli F. Epidural blockade improves substrate utilization after surgery. Am J Physiol Endocrinol Metab 2000;279:E646-53.

11. Schricker T, Wykes L, Eberhart L, Lattermann R, Mazza L, Carli F. The anabolic effect of epidural blockade requires energy and substrate supply. Anesthesiology 2002;97:943-51.

12. Lattermann R, Carli F, Wykes L, Schricker T. Epidural blockade modifies perioperative glucose production without affecting protein catabolism. Anesthesiology 2002;97:374-81.

13. Schricker T, Meterissian S, Wykes L, Eberhart L, Lattermann R, Carli F. Postoperative protein sparing with epidural analgesia and hypocaloric dextrose. Ann Surg 2004;240:916-21.

14. Askanazi J, Nordenstrom J, Rosenbaum SH, Elwyn DH, Hyman AI, Carpentier YA, Kinney JM. Nutrition for the patient with respiratory failure: glucose vs. fat. Anesthesiology 1981;54:373-7.

15. Rassias AJ, Marrin CA, Arruda J, Whalen PK, Beach M, Yeager MP. Insulin infusion improves neutrophil function in diabetic cardiac surgery patients. Anesth Analg 1999;88:1011-6.

16. Nordenstrom J, Jeevanandam M, Elwyn DH, Carpentier YA, Askanazi J, Robin A, Kinney JM. Increasing glucose intake during total parenteral nutrition increases norepinephrine excretion in trauma and sepsis. Clin Physiol 1981;1:525-34.

17. Donatelli F. Perioperative infusion of amino acids: the effect of epidural blockade. School of Dietetics and Human Nutrition. Montreal: McGill University, 2005:85.

18. Ljungqvist O, Nygren J, Soop M, Thorell A. Metabolic perioperative management: novel concepts. Curr Opin Crit Care 2005;11:295-9.

19. Hill AG. Initiators and propagators of the metabolic response to injury. World J Surg 2000;24:624-9.

20. Desborough JP. The stress response to trauma and surgery. Br J Anaesth 2000;85:109-17.

21. Lin E, Calvano SE, Lowry SF. Inflammatory cytokines and cell response in surgery. Surgery 2000;127:117-26.

22. Wilmore DW. Metabolic response to severe surgical illness: overview. World J Surg 2000;24:705-11.

23. Mannick JA, Rodrick ML, Lederer JA. The immunologic response to injury. J Am Coll Surg 2001;193:237-44.

24. Christopherson R, Beattie C, Frank SM, Norris EJ, Meinert CL, Gottlieb SO, Yates H, Rock P, Parker SD, Perler BA, et al. Perioperative morbidity in patients randomized to epidural or general anesthesia for lower extremity vascular surgery. Perioperative Ischemia Randomized Anesthesia Trial Study Group. Anesthesiology 1993;79:422-34.

25. Brownlee M. A radical explanation for glucose-induced beta cell dysfunction. J Clin Invest 2003;112:1788-90.

26. Kinney JM, Elwyn DH. Protein metabolism and injury. Annu Rev Nutr 1983;3:433-66.

27. Watters JM, Clancey SM, Moulton SB, Briere KM, Zhu JM. Impaired recovery of strength in older patients after major abdominal surgery. Ann Surg 1993;218:380-90; discussion 390-3.

28. Bonadonna R, Groop L, Simonson D, DeFronzo R. Free fatty acid and glucose metabolism in human aging: Evidence for operation of the Randle cycle. Am J Physiol 1994:E501-E509.

29. Carpentier A, Mittelman S, Lamarche B, Berman R, Giacca A, Lewis G. Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. Am J Physiol 1999:E1055-E1066.

30. Mittelman S, Bergman R. Inhibition of lipolysis causes suppression of endogenous glucose production independent of changes in insulin. Am J Physiol 2000:E630-E637.

31. Lattermann R, Carli F, Schricker T. Epidural blockade suppresses lipolysis during major abdominal surgery. Reg Anesth Pain Med 2002;27:469-75.

32. Opie L. Metabolism of free fatty acids, glucose and catecholamines in acute myocardial infarction. Relation to myocardial ischemia and infarct size. Am J Cardiol 1975:938-953.

33. Richardson AP, Tayek JA. Type 2 diabetic patients may have a mild form of an injury response: a clinical research center study. Am J Physiol Endocrinol Metab 2002;282:E1286-90.

34. Ziegler TS, RJ. Parenteral nutrition in patients with diabetes mellitus. In: Rombeau JL, Caldwell MD (Eds.). Clinical Nutrition: Parenteral Nutrition. Saunders, 1993.

35. Barker JP, Robinson PN, Vafidis GC, Burrin JM, Sapsed-Byrne S, Hall GM. Metabolic control of non-insulin-dependent diabetic patients undergoing cataract surgery: comparison of local and general anaesthesia. Br J Anaesth 1995;74:500-5.

36. Fotiadis RJ, Badvie S, Weston MD, Allen-Mersh TG. Epidural analgesia in gastrointestinal surgery. Br J Surg 2004;91:828-41.

37. Hahnenkamp K, Herroeder S, Hollmann MW. Regional anaesthesia, local anaesthetics and the surgical stress response. Best Pract Res Clin Anaesthesiol 2004;18:509-27.

38. Kehlet H. Modification of responses to surgery by neural blockade: Clinical implications. In: Cousins MJ, Bridenbaugh PS edn.: Neural blockade in clinical anesthesia and management of pain. Lippincott-Raven, 1998.

39. Carli F, Halliday D. Modulation of protein metabolism in the surgical patient. Effect of 48-hour continuous epidural block with local anesthetics on leucine kinetics. Reg Anesth 1996;21:430-5.

40. Segawa H, Mori K, Kasai K, Fukata J, Nakao K. The role of the phrenic nerves in stress response in upper abdominal surgery. Anesth Analg 1996;82:1215-24.

41. Moller IW, Dinesen K, Sondergard S, Knigge U, Kehlet H. Effect of patient-controlled analgesia on plasma catecholamine, cortisol and glucose concentrations after cholecystectomy. Br J Anaesth 1988;61:160-4.

42. Christensen P, Brandt MR, Rem J, Kehlet H. Influence of extradural morphine on the adrenocortical and hyperglycaemic response to surgery. Br J Anaesth 1982;54:23-7.

43. Hosoda R, Hattori M, Shimada Y. Favorable effects of epidural analgesia on hemodynamics, oxygenation and metabolic variables in the immediate post-anesthetic period. Acta Anaesthesiol Scand 1993;37:469-74.

44. Kouraklis G, Glinavou A, Raftopoulos L, Alevisou V, Lagos G, Karatzas G. Epidural analgesia attenuates the systemic stress response to upper abdominal surgery: a randomized trial. Int Surg 2000;85:353-7.

45. Brandt MR, Fernades A, Mordhorst R, Kehlet H. Epidural analgesia improves postoperative nitrogen balance. Br Med J 1978;1:1106-8.

46. Vedrinne C, Vedrinne JM, Guiraud M, Patricot MC, Bouletreau P. Nitrogen-sparing effect of epidural administration of local anesthetics in colon surgery. Anesth Analg 1989;69:354-9.

47. Tsuji H, Shirasaka C, Asoh T, Uchida I. Effects of epidural administration of local anaesthetics or morphine on postoperative nitrogen loss and catabolic hormones. Br J Surg 1987;74:421-5.

48. Carli F, Webster J, Pearson M, Pearson J, Bartlett S, Bannister P, Halliday D. Protein metabolism after abdominal surgery: effect of 24-h extradural block with local anaesthetic. Br J Anaesth 1991;67:729-34.

49. Barratt SM, Smith RC, Kee AJ, Carlsson AR, Mather LE, Cousins MJ. Epidural analgesia reduces the release of amino acids from peripheral tissues in the ebb phase of the metabolic response to major upper abdominal surgery. Anaesth Intensive Care 1999;27:26-32.

50. Carli F, Halliday D. Continuous epidural blockade arrests the postoperative decrease in muscle protein fractional synthetic rate in surgical patients. Anesthesiology 1997;86:1033-40.

51. Krinsley J. Perioperative glucose control. Curr Opin Anaesthesiol 2006;19:111-6.

52. Jensen CH, Berthelsen P, Kuhl C, Kehlet H. Effect of epidural analgesia on glucose tolerance during surgery. Acta Anaesthesiol Scand 1980;24:472-4.

53. Houghton A, Hickey JB, Ross SA, Dupre J. Glucose tolerance during anaesthesia and surgery. Comparison of general and extradural anaesthesia. Br J Anaesth 1978;50:495-9.

54. Lund J, Stjernstrom H, Jorfeldt L, Wiklund L. Effect of extradural analgesia on glucose metabolism and gluconeogenesis. Studies in association with upper abdominal surgery. Br J Anaesth 1986;58:851-7.

55. Ward N. Nutrition support to patients undergoing gastrointestinal surgery. Nutr J 2003;2:18.

56. Sax HC. Immunonutrition and upper gastrointestinal surgery: what really matters. Nutr Clin Pract 2005;20:540-3.

57. Woods JH, Erickson LW, Condon RE, Schulte WJ, Sillin LF. Postoperative ileus: a colonic problem? Surgery 1978;84:527-33.

58. Reissman P, Teoh TA, Cohen SM, Weiss EG, Nogueras JJ, Wexner SD. Is early oral feeding safe after elective colorectal surgery? A prospective randomized trial. Ann Surg 1995;222:73-7.

59. Braga M, Gianotti L, Gentilini O, Liotta S, Di Carlo V. Feeding the gut early after digestive surgery: results of a nine-year experience. Clin Nutr 2002;21:59-65.

60. Beier-Holgersen R, Boesby S. Influence of postoperative enteral nutrition on postsurgical infections. Gut 1996;39:833-5.

61. Schroeder D, Gillanders L, Mahr K, Hill GL. Effects of immediate postoperative enteral nutrition on body composition, muscle function, and wound healing. JPEN J Parenter Enteral Nutr 1991;15:376-83.

62. Reynolds JV. Gut barrier function in the surgical patient. Br J Surg 1996;83:1668-9.

63. Wolfe R. Carbohydrate metabolism and requirements. In: Rombeau J, Caldwell M (Eds.). Clinical nutrition: parenteral nutrition. WB Saunders Company, 1993.

64. Shaw JH, Klein S, Wolfe RR. Assessment of alanine, urea, and glucose interrelationships in normal subjects and in patients with sepsis with stable isotopic tracers. Surgery 1985;97:557-68.

65. Shaw JH, Wolfe RR. An integrated analysis of glucose, fat, and protein metabolism in severely traumatized patients. Studies in the basal state and the response to total parenteral nutrition. Ann Surg 1989;209:63-72.

66. Thorell A, Nygren J, Ljungqvist O. Insulin resistance: a marker of surgical stress. Curr Opin Clin Nutr Metab Care 1999;2:69-78.

67. Rizza RA, Mandarino LJ, Gerich JE. Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor detect of insulin action. J Clin Endocrinol Metab 1982;54:131-8.

68. Deibert DC, DeFronzo RA. Epinephrine-induced insulin resistance in man. J Clin Invest 1980;65:717-21.

69. Pacy PJ, Cheng KN, Ford GC, Halliday D. Influence of glucagon on protein and leucine metabolism: a study in fasting man with induced insulin resistance. Br J Surg 1990;77:791-4.

70. Kollind M, Adamson U, Lins PE, Efendic S. Diabetogenic action of GH and cortisol in insulin-dependent diabetes mellitus. Aspects of the mechanisms behind the Somogyi phenomenon. Horm Metab Res 1987;19:156-9.

71. Brandi LS, Frediani M, Oleggini M, Mosca F, Cerri M, Boni C, Pecori N, Buzzigoli G, Ferrannini E. Insulin resistance after surgery: normalization by insulin treatment. Clin Sci (Lond) 1990;79:443-50.

72. Sakurai Y, Aarsland A, Herndon DN, Chinkes DL, Pierre E, Nguyen TT, Patterson BW, Wolfe RR. Stimulation of muscle protein synthesis by long-term insulin infusion in severely burned patients. Ann Surg 1995;222:283-94; 294-7.

73. Woolfson AM, Heatley RV, Allison SP. Insulin to inhibit protein catabolism after injury. N Engl J Med 1979;300:14-7.

74. Hall RI, Grant JP, Ross LH, Coleman RA, Bozovic MG, Quarfordt SH. Pathogenesis of hepatic steatosis in the parenterally fed rat. J Clin Invest 1984;74:1658-68.

75. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-3C]leucine. Am J Physiol 1980;238:E473-9.

76. Picou D, Taylor-Roberts T. The measurement of total protein synthesis and catabolism and nitrogen turnover in infants in different nutritional states and receiving different amounts of dietary protein. Clin Sci 1969;36:283-96.

77. Kalhan SC. Stable isotopic tracers for studies of glucose metabolism. J Nutr 1996;126:362S-369S.

78. Gump FE, Long CL, Geiger JW, Kinney JM. The significance of altered gluconeogenesis in surgical catabolism. J Trauma 1975;15:704.

79. Landau BR, Wahren J, Chandramouli V, Schumann WC, Ekberg K, Kalhan SC. Contributions of gluconeogenesis to glucose production in the fasted state. J Clin Invest 1996;98:378-85.

80. Simmons PS, Miles JM, Gerich JE, Haymond MW. Increased proteolysis. An effect of increases in plasma cortisol within the physiologic range. J Clin Invest 1984;73:412-20.

81. Castellino P, Luzi L, Simonson DC, Haymond M, DeFronzo RA. Effect of insulin and plasma amino acid concentrations on leucine metabolism in man. Role of substrate availability on estimates of whole body protein synthesis. J Clin Invest 1987;80:1784-93.

82. Schricker T, Klubien K, Wykes L, Carli F. Effect of epidural blockade on protein, glucose, and lipid metabolism in the fasted state and during dextrose infusion in volunteers. Anesthesiology 2000;92:62-9.

83. Schricker T, Klubien K, Carli F. The independent effect of propofol anesthesia on whole body protein metabolism in humans. Anesthesiology 1999;90:1636-42.

84. Mackenzie ML, Warren MR, Wykes LJ. Colitis increases albumin synthesis at the expense of muscle protein synthesis in macronutrient-restricted piglets. J Nutr 2003;133:1875-81.

85. Hachey DL, Patterson BW, Reeds PJ, Elsas LJ. Isotopic determination of organic keto acid pentafluorobenzyl esters in biological fluids by negative chemical ionization gas chromatography/mass spectrometry. Anal Chem 1991;63:919-23.

86. Schricker T, Albuszies G, Weidenbach H, Beckh K, Ensinger H, Anhaupl T, Radermacher P, Vogt J, Adler G, Georgieff M. Effects of epinephrine on glucose metabolism in patients with alcoholic cirrhosis. Hepatology 1996;24:330-6.

87. Schwenk WF, Beaufrere B, Haymond MW. Use of reciprocal pool specific activities to model leucine metabolism in humans. Am J Physiol 1985;249:E646-50.

88. Ang B, Wade A, Halliday D, Powell-Tuck J. Insulin reduces leucine oxidation and improves net leucine retention in parenterally fed humans. Nutrition 2000;16:221-5.

89. Detsky AS, Baker JP, O'Rourke K, Johnston N, Whitwell J, Mendelson RA, Jeejeebhoy KN. Predicting nutrition-associated complications for patients undergoing gastrointestinal surgery. JPEN J Parenter Enteral Nutr 1987;11:440-6.

90. Baker JP, Detsky AS, Wesson DE, Wolman SL, Stewart S, Whitewell J, Langer B, Jeejeebhoy KN. Nutritional assessment: a comparison of clinical judgement and objective measurements. N Engl J Med 1982;306:969-72.

91. Yamanaka H, Nishi M, Kanemaki T, Hosoda N, Hioki K, Yamamoto M. Preoperative nutritional assessment to predict postoperative complication in gastric cancer patients. JPEN J Parenter Enteral Nutr 1989;13:286-91.

92. Seltzer MH, Slocum BA, Cataldi-Betcher EL, Fileti C, Gerson N. Instant nutritional assessment: absolute weight loss and surgical mortality. JPEN J Parenter Enteral Nutr 1982;6:218-21.

93. Reinhardt GF, Myscofski JW, Wilkens DB, Dobrin PB, Mangan JE, Jr., Stannard RT. Incidence and mortality of hypoalbuminemic patients in hospitalized veterans. JPEN J Parenter Enteral Nutr 1980;4:357-9.

94. Hoogwerf BJ. Perioperative management of diabetes mellitus: how should we act on the limited evidence? Cleve Clin J Med 2006;73 Suppl 1:S95-9.

95. Wilmore DW. Catabolic illness. Strategies for enhancing recovery. N Engl J Med 1991;325:695-702.

96. Shaw JH. Influence of stress, depletion, and/or malignant disease on the responsiveness of surgical patients to total parenteral nutrition. Am J Clin Nutr 1988;48:144-7.

97. Shaw JH, Wolfe RR. Glucose and urea kinetics in patients with early and advanced gastrointestinal cancer: the response to glucose infusion, parenteral feeding, and surgical resection. Surgery 1987;101:181-91.

98. Veterans Administration cooperative trial of perioperative total parenteral nutrition in malnourished surgical patients. Background, rationale, and study protocol. Am J Clin Nutr 1988;47:351-91.

99. Hawkins M, Tonelli J, Kishore P, Stein D, Ragucci E, Gitig A, Reddy K. Contribution of elevated free fatty acid levels to the lack of glucose effectiveness in type 2 diabetes. Diabetes 2003;52:2748-58.

100. Mevorach M, Giacca A, Aharon Y, Hawkins M, Shamoon H, Rossetti L. Regulation of endogenous glucose production by glucose per se is impaired in type 2 diabetes mellitus. J Clin Invest 1998;102:744-53.

101. Basu A, Caumo A, Bettini F, Gelisio A, Alzaid A, Cobelli C, Rizza RA. Impaired basal glucose effectiveness in NIDDM: contribution of defects in glucose disappearance and production, measured using an optimized minimal model independent protocol. Diabetes 1997;46:421-32.

102. Chandramouli V, Ekberg K, Schumann WC, Kalhan SC, Wahren J, Landau BR. Quantifying gluconeogenesis during fasting. Am J Physiol 1997;273:E1209-15.

103. Basu R, Schwenk WF, Rizza RA. Both fasting glucose production and disappearance are abnormal in people with "mild" and "severe" type 2 diabetes. Am J Physiol Endocrinol Metab 2004;287:E55-62.

104. Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI. Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. J Clin Invest 1992;90:1323-7.

105. Umpleby AM, Scobie IN, Boroujerdi MA, Carson ER, Sonksen PH. Diurnal variation in glucose and leucine metabolism in non-insulin-dependent diabetes. Diabetes Res Clin Pract 1990;9:89-96.

106. Shaw JH, Humberstone DM, Wolfe RR. Energy and protein metabolism in sarcoma patients. Ann Surg 1988;207:283-9.

107. Humberstone DA, Shaw JH. Metabolism in hematologic malignancy. Cancer 1988;62:1619-24.

108. Donatelli F, Schricker T, Mistraletti G, Asenjo F, Parrella P, Wykes L, Carli F. Postoperative Infusion of Amino Acids Induces a Positive Protein Balance Independently of the Type of Analgesia Used. Anesthesiology 2006;105:253-259.

109. Humberstone DA, Koea J, Shaw JH. Relative importance of amino acid infusion as a means of sparing protein in surgical patients. JPEN J Parenter Enteral Nutr 1989;13:223-7.

110. Charlton M, Nair KS. Protein metabolism in insulin-dependent diabetes mellitus. J Nutr 1998;128:323S-327S.

111. Luzi L, Petrides AS, De Fronzo RA. Different sensitivity of glucose and amino acid metabolism to insulin in NIDDM. Diabetes 1993;42:1868-77.

112. Halvatsiotis P, Short KR, Bigelow M, Nair KS. Synthesis rate of muscle proteins, muscle functions, and amino acid kinetics in type 2 diabetes. Diabetes 2002;51:2395-404.

113. Gougeon R, Marliss EB, Jones PJ, Pencharz PB, Morais JA. Effect of exogenous insulin on protein metabolism with differing nonprotein energy intakes in Type 2 diabetes mellitus. Int J Obes Relat Metab Disord 1998;22:250-61.

114. Gougeon R, Pencharz PB, Sigal RJ. Effect of glycemic control on the kinetics of whole-body protein metabolism in obese subjects with non-insulin-dependent diabetes mellitus during iso- and hypoenergetic feeding. Am J Clin Nutr 1997;65:861-70.

115. Charlton MR, Nair KS. Role of hyperglucagonemia in catabolism associated with type 1 diabetes: effects on leucine metabolism and the resting metabolic rate. Diabetes 1998;47:1748-56.

116. Windsor JA, Hill GL. Weight loss with physiologic impairment. A basic indicator of surgical risk. Ann Surg 1988;207:290-6.

117. Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. Ann N Y Acad Sci 1959;82:420-30.

118. Abu-Lebdeh HS, Nair KS. Protein metabolism in diabetes mellitus. Baillieres Clin Endocrinol Metab 1996;10:589-601.

119. Inchiostro S, Biolo G, Bruttomesso D, Fongher C, Sabadin L, Carlini M, Duner E, Tiengo A, Tessari P. Effects of insulin and amino acid infusion on leucine and phenylalanine kinetics in type 1 diabetes. Am J Physiol 1992;262:E203-10.

120. Halvatsiotis PG, Turk D, Alzaid A, Dinneen S, Rizza RA, Nair KS. Insulin effect on leucine kinetics in type 2 diabetes mellitus. Diabetes Nutr Metab 2002;15:136-42.

121. Krebs M, Krssak M, Bernroider E, Anderwald C, Brehm A, Meyerspeer M, Nowotny P, Roth E, Waldhausl W, Roden M. Mechanism of amino acid-induced skeletal muscle insulin resistance in humans. Diabetes 2002;51:599-605.

122. Staten MA, Matthews DE, Bier DM. Leucine metabolism in type II diabetes mellitus. Diabetes 1986;35:1249-53.

September 15, 2006

To Whom It May Concern:

The purpose of the present letter is to confirm that the co-authors (Francesco Donatelli, Thomas Schricker, Linda Wykes and Franco Carli) agree that the candidate (Andrea Kopp Lugli) includes the two manuscripts entitled *The effect of amino acids on the catabolic response to surgery in diabetes mellitus type 2 patients in comparison to dextrose* and *Can epidural analgesia mitigate the catabolic response to surgery in diabetes mellitus type 2 patients receiving amino acid infusion?* in her thesis.

The candidate's roles in these studies included developing the study designs, recruiting the patients, performing the study sessions according to the protocol, assessing, derivatizing and analyzing the samples with gas chromatography and mass spectrometry. The candidate wrote the manuscripts under the guidance of the co-authors and made modifications to it in response to their comments.

Andrea Kopp Lugli

I, the co-author, agree that the candidate, Andrea Kopp Lugli, includes the manuscripts entitled *The effect of amino acids on the catabolic response to surgery in diabetes mellitus type 2 patients in comparison to dextrose* and *Can epidural analgesia mitigate the catabolic response to surgery in diabetes mellitus type 2 patients receiving amino acid infusion?* in her thesis.

Francesco Donatelli_____

Thomas Schricker_____

Linda Wykes_____

Franco Carli_____

Informed consent form

Can epidural analgesia revert the catabolic response to surgery in type 2 diabetic patients?

Principal Investigator: Dr Franco Carli, Dr Andrea Kopp Lugli, Dr Francesco Donatelli, Department of Anesthesia
Collaborators: Dr B Stein, Dr P Charlebois, Department of Surgery
Participating Institutions: McGill University Health Centre

Introduction

You are being invited to participate in this study and you will soon undergo surgery and you are known to be diabetic, and you have discussed with your treating surgeon, and are prepared to receive either patient controlled analgesia (PCA) with intravenous morphine or epidural analgesia.

Before deciding to participate in the study, you should clearly understand its requirements, risks and benefits. This document provides information about the study, and it may contain words you do not fully understand. Please read it carefully and ask the study staff any questions you may have. They will discuss the study with you in detail. You may take this form with you and discuss the study with anyone else before making any decision. If you decide to participate, you will be asked to sign this form and a copy will be given to you.

Study Description

Loss of muscle mass and body protein is the principal cause of fatigue and decreased muscle strength after surgery. This protein catabolism is even more intense in type 2 diabetic patients. There is evidence that a specialist in anesthesia can influence these by choosing the type of pain treatment (analgesics) and by providing nutrients (food) after the operation. Patients who are treated with local anesthetics (freezing medication) through epidural catheters for analgesia after surgery and are fed with **amino acids (proteins)** over a short period (3 hours) lose less proteins than patients without epidural analgesia (PCA with morphine). An epidural catheter is a little plastic tube that is placed in a space surrounding the spinal cord under the skin of the back. PCA means that morphine is given intravenously (IV) with a pump that you control when you have pain by pressing a button.

Purpose of the study

The purpose of this study is to assess if epidural analgesia compared to patient controlled analgesia (PCA) with morphine increase protein conservation (this means that your body will be less inclined to digest muscle to derive energy) in patients who suffer from diabetes, and to determine if the same benefit seen in non-diabetic patients will be shown in diabetic patients.

A total of twenty (20) subjects undergoing elective colorectal surgery will be enrolled in this study at the Montreal General Hospital for two study period of six (6) hours each, the day before surgery and two days after surgery. The overall research study will last within twelve (12) months.

Study procedures

If you agree to participate in this study, and you have accepted to receive either PCA or epidural analgesia for pain control after your surgery, you will be randomly assigned (like a flip of a coin) to one of the following two groups:

Group 1: you will receive epidural local anesthetic bupivacaine (freezing solution) with fentanyl (morphine-like) through the epidural catheter.

Group 2: you will receive PCA morphine where the pump is programmed in order to avoid an overdose of morphine.

On the day before surgery we will conduct the study (6 hours) in the anesthesia laboratory during the morning hours. You will be fasting from midnight and taking only water during the study period (6 hours). Once the study is over (around 13:00) you will be able to take clear fluids as needed. Once the study is finished the nurse will provide you with instruction to take some medication to clear your bowels for surgery. If you live far from the hospital, or you are diabetic on insulin, you will stay in hospital after the study and go to the surgical ward to receive the bowel prep. After the first 3 hours of the study you will receive a venous infusion (IV infusion) of **amino acids (like proteins)** for 3 hours.

In order to assess the efficiency of analgesia and feeding to reduce body protein losses, the use of a "stable isotope" (a non radioactive tracer technique) will be applied and the isotopes will be infused into a vein in your hand for 6 hours the day before surgery and on the second day after surgery. The stable isotopes used for the study have been developed for use in humans.

Changes in glucose and protein metabolism will be measured to determine whether epidural analgesia, instead of PCA morphine, facilitates the use of infused **amino acids (like proteins)**, therefore minimizing the loss of proteins from your body.

Blood samples (100 cc/ half of cup) will be drawn after 3 and 6 hours of isotope infusion from a second plastic tube that will be inserted in one of your veins of your arm. This amount is approximately a quarter of the amount that is usually taken when giving a blood donation, and less than 3% of your total blood.

Whether or not you are part of the study, this will not influence your standard of care for surgery. Following surgery you will be transferred to the recovery room, where the study doctor and nurse will monitor you until your cardiorespiratory system and sufficient pain control will be stabilized. You will then be transferred to the ward and seen daily by the acute Pain Team to make sure that pain treatment is effective and your pain relief is satisfactory.

Possible Risks and Discomforts

Blood tests: The taking of blood samples may cause some discomfort, fainting, formation of a small blood clot or swelling of the vein on surrounding tissue, bleeding from the puncture site, and /or rarely an infection. There is a possibility that you may faint, however, precautions will be taken to ensure your safety should this occur.

Epidural catheter: The risks associated with the use of an epidural catheter include the following: a temporary fall in blood pressure, temporary headache and possible bruising in the back. However, this is a technique that is used on an everyday basis by the anesthesiologists of the Montreal General Hospital and, therefore, these complications rarely occur and do not cause permanent damage.

Patient Controlled Analgesia: The risks associated with the use of intravenous morphine include the following: nausea and vomiting (10-70%- controlled with medications) and deep sedation (where your respiration decreases and you are heavily sedated). However, this is a technique that is used on an everyday basis by the anesthesiologists of the Montreal General Hospital and, therefore, these complications rarely occur and do not cause permanent damage.

Possible benefits

You should not expect any direct benefit from participating in the study. The information from the study will help further our knowledge of this disease, and potentially help improve future patient care.

Costs and compensation for participation

You will not be compensated for your participation in this study.

Indemnification

The McGill University Health Centre, the MUHC Research Institute, and the investigators would not be able to offer compensation in the unlikely event of any injury resulting from your participation in this research study. However, you are not giving up any of your legal rights by signing this consent form and agreeing to participate to this study.

Confidentiality

The team of researchers of the Montreal General Hospital will consult your medical file to take note of the relevant data to this research project. All information (medical history, physical examinations, laboratory results) will be kept strictly confidential by identifying you by a code to which only authorized personnel will have access. The results from this study may be published, and other physicians participating in this research study may have access to your records related to this research study; however, your identity will not be revealed in the combined results. In order to verify the research study data, monitors from one of the McGill University Health Centre Research Ethics Boards may review these records.

By signing this consent form, you give us permission to release information regarding your participation in this study to these individuals, and to inform your treating physician of your participation in the research study. Your confidentiality will be protected to the extent permitted by applicable laws and regulations.

Voluntary participation and/or withdrawal

Your participation in this study is strictly voluntary. You may refuse to participate or you may discontinue your participation at any time without explanation, and without penalty or loss of benefits to which you are otherwise entitled. If you decide not to participate, or if you discontinue your participation, appropriate alternative therapies will be made available, and you will suffer no prejudice regarding medical care or your participation in any other research studies. If you decide to discontinue your participation, the study doctor will ask you to return for a final visit. You will be informed of any new findings that may affect your willingness to continue your participation.

The study doctor may end your participation if you experience excessive side effects or deterioration in your health, if you do not follow study procedures, or if you need a medication that is not allowed during the study, if the findings indicate your continuation in the study would be dangerous or detrimental to your health, or for administrative reasons unrelated to the purpose of the study. In addition, one of the McGill University Health Centre Research Ethics Boards may terminate the study.

Formulaire de consentement éclairé

Est-ce qu'une analgésie péridurale peut renverser la réponse catabolique à la chirurgie chez les patients souffrant de diabète de type 2?

Chercheur principal : Dr Franco Carli, Dr Andrea Kopp Lugli, Dr Francesco Donatelli, Service d'anesthésie

Collaborateurs : Dr B. Stein, Dr P. Charlebois, Département de chirurgie

Institutions participantes : Centre universitaire de santé McGill

Introduction

Vous avez été invité(e) à participer à cette étude parce que vous devez bientôt subir une chirurgie et que vous êtes diabétique; vous avez discuté avec votre chirurgien et êtes préparé à recevoir une analgésie contrôlée par le patient (PCA) en recevant de la morphine par voie intraveineuse ou une analgésie péridurale.

Avant de décider de participer à cette étude, vous devriez comprendre clairement ses exigences, ses risques et ses bénéfices. Ce document procure de l'information sur l'étude et peut contenir des termes qui ne vous sont pas familiers. Veuillez le lire attentivement et poser toute question au personnel de l'étude. Ils discuteront de l'étude en détail avec vous. Vous pouvez apporter ce formulaire avec vous et discuter de cette étude avec toute autre personne avant de prendre votre décision. Si vous décidez d'y participer, on vous demandera de signer ce formulaire et on vous en remettra une copie.

Description de l'étude

La perte de masse musculaire et de protéine organique est la cause principale de la fatigue et de la diminution de la force musculaire suite à une chirurgie. Ce catabolisme protéique est encore plus intense chez les patients souffrant de diabète de type 2. Il a été démontré que les anesthésiologistes peuvent influencer ces changements en choisissant le type de traitement de la douleur (analgésiques) et en administrant des nutriments (substances alimentaires) après la chirurgie. Les patients qui sont traités avec des agents anesthésiques locaux (médicaments pour geler) via un cathéter péridural pour l'analgésie suite à une chirurgie et qui reçoivent **des acides aminés (protéines)** pour une courte période de temps (3 heures) perdent moins de protéines organiques que les patients n'ayant pas reçu d'analgésie par voie péridurale (PCA avec morphine). Un cathéter péridural est une petite canule de plastique qui est insérée sous la peau dans votre dos dans l'espace entourant votre moelle épinière. La PCA implique que vous receviez de la morphine par voie intraveineuse (IV) via une pompe que vous contrôlez en pressant un bouton lorsque vous ressentez de la douleur.

But de l'étude

Le but de cette étude est d'évaluer si le fait de recevoir une analgésie péridurale plutôt qu'une analgésie contrôlée par le patient (PCA) avec morphine augmente la conservation protéique (ce qui veut dire que votre corps sera moins enclin à digérer les muscles qui génèrent l'énergie) chez les patients souffrant de diabète et aussi de déterminer si les mêmes bénéfices observés chez les patients non diabétiques pourraient être démontrés chez les patients diabétiques.

Un total de vingt (20) patients devant subir une chirurgie colorectale élective seront inscrits à cette étude à l'hôpital général de Montréal pour deux périodes d'étude de 6 heures chacune, la journée de la chirurgie et 2 jours suivant la chirurgie. La recherche associée à cette étude sera complétée au cours des douze (12) prochains mois.

Procédure de l'étude

Si vous acceptez de participer à cette étude et de recevoir soit une PCA ou une analgésie péridurale pour le contrôle de la douleur suite à votre chirurgie, vous serez assigné(e) au hasard à l'un des deux groupes suivants :

Groupe 1. Vous recevrez un agent anesthésique local, la bupivacaine (médicament pour geler) avec du fentanyl (médicament morphinique) via le cathéter péridural.

Groupe 2. Vous recevrez de la morphine par PCA où la pompe est programmée afin d'éviter une surdose de morphine.

L'étude débutera la journée précédent votre chirurgie durant l'avant-midi (6 heures) au laboratoire d'anesthésie. Vous serez à jeun depuis minuit et ne pourrez boire que de l'eau durant la période d'étude (6 heures). Une fois l'étude terminée (vers 13 : 00 heures), vous pourrez prendre des liquides au besoin; l'infirmière vous indiquera comment prendre des médicaments afin de vider vos intestins avant la chirurgie. Si vous demeurez loin de l'hôpital ou si vous êtes diabétique et devez prendre de l'insuline, vous resterez à l'hôpital après l'étude et vous vous rendrez à l'unité chirurgicale afin d'y recevoir votre lavement. Trois heures après le début de l'étude, vous recevrez une infusion intraveineuse (infusion IV) **d'acides aminés (protéines)** pendant les trois prochaines heures.

Afin d'évaluer l'efficacité de l'analgésie et de l'alimentation sur les pertes protéiques organiques, on utilise des isotopes stables (une technique de traceurs non-radioactive) qui seront administrés dans une veine de la main pendant six heures au cours de la journée précédant votre chirurgie et au cours de la deuxième journée après votre chirurgie. Les isotopes stables utilisés pour cette étude ont été développés à des fins d'études sur les humains.

On mesurera ensuite les changements du métabolisme protéique et du glucose afin de déterminer si le fait de recevoir une analgésie péridurale au lieu de morphine par PCA facilite l'utilisation **des acides aminés infusés (protéines)**, réduisant donc la perte de protéines organiques.

Des échantillons sanguins (100 cc/une demi-tasse) seront prélevés d'une deuxième canule veineuse insérée dans une veine de votre bras, 3 et 6 heures suivant l'infusion des isotopes. Cette quantité de sang correspond approximativement au quart de ce qui est habituellement prélevé lorsque vous donnez du sang et moins de 3% de votre volume sanguin total.

Le fait de participer ou non à cette étude n'influencera en aucun cas les standards de soins chirurgicaux. Après votre chirurgie, on vous transférera à la salle de réveil où le médecin responsable de l'étude et une infirmière vous surveilleront jusqu'à ce que votre système cardiorespiratoire et le contrôle de votre douleur soient stables. Vous serez ensuite transféré(e) à l'étage et recevrez quotidiennement la visite d'un membre de l'équipe de la douleur aiguë qui s'assurera que le traitement et le soulagement de votre douleur est efficace et satisfaisant.

Risques et malaises possibles

Prélèvement sanguin : Les prélèvements sanguins peuvent occasionner des inconforts, la possibilité de perte de conscience, la formation d'un petit caillot de sang ou l'enflure d'une veine des tissus environnants, un saignement au site de ponction et/ou plus rarement, une infection. Il y a une possibilité que vous perdiez conscience, cependant, toutes les précautions seront prises afin d'assurer votre sécurité si cela se produisait.

Cathéter péridural : Les risques associés à l'utilisation d'un cathéter péridural incluent : une chute temporaire de la tension artérielle, un mal de tête temporaire et la possibilité d'ecchymose au dos. Cependant, cette technique est utilisée sur une base quotidienne par les anesthésiologistes de l'hôpital général de Montréal et ces complications surviennent que très rarement et ne laissent aucunes séquelles permanentes.

Analgésie contrôlée par le patient : Les risques associés à l'utilisation de morphine par voie intraveineuse incluent : nausée et vomissements (10-70%, contrôlé à l'aide de médicaments) et sédation profonde (où votre respiration diminue et vous êtes sous sédation profonde). Cependant, cette technique est utilisée sur une base quotidienne par les anesthésiologistes de l'hôpital général de Montréal et ces complications surviennent que très rarement et ne laissent aucunes séquelles permanentes.

Bénéfices potentiels

Vous ne devriez vous attendre à retirer aucun bénéfice suite à votre participation à cette étude. Cependant, les informations recueillies aideront à améliorer nos connaissances de cette maladie et potentiellement améliorer les soins prodigués aux futurs patients.

Coûts et compensation associés à votre participation

Vous ne recevrez aucune compensation suite à votre participation à cette étude.

Indemnité

Le centre universitaire de santé McGill, l'Institut de recherche du CUSM et les chercheurs ne seraient pas en mesure de vous compenser dans l'éventualité peu probable où vous subiriez une lésion suite à votre participation à cette étude. Cependant, le fait de participer à cette étude et de signer ce formulaire de consentement ne vous empêchent pas de vous prévaloir de vos droits légaux.

Confidentialité

L'équipe de chercheurs de l'hôpital général de Montréal pourront consulter votre dossier médical afin de noter des informations pertinentes à cette étude. Tous les renseignements (antécédents médicaux, examens physiques, résultats de laboratoires) sont confidentiels et votre identité sera protégée en vous identifiant par un code auquel seulement le personnel autorisé aura accès. Les résultats de cette recherche peuvent être publiés et d'autre médecins participant à cette recherche peuvent avoir accès à votre dossier de recherche; cependant, votre identité ne sera pas dévoilée lors de la publication des résultats. Afin de vérifier certaines données associées à cette recherche, des membres de l'un des comités d'éthique sur la recherche du centre universitaire de santé McGill peuvent réviser ces dossiers.

En signant ce formulaire de consentement, vous nous donnez la permission de divulguer des informations concernant votre participation à cette étude à ces personnes et à votre médecin traitant. Votre anonymat sera protégé dans la mesure des lois et règles applicables.

Participation volontaire et/ou retrait

Votre participation à cette étude est strictement volontaire. Vous pouvez refuser d'y participer ou vous en retirer en tout temps, sans explications et sans que cela n'affecte les soins de santé auxquels vous avez droit. Si vous décidez de ne pas y participer ou de vous retirer, on vous offrira des traitements alternatifs et vous ne subirez aucun préjudice quant à vos soins de santé ou votre participation à toute autre étude. Si vous décidez de vous retirer, le médecin responsable vous demandera de retourner pour une dernière visite. Vous serez avisé de tout nouveau résultat pouvant modifier votre décision de continuer à participer à cette étude.

Le médecin responsable peut interrompre votre participation à l'étude si vous souffrez d'effets secondaires graves, si votre état de santé se détériore, si vous ne suivez pas les procédures associées à l'étude, si vous avez besoin d'un médicament qui ne doit pas être pris au cours de cette étude, si les résultats indiquent que votre participation à cette étude pourrait être dangereuse ou au détriment de votre santé ou pour des raisons administratives non reliées à l'étude. De plus, un membre du comité d'éthique sur la recherche du centre universitaire de santé McGill peut mettre fin à cette étude.

Questions et informations

Pour toutes questions reliées à cette étude, vous pouvez contacter le chercheur principal Dr Franco Carli au (514) 934-1934 poste 43261 ou le signaler au (514) 406-0905 ou le moniteur